Acute kidney injury in patients with acute-on-chronic liver failure: clinical significance and management

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Risk of ventricular tachycardia and its outcomes in patients undergoing continuous renal replacement therapy due to acute kidney injury

Dialysis specialist care and patient survival in hemodialysis facilities: a Korean nationwide cohort study
Aims and Scope

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

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

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

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The image on the front cover: Kong et al reported the shortening of primary cilia in distal tubule cells and principal cells of water-deprived mice. Please see the text for more details (pp. 312-324).
Most cells in the human body have either one or multiple cilia. The cilia are specialized microtubule-based projections from the cells’ apical surface, and they can be classified into motile and primary cilia. Motile cilia are typically distributed in the epithelial cells of the respiratory tract and reproductive system, and these cells have multiple cilia on their apical side [1]. In the kidney, the primary or non-motile cilium, a single or monocilium in one cell, is found on the parietal cells of the Bowman’s capsule and all tubular epithelial cells, with the exception of the intercalated cells in the collecting duct [2].

What roles do primary cilia play in the kidney? They detect chemical and mechanical cues and transduce extracellular information into downstream intracellular signaling mechanisms to maintain homeostasis, and the primary cilia are often referred to as cellular antennae [3]. It is unclear what the primary cilium specifically sense in the tubular lumen or urine flow. Instead, specific downstream signaling transduction pathways, including Hedgehog, Wnt, Notch, calcium, transforming growth factor-β, platelet-derived growth factor, G protein-coupled receptor, and mammalian target of rapamycin, were identified in the primary cilium [1,4]. Moreover, dysfunction of the primary cilia, which contributes to the pathogenesis of a large spectrum of human genetic (e.g., polycystic kidney disease, nephronophthisis, and Bardet-Biedl syndrome) and acquired (e.g., hypertension and diabetes mellitus) diseases are known as ciliopathies. Renal ciliopathies caused by mutations in cilia-associated proteins are characterized by the presence of kidney cysts that develop due to uncontrolled epithelial cell proliferation, growth, and polarity [3]. Table 1 summarizes the major renal ciliopathies that occur as a result of dysregulated primary cilium-dependent signaling.

The structure of the primary cilium is based on an axoneme, which is encased within a membranous sheath that is continuous with the plasma membrane. The axoneme consists of nine bundles of microtubules anchored to the basal body [5]. The biogenesis of cilium can simply be explained by the process of axoneme elongation, and cilium length is modulated by both intrinsic and extrinsic factors. Intrinsic regulators of cilium length (e.g., Kif3a and Pitchfork) are initiated by structural and signaling molecules inside the cell. Extrinsic factors (e.g., fluid shear stress) originate from the extracellular environment, and they most likely regulate cilium length by affecting intracellular signaling [1].

In the current issue of Kidney Research and Clinical Practice, Kong et al. [6] reported an association between water balance and primary cilia length. As expected, they found that water deprivation enhanced urine concentration and
upregulated aquaporin-2 (AQP2) in mice. In addition, the primary cilia length was shortened by water deprivation in association with deacetylation of α-tubulin and increased histone deacetylase 6 (HDAC6) activity. All these changes were blocked by tubastatin, a selective HDAC6 inhibitor.

For the stability and dynamics of primary cilia, axonemal microtubules, consisting of α- and β-tubulins, undergo a wide range of posttranslational modifications, including acetylation, glutamylation, glycosylation, ubiquitination, methylation, and phosphorylation. In particular, acetylation is a characteristic of α-tubulin in the cilia, but not in the cytoplasm [1]. Acetylated α-tubulin was also used in the current study [6] as the standard marker of cilia. Regulators of cilium disassembly, such as the scaffolding protein HEF1, can activate Aurora A kinase, which in turn phosphorylates and stimulates HDAC6, thereby promoting the deacetylation of modified, stabilized tubulins within the axoneme [7]. According to Kong et al. [6], water deprivation acted as a regulator of cilium disassembly in mice. Arginine vasopressin might be a critical initiator of this process (Fig. 1). In polycystic kidney disease, the increased intracellular cyclic adenosine monophosphate (cAMP) level may lead to overexpression of HDAC6 [8]. Cilioplasmic and cytoplasmic cAMP levels may be affected by ciliary and intracellular signaling mechanisms, respectively [9].

However, evidence is lacking to support the authors’ assumption that the shortening of cilia length is related to the antenna function of the primary cilia, sensing urine flow or osmolality. Regarding the AQP2 expression, the role of histone acetylation in AQP2 transcription might have been involved [10]. Consistent with the current study, vasopressin receptor 2 (V2R) was reported to be localized to the cilia in kidney epithelial cells, and the V2R antagonist tolvaptan increased the ciliary length in vitro [9]. Whether changes in primary cilium length occur in animals with water disturbance, including Brattleboro rats and V2R knockout mice,

### Table 1. Major renal ciliopathies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Main associated genes</th>
<th>Protein (localization in cilium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADPKD</td>
<td>PKD1, PKD2</td>
<td>Polycystin complex (ciliary membrane)</td>
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<tr>
<td>ARPKD</td>
<td>PKD1H1</td>
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</tr>
<tr>
<td>BBS</td>
<td>BBS1, BBS10, BBS2</td>
<td>BBsome (basal body)</td>
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<tr>
<td>J BTS</td>
<td>AH1</td>
<td>AH1 (axoneme)</td>
</tr>
<tr>
<td>MKS</td>
<td>MKS1, TMEM216, TMEM67</td>
<td>MKS (axoneme)</td>
</tr>
<tr>
<td>NPHP</td>
<td>NPHP1</td>
<td>NPHP 1.4-8 (axoneme)</td>
</tr>
<tr>
<td>SLS</td>
<td>IQCB1/NPHP5</td>
<td>NPHP 5-6 (basal body)</td>
</tr>
</tbody>
</table>

ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; BBS, Bardet-Biedl syndrome; J BTS, Joubert syndrome; MKS, Meckel-Gruber syndrome; NPHP, nephronophthisis; SLS, Senior-Loken syndrome.
are needed to be investigated.

**Conflicts of interest**

The author has no conflicts of interest to declare.

**Data sharing statement**

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

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**References**

Ureteral obstruction (UO) has a significant impact on renal function by altering hemodynamics, decreasing glomerular filtration, and inducing renal transcriptomic and metabolomic changes. It can also cause structural changes in the kidney parenchyma, most notably hydronephrosis and renal fibrosis. Renal fibrosis, a common feature of chronic kidney disease (CKD), is a complex process characterized by loss of capillary networks, progressive accumulation of fibrous collagens, and activation of myofibroblasts and inflammatory cells. The process results in a substantial decline in renal blood flow and tissue perfusion, impaired tubular handling of water and electrolytes, and an increase in urinary protein excretion. CKD can be caused by many diseases, including diabetic nephropathy, hypertensive nephrosclerosis, glomerulonephritis, chronic obstructive nephropathy, and others. Globally, the prevalence of CKD is increasing, which poses a significant clinical challenge as CKD can progress to end-stage renal disease (ESRD), which requires dialysis and kidney transplantation. Approximately 9.1% of the world’s population, or 700 million individuals, have CKD.

Chronic obstruction of the urinary tract and urine flow leads to obstructive nephropathy and ESRD. Animal models have contributed significantly to the comprehension of the mechanisms underlying structural and functional alterations. In particular, various species with unilateral UO (UUO), a model for experimental hydronephrosis, have revealed novel mechanisms underlying renal fibrosis. However, animal models of complete and irreversible UO, such as UUO, have limitations for examining tubule damage as well as hemodynamic alterations, as the majority of clinical UO in human patients involves partial and chronic UO as opposed to acute and completely irreversible UO.

The pathogenesis of fibrosis is an imbalance between the synthesis, deposition, and degradation of the extracellular matrix. Diverse molecular mechanisms are involved, including growth factors (transforming growth factor-β [TGF-β], platelet-derived growth factor, connective tissue growth factor), endothelin-1, and integrins. Among profibrotic molecules, TGF-β is critical for cell growth, differentiation, proliferation, apoptosis, immune response, and fibrosis. TGF-β promotes fibrosis by both canonical and non-canonical pathways. TGF-β1 binds to TβRII, which activates TβRI by phosphorylation, thereby activating TGF-β1 downstream effectors. Suppressor of mothers against decapentaplegic (Smad) is associated with the canonical pathway. TGF-β1 signaling transduction occurs via the receptor-regulated Smads (R-Smads), e.g., Smad2 and Smad3, both of which are overexpressed in human fibrotic
Phosphorylated Smad2 and Smad3 complexes with Smad4 translocate into the nucleus and modulate the transcription of profibrotic genes, such as collagen I and III. Inhibiting Smad2 in kidney cells (Smad2 knockouts are embryonically lethal) exacerbates fibrosis. In contrast, mice lacking the Smad3 gene exhibit slower progression of renal fibrosis, indicating that Smad2 and Smad3 function in opposition in the progression of renal fibrosis [2]. The non-canonical Smad-independent signaling pathways include mitogen-activated protein kinase (MAPK), Jun N-terminal kinases, Rho-like GTPase, and PI3k/AKT. These pathways influence the transcription of target genes that induce an epithelial-to-mesenchymal transition (EMT) or apoptosis [3].

As treatment strategies for CKD after the development of tubulointerstitial fibrosis are ineffective, researchers should instead focus on elucidating the early disease process. Treatments typically involve reducing the production of fibrotic and inflammatory proteins, suppressing fibroblast proliferation, preventing EMT, reducing oxidative stress, inhibiting the action of nuclear factor-κB (NF-κB), reducing the phosphorylation of Smad2/3 or MAPKs, correcting metabolic acidosis, and inhibiting the activation of the renin-angiotensin system. Fig. 1 provides examples of potential therapeutic targets for inhibiting renal fibrosis progression in CKD [4,5].

In the current issue of *Kidney Res Clin Pract*, Ju et al. [6] reported that treatment with fisetin (3,3’4’,7-tetrahydroxy-flavone), a flavonol found in smoketree (*Cotinus coggygria*) and various fruits and vegetables (e.g., strawberry, apple, grape, persimmon, cucumber, and onion at concentrations in the range of 2–160 μg/g) inhibited the progression of renal fibrosis in the kidneys of C57BL/6 female mice subjected to UUO for 7 days. The proposed mechanisms were inhibition of phosphorylation of Smad3, oxidative damage, inflammation, apoptotic cell death, and decreasing the accumulation of profibrotic M2 macrophages in the obstructed kidneys. Intraperitoneal injections of fisetin (25 mg/kg) were administered one hour prior to surgery and every other day for seven days following surgery. In addition, pretreatment with fisetin (40 μM) substantially decreased the TGF-β1–induced phosphorylation of Smad2 and Smad3 in HK-2 cells, the human proximal tubular cell line. Due to their potential therapeutic pharmacological

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**Figure 1.** Potential therapeutics for the inhibition of renal fibrosis (based on findings from animal studies).

Specific inhibition of transforming growth factor-β (TGF-β)/Smad3 signaling
Angiotensin converting enzyme (ACE) inhibitors, angiotensin type 1 (AT1) receptor antagonists
Introduction of exogenous recombinant bone morphogenetic protein-7 (BMP-7)
Reactivation of endogenous BMP-7 signaling pathways
Inhibition of the integrin-linked kinase (ILK) activity
Inhibition of multiple αv integrins
Blockade of cellular communication network factor 2 (CCN2)
Interleukin-1 (IL-1) receptor antagonist
Purinergic P2X7 receptor antagonist
Adenosine receptor (A2A agonist and an A3 antagonist)
NLR family pyrin domain containing 3 (NLRP3) depletion or inhibitor
Tumor necrosis factor-α (TNF-α) inhibitor
Prostaglandin E2 (EP2) receptor agonist
Prostaglandin PGF2 receptor agonist
Endothelin ETa and ETb receptor antagonist
Overexpression of microRNA (miRNA)-29, miR-9-5p, miR-27b-3p
Sodium-glucose cotransporter 2 (SGLT-2) inhibitor
Glucagon-like peptide-1 (GLP-1) analog
Overexpression of carnitine palmitoyl-transferase 1A (Cpt1a)
Pyruvate dehydrogenase kinase-1 inhibitor
Ca2+/calmodulin-dependent protein kinase type II β-chain (CaMKIIβ) small interfering RNA or overexpression of miR34c-5p
Silencing of secreted modular calcium-binding protein 2 (SMOC2)
Silencing of DNA-dependent protein kinase catalytic subunit (DNA-PKcs)
Silencing of disabled-2 (Dab2)
properties, flavonoids have received considerable attention as pharmaceuticals and health food supplements. Antioxidant, anti-inflammatory, and antineoplastic actions are some of the biological functions attributed to fisetin. Sahu et al. [7] used a cisplatin-induced nephrotoxicity model to show that fisetin protects kidney function by reducing oxidative stress, restoring mitochondrial respiratory enzyme activities, decreasing apoptosis in renal tubular cells, and inhibiting NF-κB activation. Moreover, fisetin improves renal fibrosis in the hyperuricemic nephropathy model, as shown by Ren et al. [8] via modulation of STAT3 and TGF-β1/Smad3 signaling.

Based on their chemical structure, flavonoids can be classified into flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones. Fisetin, the most prevalent bioactive flavonol, exerts a variety of biological effects, including anti-inflammatory properties and decreases in oxidative stress and apoptotic cell death [9]. Fisetin forms complexes with Al(III), Cu(II), Zn(II), and Pb(II) at its ligand chelation sites (3-hydroxy-4-Oxo and 3’,4’-dihydroxy group). Due to its ability to accept free radicals, it was hypothesized that the association of flavonoids with a metal ion would enhance their biological activity. In addition, it was demonstrated that metal complexation promotes the antioxidant properties of flavonoids.

An acute oral toxicity study revealed that the complex has an lethal dose 50% of 500 mg/kg; however, there were no treatment-related alterations in hematological and serum biochemical parameters in the 50, 100, and 200 mg/kg groups [10]. Interestingly, a previous study showed moderate infiltration of inflammatory cells in the kidney tissues of rats administered 2.5 mg/kg fisetin intraperitoneally [6]. In the current issue, however, Ju et al. [6] employed intraperitoneal injections of fisetin (25 mg/kg) in mice, where the number of F4/80-positive cells in the kidneys was comparable between vehicle-treated control mice and fisetin-treated control mice. Further studies are needed to examine the dose-dependent response in the kidney.

There is evidence that fisetin has anti-inflammatory, anti-neoplastic, and antioxidant properties. More in vitro and in vivo research is needed to confirm that fisetin has renoprotective effects and to determine the precise molecular mechanisms by which it slows the progression of CKD, including obstructive nephropathy. Patients with renal disease, especially those at a more advanced stage of renal fibrosis or CKD, have rarely been cured by single-agent-targeted therapies. There is a need for additional research into target identification and pathway analysis.

**Conflicts of interest**

The author has no conflicts of interest to declare.

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**Data sharing statement**

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

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Acute kidney injury in patients with acute-on-chronic liver failure: clinical significance and management

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Acute-on-chronic-liver failure (ACLF) refers to a phenomenon in which patients with chronic liver disease develop multiple organ failure due to acute exacerbation of underlying liver disease. More than 10 definitions of ACLF are extant around the world, and there is lack of consensus on whether extrahepatic organ failure is a main component or a consequence of ACLF. Asian and European consortia have their own definitions of ACLF. The Asian Pacific Association for the Study of the Liver ACLF Research Consortium does not consider kidney failure as a diagnostic criterion for ACLF. Meanwhile, the European Association for the Study of the Liver Chronic Liver Failure and the North American Consortium for the Study of End-stage Liver Disease do consider kidney failure as an important factor in diagnosing and assessing the severity of ACLF. When kidney failure occurs in ACLF patients, treatment varies depending on the presence and stage of acute kidney injury (AKI). In general, the diagnosis of AKI in cirrhotic patients is based on the International Club of Ascites criteria: an increase of 0.3 mg/dL or more within 48 hours or a serum creatinine increase of 50% or more within one week. This study underscores the importance of kidney failure or AKI in patients with ACLF by reviewing its pathophysiology, prevention methods, and treatment approaches.

Keywords: Acute kidney injury, Acute-on-chronic-liver failure, Renal insufficiency

Introduction

Broadly, there are three clinical stages of liver cirrhosis: compensated cirrhosis, decompensated cirrhosis, and late decompensated cirrhosis (DC) [1]. However, in 1997, Jalan et al. [2] reported several cases of young cirrhotic patients who died shortly after a transjugular intrahepatic porto-systemic shunt procedure for variceal bleeding due to increased intracranial pressure and rapid liver function deterioration. It is inappropriate to classify these cases into the extant cirrhosis stages because increased intracranial pressure is frequently found in acute liver failure rather than in DC. Accordingly, acute exacerbation of existing chronic liver disease has been officially referred to as acute-on-chronic-liver failure (ACLF) [3].

As suggested, ACLF refers to a specific condition of preexisting chronic liver disease (with or without liver cirrhosis) that is associated with rapid deterioration of liver function, multiorgan failure, and increased mortality [4-9]. The prognosis of ACLF is notably different from that of cirrhosis.
rhosis in that ACLF tends to get worse suddenly [4,10]. The socioeconomic burden of ACLF is estimated to be much larger than that of liver cirrhosis. In the United States, significantly greater per capita hospitalization costs ($51,000 vs. $14,000), hospitalization days (16 days vs. 7 days), and mortality rates (50% vs. 7%) were reported in ACLF patients than in liver cirrhosis patients [11]. ACLF is comparable to acute kidney injury (AKI) in chronic kidney disease (CKD).

**Various definitions of acute-on-chronic-liver failure**

Within the field of hepatology, ACLF has been extensively studied, and more than 1,000 papers have been published [9]. The classification and prognosis of ACLF vary across countries, race and ethnic groups, and etiologies, reflecting inconsistent views, and there are more than 10 definitions of ACLF around the world. The most widely accepted clinical definitions are those outlined by the Asian Pacific Association for the Study of the Liver (AARC) [12], the European Association for the Study of the Liver Chronic Liver Failure (EASL-CLIF) [13], and the North American Consortium for the Study of End-stage Liver disease (NACSELD) [14]. They are consistent in defining ACLF as a high-mortality disease that involves acute exacerbation of existing liver disease. However, their diagnostic considerations differ in terms of 1) liver condition, 2) organ failures, and 3) precipitating factors. Table 1 presents the most representative diagnostic criteria of the AARC, the EASL-CLIF, and the NACSELD [12–14].

**Multiple perspectives on organ failure and the importance of kidney failure**

As shown in Table 1, the AARC considers extrahepatic organ failure a consequence of ACLF and does not include it in the diagnostic criteria. The EASL-CLIF and the NACSELD, however, incorporate extrahepatic organ failure as an important component of the diagnostic criteria for ACLF. To evaluate organ failure, the AARC, the EASL-CLIF, and the NACSELD respectively consider one, five, and four organs (Table 1). The definitions of organ failure of the EASL-CLIF and the AARC are presented in Table 2 [5–7]. The ACLF grades are classified depending on the number of organ failures, which is directly related to mortality (Table 3). With a single organ failure, the 28-day mortality rate of ACLF was 6.3%, whereas that for failure of four or more organs reached 88.9% [6,15]. Kidney is known to be easily affected by extrahepatic organ failure in ACLF patients, and the incidence of kidney failure (55.8%) was higher than that

<table>
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<th>Table 1. Comparison of ACLF diagnostic criteria</th>
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<td>Criteria</td>
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<td>Liver condition</td>
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<td>Precipitating factor</td>
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<td>Exclusion criteria</td>
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<td>Organ failure</td>
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</tbody>
</table>

AARC, Asian Pacific Association for the Study of the Liver ACLF Research Consortium; ACLF, acute-on-chronic-liver failure; EASL-CLIF, European Association for the Study of the Liver Chronic Liver Failure; HIV, human immunodeficiency virus; NACSELD, North American Consortium for the Study of End-stage Liver disease.
of liver failure (43.6%) in the CANONIC study [15,16]. Also, kidney failure can more adversely affect the prognosis of ACLF than can failure of any other single organ [17–19]. In non-kidney organ failure, the 28-day mortality rate of ACLF patients was 6.3%, while that of patients with kidney failure alone reached as high as 18.6% [15]. Therefore, failure of a single organ other than the kidney is considered ACLF grade 0, whereas failure of the kidney alone is considered ACLF grade 1 (Table 3).

### Definitions of acute kidney injury and hepatorenal syndrome in patients with cirrhosis

For diagnosis of AKI in cirrhosis patients, the definition of the International Club of Ascites (ICA) and KDIGO (Kidney Disease: Improving Global Outcomes) AKI guidelines have been most widely used [20,21]. In 2007, the ICA introduced the concepts of hepatorenal syndrome (HRS)-1 and -2, characterized by rapid deterioration and refractory ascites, respectively [22]; the concepts of AKI and HRS were updated in 2015 [20]. AKI was defined as serum creatinine level of 1.5 mg/dL or greater or a serum creatinine increase of 50% or greater compared to the baseline value [22]. However, AKI was redefined as a serum creatinine increase by 0.3 mg/dL or greater within 48 hours or a serum creatinine increase by 50% or greater compared to the baseline value within 1 week. This was an important change that eliminated the absolute standard of 1.5 mg/dL of serum creatinine level in the diagnosis of AKI. Table 4 summarizes the most commonly used diagnostic standards for HRS and guidelines for determining AKI stages (adapted from the ICA definition) [20]. Due to poor nutritional status and low muscle mass in cirrhotic patients, there have been

---

### Table 2. Outlines and definitions of organ failure

<table>
<thead>
<tr>
<th>Organ</th>
<th>Measurement</th>
<th>Point</th>
<th>EASL-CLIF (CLIF-C OF score)</th>
<th>AARC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Total bilirubin (mg/dL)</td>
<td>1</td>
<td>&lt;6</td>
<td>&lt;15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6–11.9</td>
<td>15–25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>≥12</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Kidney</td>
<td>Serum creatinine (mg/dL)</td>
<td>1</td>
<td>&lt;2</td>
<td>&lt;0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.0–3.4</td>
<td>0.7–1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>≥3.5 or RRT</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Brain</td>
<td>Hepatic encephalopathy (grade)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1–2</td>
<td>1–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3–4</td>
<td>3–4</td>
</tr>
<tr>
<td>Coagulation</td>
<td>PT INR</td>
<td>1</td>
<td>&lt;2.0</td>
<td>&lt;1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.0–2.4</td>
<td>1.8–2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>≥2.5</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>Circulation</td>
<td>Mean arterial pressure (mmHg)</td>
<td>1</td>
<td>≥70</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>&lt;70</td>
<td>Not stated</td>
</tr>
<tr>
<td>Respiration</td>
<td>PaO_{2}/FiO_{2} &amp; SpO_{2}/FiO_{2}</td>
<td>1</td>
<td>&gt;300 &amp; &gt;357</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>201–300 &amp; 215–357</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>≤200 &amp; ≤214</td>
<td></td>
</tr>
</tbody>
</table>

AARC, Asian Pacific Association for the Study of the Liver ACLF Research Consortium; CLIF-C OF, CLIF Consortium Organ Failure; EASL-CLIF, European Association for the Study of the Liver Chronic Liver Failure; FiO_{2}, fraction of inspired oxygen; PaO_{2}, partial pressure of oxygen; SpO_{2}, peripheral oxygen saturation.

### Table 3. ACLF grade

<table>
<thead>
<tr>
<th>ACLF grade</th>
<th>EASL-CLIF</th>
<th>AARC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>No organ failure or single non-kidney organ failure, no hepatic encephalopathy, serum creatinine level of &lt;1.5 mg/dL</td>
<td>Not stated</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Single kidney failure OR Single non-kidney organ failure with serum creatinine level of 1.5–1.9 mg/dL and/or hepatic encephalopathy grade 1–2</td>
<td>5–7</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Two organ failures</td>
<td>8–10</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Three or more organ failures</td>
<td>11–15</td>
</tr>
</tbody>
</table>

ACLF, acute-on-chronic-liver failure; AARC, Asian Pacific Association for the Study of the Liver ACLF Research Consortium; EASL-CLIF, European Association for the Study of the Liver Chronic Liver Failure.
persistent concerns that the creatinine-based glomerular filtration rate (GFR) formula might overestimate actual kidney function [23]. In particular, overestimation of renal function with the creatinine-based GFR formula reached up to about 50% in patients with severely impaired liver function [24]. Thus, clinicians should pay attention to deterioration of renal function and start treatment for AKI in a timely manner, well before serum creatinine level increases and reaches 1.5 mg/dL. Studies have shown that high AKI grade is associated with poor prognosis in cirrhotic patients. Even in cirrhotic patients with AKI grade I, 3-month survival rate was reduced to 84% [25–27].

Meanwhile, as the definition of AKI in cirrhosis patients was updated and the absolute value of serum creatinine is no longer involved, HRS-1, which was previously determined by an increase of serum creatinine level 2.5 mg/dL or greater, was renamed HRS-AKI [20]. These changes are primarily based on the low response rate of patients with high serum creatinine to treatment with terlipressin plus albumin for HRS [28–30]. The newly revised definition recommends starting treatment with vasoconstrictor and albumin in the early stage of AKI, even if serum creatinine level is less than 2.5 mg/dL. The level of serum creatinine tends to overestimate renal function in cirrhotic patients and varies greatly by day. Hence, absolute standards such as serum creatinine level of 1.5 mg/dL (AKI) or 2.5 mg/dL (HRS) should be avoided, and clinicians need to start treating AKI and HRS as early as possible in consideration of the dynamic fluctuation present in serum creatinine level.

In the same vein, attempts have been made to rename HRS type 2, which is characterized by a slow increase of serum creatinine and refractory ascites, to HRS-non-AKI (NAKI) and to have it be divided into HRS-acute kidney disease and HRS-CKD depending on the rate of exacerbation [31]. However, HRS-NAKI is rarely reported and analyzed in ACLF patients, so we will not discuss it in detail in this review.

### Prevalence of acute kidney injury in patients with acute-on-chronic-liver failure

According to the CANONIC study, the prevalence of AKI in ACLF patients is 69% [15]. Among them, the proportions of kidney dysfunction (serum creatinine level of 1.5–1.9 mg/dL) and kidney failure (serum creatinine level of >2 mg/dL) cases were respectively 19.1% and 80.9% [15]. These reports were made prior to revision of the ICA-AKI standard in 2015. Thus, the values are likely to be higher if the current standards of AKI are applied. Even in the ACLF patient group based on the AARC criteria, the prevalence of AKI was estimated to be around 22.8% to 51%, much higher than that (20%) in cirrhotic patients without ACLF [12]. In research conducted in South Korea, 340 among 1,470 patients (23.1%) hospitalized for chronic liver disease had ACLF, and 49% of ACLF patients had kidney failure [32].

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**Table 4. The (ICA-AKI) definitions of AKI and HRS in patients with cirrhosis**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition of AKI</strong></td>
<td>An increase in sCr ≥0.3 mg/dL within 48 hours OR A percentage increase in sCr ≥50% from baseline within the prior 7 days</td>
</tr>
</tbody>
</table>
| **Stage of AKI**  | Stage 1: an increase in sCr ≥0.3 mg/dL or an increase in sCr ≥1.5- to 2-fold from baseline  
Stage 2: an increase in sCr >2- to 3-fold from baseline  
Stage 3: an increase in sCr >3-fold from baseline or sCr ≥4.0 mg/dL with an acute increase ≥0.3 mg/dL or initiation of renal replacement therapy |
| **Definition of HRS-AKI** | Diagnosis of cirrhosis and ascites  
Diagnosis of AKI according to the ICA-AKI criteria  
No response after 2 consecutive days of diuretic withdrawal and plasma volume expansion with albumin 1 g per kg of body weight  
Absence of shock  
No current or recent use of nephrotoxic drugs (NSAIDs, aminoglycosides, iodinated contrast media, etc.)  
No macroscopic sign of structural kidney injury such as proteinuria, microhematuria, or abnormal findings on renal ultrasonography |

AKI, acute kidney injury; HRS, hepatorenal syndrome; ICA, International Club of Ascites; sCr, serum creatinine; NSAIDs, nonsteroidal anti-inflammatory drugs.
Differences in characteristics of acute kidney injury between acute-on-chronic-liver failure and decompensated cirrhosis

In the past, the concept of ACLF was not well established, and there was a lack of distinction between cirrhotic patients with DC and those with ACLF in terms of reported prognosis and clinical outcome. Accumulating evidence suggests that the pattern of organ failure between ACLF and DC is different [33]. Even though the incident rate of AKI was not significantly different between ACLF and DC patient groups (around 13%–25%), the phenotype of AKI was notably different [34,35]. The DC-AKI group tended to be functional and volume-responsive, while the ACLF-AKI group had more severe structural damage [33]. In addition, the probability of AKI progression was relatively low in the DC-AKI group, and resolution was rare [33]. On the other hand, the ACLF-AKI group had a high possibility of AKI progression, as well as of resolution [33,34]. The ACLF-AKI group required renal replacement therapy (RRT) more frequently and had lower response rate to terlipressin than the DC-AKI group, possibly due to severe and extensive systemic inflammation. Table 5 summarizes the differences in characteristics of the ACLF-AKI and DC-AKI groups. However, more studies are needed to better understand the difference between DC-AKI and ACLF-AKI groups.

A prior study on the difference between DC and ACLF patient groups suggests that they have different metabolic profiles such that ACLF patients showed increased skeletal muscle catabolism resulting in release of amino acid to increase nonessential amino acid/glucose and methionine compared with DC patients [36]. Also, the level of spermidine, an inducer of anti-inflammatory autophagy, was decreased in ACLF patients [36]. Another difference between DC and ACLF patient groups is the gut microbiome. Progression of cirrhosis was associated with a reduction of gene and metagenomic species richness in the gut, with maximal changes in ACLF patients [37].

Precipitating factors

Common causes of AKI in general cirrhotic patients include use of diuretics, gastrointestinal bleeding, large volume paracentesis, infection, and use of nephrotoxic drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) [38–40]. The precipitating factors of AKI and ACLF are similar. ACLF can be precipitated by both hepatic and nonhepatic factors [41,42]. In the AARC criteria, acute exacerbation caused by nonhepatic precipitating factors such as infection and gastrointestinal bleeding is excluded from the ACLF definition. However, both nonhepatic and hepatic precipitating factors are considered in the EASL-CLIF criteria. According to studies conducted in Western countries (CANONIC study, EASL-CLIF cohort), the most important causes of acute exacerbation were active alcohol drinking and bacterial infection [15]. On the other hand, according to studies conducted in Eastern countries, hepatitis B virus reactivation, hepatitis A or E virus infection, active alcohol drinking, and bacterial infection were major precipitating factors [43,44]. In South Korean ACLF patients, the most common precipitating factor was active alcohol drinking (41%), followed by gastrointestinal bleeding (31%) and bacterial infection (18.5%) [32]. However, the cause of acute exacerbation in about 40% to 50% of ACLF patients is not clearly elucidated in many studies [15,45]. In patients with unclear precipitating factors, drug-induced liver injury might be one viable reason, but studies on precipitating factors of ACLF-AKI are lacking [46].

Pathogenesis of acute kidney injury in acute-on-chronic-liver failure

The mechanism of AKI in patients with ACLF is complex

Table 5. Differences in characteristics of AKI between ACLF and DC

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACLF</th>
<th>DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of AKI</td>
<td>13%–25.4%</td>
<td>13%–21.2%</td>
</tr>
<tr>
<td>Phenotype of AKI</td>
<td>Structural &gt; functional</td>
<td>Functional &gt; structural</td>
</tr>
<tr>
<td></td>
<td>More volume-responsive</td>
<td></td>
</tr>
<tr>
<td>AKI progression</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>AKI resolution</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Duration of AKI</td>
<td>Shorter</td>
<td>Longer</td>
</tr>
<tr>
<td>Requirement for RRT</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Response to terlipressin</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Worse</td>
<td>Better</td>
</tr>
</tbody>
</table>

ACLF, acute-on-chronic-liver failure; AKI, acute kidney injury; DC, decompensated cirrhosis; RRT, renal replacement therapy.
patients with ACLF have been reported: cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), and serum creatinine. The analysis of 429 patients in the CANONIC study cohort showed that baseline cystatin C was a useful biomarker predicting the development of renal dysfunction and ACLF, which was directly related to mortality [61]. Also, cystatin C was a more effective predictor of renal dysfunction than creatinine in ACLF of HBV patients [62]. However, NGAL did not predict the occurrence of renal dysfunction or ACLF although it was associated with short-term mortality [61]. Interleukin-18 and kidney injury molecule-1 have been examined in cirrhosis patients without ACLF but not in ACLF patients [63]. One of the major limitations of the studies on AKI biomarkers is that cystatin C or NGAL may be affected by the degree of inflammation [24]. In addition, specific cut-off values for the overlapping phenotypes were not available, limiting clinical utility [63].

Last, serum creatinine, which has been traditionally used, can also be an important biomarker in HRS-AKI patients. In HRS-AKI patients with ACLF, a higher level of serum creatinine was associated with lower response rate to terlipressin [64]. Therefore, serum creatinine is an important predictor of response to treatment when HRS-AKI occurs in ACLF patients.

**Prevention of acute kidney injury**

In cirrhosis patients, AKI prevention plays a critical role in prognosis. Avoidance of nephrotoxic drugs and appropriate control of volume status are crucial. The problem is that it is very difficult to control the volume status in patients with cirrhosis and decreased renal function [65]. NSAIDs and antibiotics are highly likely to promote AKI in cirrhosis patients should be administered with extra caution and careful determination of dose [66]. There is no evidence that contrast-induced nephropathy occurs more frequently in cirrhotic patients than in the general population. Nevertheless, caution is always required because cirrhotic patients require more frequent surveillance or examination than the general population [67]. In addition, frequent follow-up with serum creatinine is necessary in cirrhotic patients with ascites because of the high risk of AKI.

Antibiotic prophylaxis may help prevent AKI. Prophylactic antibiotics are administered in patients with frequent spontaneous bacterial peritonitis (SBP) or variceal bleed-
ing. Systemic inflammation frequently caused by bacterial translocation has been pointed out as the main cause of acute exacerbation; hence, antibiotic prophylaxis that prevents repeated SBP can also prevent AKI [68,69]. Antibiotic prophylaxis is also recommended in patients with variceal bleeding to reduce the likelihood of systemic infection and to increase survival [70,71]. In both instances (i.e., SBP and variceal bleeding), there were promising findings that antibiotic prophylaxis could lower the incidence of AKI [72,73].

Albumin infusion may help prevent AKI in two ways. First, large volume paracentesis can induce AKI by causing circulatory dysfunction and hypovolemia; hence, albumin replacement is essential to prevent AKI during large volume paracentesis [74]. Second, albumin is also recommended to prevent AKI in patients with SBP [75]. However, the clinical utility of albumin might be limited in systemic infections other than SBP [8]. A recent study showed that weekly albumin administration can be helpful for long-term prognosis of cirrhosis, demonstrating the anti-inflammatory effect of albumin beyond its volume replacing effect [76]. However, more empirical evidence is needed to safely administer weekly albumin in patients with liver cirrhosis considering cost-effectiveness.

Last, there was a study showing that administration of N-acetylcysteine (NAC) can help prevent AKI in alcoholic hepatitis patients [77]. In addition, one study reported that short-term granulocyte colony-stimulating factor (G-CSF) plus erythropoietin (darbepoetin) can decrease AKI, sepsis, and mortality in DC patients [78]. The effectiveness of NAC or G-CSF has not been clearly demonstrated, and additional results are needed.

**Treatment of acute kidney injury in acute-on-chronic-liver failure**

First, if the underlying cause of AKI is evident, treatment of the underlying condition needs to be prioritized. For example, antibiotic treatment can be considered for AKI caused by bacterial infection, volume replacement can be administered to treat hypovolemia due to gastrointestinal bleeding, and any causative agent needs to be discontinued to prevent AKI. Also, prednisolone therapy can be considered for severe alcoholic hepatitis [79]. If these underlying conditions are addressed as early as possible, AKI progression may be suppressed. Subsequently, albumin infusion is recommended for volume expansion. Usually, 1 g of albumin per kg of body weight is recommended for 1 to 2 days, after which the improvement of AKI can be evaluated [20]. If there is no response to albumin infusion, additional biomarker testing such as urine NGAL might be considered [80]. If the level of urine NGAL is elevated, it is most likely a phenotype of acute tubular necrosis [63,81].

When AKI continues to progress despite these treatments, vasoconstrictors such as terlipressin with albumin may be considered. However, the use of vasoconstrictor is not recommended for stage I AKI [8]. Treatment response to terlipressin in patients with ACLF accompanying HRS-AKI is reported to be around 35% [19,82]. The response rate to terlipressin is particularly low in patients with HRS-AKI when the baseline serum creatinine is elevated, bilirubin level is heightened, or mean arterial pressure is not elevated despite terlipressin administration [28,83]. If there is no improvement in serum creatinine after 7 to 10 days of terlipressin treatment, it should be discontinued.

ACLF patients with AKI have a high mortality rate; hence, liver transplantation (LT) should be considered. Usually, the prognosis after LT in the presence of one or two organ failures is not different from that of patients without ACLF [84]. However, when there are three or more organ failures, the 1-year posttransplantation survival rate tends to decrease slightly to 80%. Nevertheless, it is recommended to consider LT first in patients with grade 3 ACLF, because the survival rate is less than 20% if LT is not performed [85,86].

Liver-kidney co-transplantation might be a viable option for patients with estimated GFR (eGFR) of <30 mL/min/1.73 m² or underlying CKD, who are highly likely to need dialysis later [80,87]. However, studies on liver-kidney co-transplantation in ACLF patients are lacking due to donor shortage. In patients who underwent LT only, the rate of renal dysfunction after transplantation was increased proportionally to the pretransplantation AKI duration [88,89]. In particular, patients with AKI for more than 6 weeks were more likely to experience renal dysfunction after LT [90]. Therefore, liver-kidney co-transplantation should be considered first in the earlier-mentioned conditions [16]. In updated Organ Sharing/Organ Procurement and Transplantation Network criteria, liver-kidney co-transplantation is recommended for the following three conditions [91–93]: 1) CKD with a measured or eGFR of <60 mL/min/1.73 m² for more than 90 consecutive days, 2)
sustained AKI, or 3) accompanying metabolic disease such as hyperoxaluria or atypical hemolytic uremic syndrome.

RRT can be performed as a bridging therapy if AKI continues to worsen in patients for whom immediate LT is not available [94,95]. Studies reported that continuous RRT rather than intermittent RRT improves cardiovascular stability and reduces intracranial pressure in patients with cirrhosis [96–98]. However, there is no randomized controlled trial comparing continuous and intermittent RRT, and RRT by itself does not reduce mortality in ACLF or cirrhotic patients.

Last, artificial liver treatment such as that with a Molecular Adsorbent Recirculating System (MARS) can be considered as a bridging therapy in ACLF patients, although it may not improve survival [99,100]. Due to its limited effectiveness and high cost, MARS is not widely used.

**Conclusion**

ACLF is an acute exacerbation of preexisting liver disease, resulting in high mortality and high socioeconomic burden. In patients with ACLF, the kidney is the most common site of organ failure, and the prognosis is poor when AKI occurs. AKI in patients with ACLF is clinically different from AKI in patients with decompensated liver cirrhosis, but more research is needed to elucidate its mechanism. AKI associated with ACLF develops through multifactorial pathogeneses involving both hemodynamic abnormality and inflammation, especially systemic inflammation, bacterial translocation, and infection. Prophylactic antibiotic administration or albumin replacement might be effective in preventing AKI. Treatment for AKI includes management of underlying disease, volume replacement, vasoconstrictor with albumin, RRT, and LT. More studies are needed to advance the understanding of ACLF-AKI and its prevention and treatment.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

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

**Authors’ contributions**

Conceptualization, Funding acquisition: SGK
Investigation: JJY
Resources: MYP
Writing–original draft: JJY
Writing–review & editing: SGK
All authors read and approved the final manuscript.

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Expanding the potential therapeutic options of hemoperfusion in the era of improved sorbent biocompatibility

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Hemoperfusion has been considered a promising adjuvant treatment for chronic diseases and some acute states when specific removal of pathogenic factors from the bloodstream is desired. Over the years, advances in adsorption materials (e.g., new synthetic polymers, biomimetic coating, and matrices with novel structures) have renewed scientific interest and expanded the potential therapeutic indications of hemoperfusion. There is growing evidence to suggest a prominent place for hemoperfusion as an adjuvant treatment in the setting of sepsis or severe coronavirus disease 2019 and as a therapeutic option for chronic complications associated with accumulated uremic toxins in patients with end-stage renal disease. This literature review will describe the principles, therapeutic perspectives, and the emerging role of hemoperfusion as a complementary therapy for patients with kidney disease.

Keywords: Adsorption, COVID-19, Hemodialysis, Renal dialysis, Kidney diseases

Introduction

The removal of unwanted plasma solutes and pathogens can be life-saving under certain conditions, such as sepsis, intoxication, and organ failure. Thus, the unique ability of hemoperfusion (HP) to adsorb specific molecules with large molecular weight (MW) and/or a high protein-binding affinity could explain why HP has been allured as a promising treatment for several diseases [1].

Whereas poisoning was once considered the classical indication of HP, advances in sorbents’ biocompatibility and design have helped to expand its potential clinical indications to the treatment of inflammatory conditions (e.g., sepsis, pancreatitis, and hepatitis), autoimmune diseases, and chronic uremic symptoms [2].

The European Uremic Toxin Group classifies uremic toxins into three groups: small water-soluble toxins with an MW of <500 Da (e.g., urea and creatinine), middle molecules with an MW of ≥500 Da (e.g., parathyroid hormone [PTH], C-reactive protein, and β2-microglobulin [B2M]) that can be successfully removed by hemofiltration (HF) and high-flux hemodialysis (HD), and protein-bound solutes (e.g., homocysteine) which are not removed by classic HD or HF [3]. Moreover, as current dialysis techniques based on diffusion and convection show limitations due to membrane permeability characteristics [4], HP can be an attractive adjuvant modality for blood purification either alone or in combination with other renal-replacement therapies (RRTs). Besides, the high mortality rate attributed to cardiovascular disease and the outcomes of end-stage renal disease (ESRD) patients on HD have been correlated with blood levels of medium/large molecules insufficiently
cleared by RRT [5]. Novel sorbents with greater biocompatibility and safety than before have renewed scientific interest in the broader implementation of HP [6]. Fig. 1 summarizes the advantages, disadvantages, and clinical conditions where HP can be considered.

Given the accumulation of encouraging data and the emerging new perspectives derived from research in HP as well as the current lack of consensus clinical guidelines, we aimed to conduct a literature review of the principles of HP, the evolution of sorbent materials, and the promising applications of HP in different clinical settings with a special focus on ESRD patients.

Principles of function and adsorption materials

According to the Consensus Conference on Biocompatibility, adsorption is the process of removing particles and toxins from the blood or plasma through their connection to the surface of the adsorbent, which lies in an extracorporeal purification machine [7].

Chemical and physical attraction forces are responsible for retaining the adsorbed molecules on the adsorbent. Physical forces include Van der Waals and hydrophobic interactions, whereas chemical interactions involve the formation of chemical bonds between the surface and the adsorbed species.

Adsorption materials can be found in nature or can be manufactured (e.g., synthetic polymers). Activated carbon produced from natural raw materials has shown a good adsorption capacity but poor biocompatibility [8,9]. Activated encapsulation technology or the use of activated carbon from other sources (resin-based) was considered for overcoming safety issues due mostly to the latter’s rough surface [10]. With these modifications and alternatives, however, the adsorption capacity was influenced [11].

Inorganic porous materials present some important advantages, such as reusability and pore size variability, but adverse effects have been reported with their use [12–14]. Synthetic polymeric materials show remarkable functions, stability, and biocompatibility due to the tailor-made molecular design and/or surface modifications compared to natural polymeric materials. Notably, their low cost, hemocompatibility, and structure designability are their main advantages (Table 1) [15].

In general, adsorption materials can be found as gran-

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**Figure 1. Overview of hemoperfusion technique.**
HD, hemodialysis; COVID-19, coronavirus disease 2019; ESRD, end-stage renal disease.
ules, spheres, fibers, cylindrical pellets, flakes, and powders. They are solid particles with diameters ranging from 50 μm to 1.2 cm. Their surface area to volume ratio is extremely high, with the surface area ranging from 300 to 1,200 m²/g. Further classification of sorbents is based on their pore size, i.e., >500 Å (50 nm) (macroporous), 20 to 500 Å (mesoporous), and <20 Å (microporous).

Sorbents should also have favorable kinetics and transport properties. Isotherm equations and data from plotting curves known as adsorption isotherms during laboratory experiments provide information about the amount of sorbent required to remove a given amount of solute (Fig. 2) [1]. Moreover, packing sorbent particles into a cartridge requires a tortuous pathway (sorbent bed) through which blood or fluid must flow and be distributed uniformly. The mechanisms of solute adsorption in porous media include: 1) the external (interphase) mass transfer of the solute by convection from the bulk fluid and by diffusion through a thin film or boundary layer to the outer surface of the sorbent, 2) the internal (intra-phase) mass transfer of the solute by convection from the outer phase of the sorbent into the internal porous structure, and 3) surface diffusion along the surface of the internal pores and adsorption of the solute onto the porous surface (Fig. 3) [16].

### Biocompatibility

The ideal sorbent material for extracorporeal therapies is one that is biocompatible. Moreover, it should be characterized by hardness and mechanical strength to prevent crushing and erosion and to avoid any release of fragments into the systemic circulation. Additionally, since the blood is exposed to a larger surface in this context compared to other extracorporeal therapies, any cytotoxic reaction and immune system activation—clinically identifiable with the onset of rashes, shivers, leukopenia, and thrombocytopenia—must be prevented [17].

Therefore, surface coating is an attractive method to increase biocompatibility. Coating materials such as cellulose nitrate, albumin-collodion, cellulose acetate, and polyamide were initially evaluated by Chang [18], whereas

<table>
<thead>
<tr>
<th>Adsorbent type</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Example</th>
<th>Clinical availability</th>
<th>Clinical indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated carbon (natural or resin-based) [8–11,15]</td>
<td>Low cost, high adsorption capacity</td>
<td>Poor biocompatibility</td>
<td>Adsorba (coated with cellulose acetate)</td>
<td>–</td>
<td>Intoxication</td>
</tr>
<tr>
<td>Inorganic porous material (e.g., mesoporous silica, silica gel) [12–15]</td>
<td>Reusability, pore size highly variable for different sizes of toxin molecules</td>
<td>High cost, variable results in biocompatibility, poor modifiability, limited structural design possibility</td>
<td>LiChroprep RP-18</td>
<td>–</td>
<td>Removal of bilirubin and uric acid, medicine removal</td>
</tr>
<tr>
<td>Natural (e.g., polysaccharide) or synthetic polymeric materials [15]</td>
<td>Hemocompatibility, high stability, bioinertia, structure designability and modifiability, low cost</td>
<td>Selectivity of natural polymeric materials not advantageous</td>
<td>CytoSorb, HA 330, Toraymyxin</td>
<td>+</td>
<td>Wide range</td>
</tr>
</tbody>
</table>

Data reproduced from references.
Figure 3. Mechanism of solute adsorption in porous media. Mechanisms of mass transport from the bulk solution to the sorbent surface. (A) External (interphase) mass transfer of the solute by convection from the bulk fluid by diffusion through a thin film of boundary layer to the outer surface of the sorbent. (B) Internal (intra-phase) mass transfer of the solute by pore convection from the outer surface of the adsorbent to the inner surface of the internal porous structure. (C) Surface diffusion along the porous surface and adsorption of the solute onto the porous surface. Reproduced from Clark et al. [16] with permission of Karger Publishers.

Anti-adhesion properties are also important for bio-compatibility since the contact of blood with an artificial surface triggers a number of processes, including protein and cell adsorption and platelets’ adhesion to the artificial surface. Thus, anti-adhesion modifications and surface coating using new materials like zwitterionic groups have received increasing interest [20]. Indeed, zwitterionic materials are highly resistant to non-specific protein adsorption, bacterial adhesion, and biofilm formation [21].

Selective targeting

Selective targeting of a key molecule as an endotoxin has promoted the concept of surface grafting. Besides, in complex biological media such as blood, they will always exist molecules of different origins that compete for the chemical adsorption site against the target molecules. By immobilizing a molecule with a specific affinity for the target molecule on the surface, a high affinity is obtained, mainly via a combination of electrostatic and hydrophobic interactions plus steric complementarity of both molecules rather than a covalent chemical bond formation [22].

Polymyxin B, an antibiotic derived from Bacillus polymyxa that binds and neutralizes endotoxin (an outer membrane component of gram-negative bacteria), and protein A, which binds anthrax toxin (a specific exotoxin secreted by virulent strains of the bacterium Bacillus anthracis), are typical applications of the surface grafting concept in cases of sepsis [23,24]. Except for antibiotics, other ligands (e.g., amino acids) have also been used [25]. Technological achievements in the genomic area have inspired the use of grafted nucleic acid-based ligands on adsorbents for the treatment of patients with systemic lupus erythematosus and hepatitis B [26,27]. Synthetic ligands also exhibit resistance to biological degradation and similar selectivity [28]. Finally, computation simulation has enabled the design of affinity ligands based on the structure of Asp-Phe-Leu-Ala-Glu (DE5), a sequence of toxic peptides frequently found in uremic patients [29].

Technical aspects of hemoperfusion

HP alone does not achieve sufficient removal of small uremic toxins and fluid balance; however, when combined with other HD techniques, a synergistic effect can be ob-
In HP, the cartridge is placed in direct contact with the patient’s blood, with the basic requirements being an HP cartridge, double lumen catheter, vascular access, and an anticoagulant (heparin or citrate) [30]. HP is effective in removing uncharged molecules through competitive binding, especially those that are significantly plasma protein-bound and lipophilic. Indeed, HP targets molecules that tend to be more difficult to remove with conventional HD or with continuous RRTs (CRRT) and has the capacity to remove molecules with MW up to 30,000 Da depending on the characteristics of the sorbent material [31]. When combined with HD/CRRT, the sorbent can be placed before or after the dialyzer and enhance the removal of middle molecules that are not sufficiently removed by HD, such as B2M [32].

In the process of plasma filtration adsorption, plasma is first separated from the whole blood and circulates through the sorbent [33], then is returned to the whole blood, which can be subjected to HD or CRRT in order to expand the clearance of small solutes such as urea and creatinine. In this case, the use of both methods maximizes solutes’ removal [34].

In double plasma filtration molecular adsorption system, different cartridges exhibiting specific characteristics can be placed in the plasma filtration circuit [35]. Finally, HP can be combined with extracorporeal membrane oxygenation [36].

**Potential clinical applications of hemoperfusion and ongoing trials**

Poisoning

Extracorporeal therapies for drug or chemical intoxication are indicated when there is life-threatening toxicity, an inadequate response to standard supportive measures, or the poison’s endogenous clearance is <4 mL/min/kg and the poison’s volume distribution is <1–2 L/kg [37].

Nowadays, the use of high-flux and high-efficiency dialyzers and the higher blood flow rates achieved, have established intermittent HD as the preeminent extracorporeal modality for poisoning. Moreover, HD is easily accessible; it removes poisons rapidly and simultaneously corrects any electrolyte and acid-base disorders [38]. In contrast, HP requires greater system anticoagulation; flows that do not exceed 350 mL/min so as to avoid the risk of hemolysis; and nonselectively adsorbs platelets, calcium, glucose, and white blood cells [39,40]. The higher cost and the need to replace the cartridge every 2 hours due to saturation are also important disadvantages of HP [41]. Finally, for some metals like lithium and alcohols (e.g., methanol, ethylene glycol), HP is not indicated due to less efficiency [42,43].

While HP use for poisoning has declined to roughly 1% of HD utilization in the United States [44], HP seems to be more effective than HD for paraquat poisoning [45]. HP achieves enhanced clearance of paraquat, leading to higher survival rates compared to high-flux HD [46]. With paraquat poisoning being an important concern mostly in Asia, current recommendations do not mention the use of extracorporeal treatment for it [37].

Currently, the strongly recommended method by the EXTRIP (Extracorporeal Treatments in Poisoning) group for the removal of most drugs is intermittent HD (https://www.extrip-workgroup.org/recommendations). In some cases, HP is an alternative option (1C or 1D) when HD cannot be performed (Table 2).

Sepsis

CRRT methods are widely used due to their capacity to retain body fluid balance and to correct electrolytic and acid-base imbalances in patients with sepsis and acute kidney injury (AKI). However, the removal of proinflammatory cytokines and complement fragments that promote kidney dysfunction and aggravate multiorgan dysfunction in the setting of septic shock is limited due to the limited permeability of the membranes [47]. Consequently, the use of high-flux HF and/or high cut-off membranes has been encouraged due to their removal capacity reaching up to 65 kDa. Unfortunately, their main disadvantage is the concomitant removal of important amounts of albumin. Therefore, HP and the design of biocompatible cartridges with the potential for customizing the target solutes have led to the increasing application of adsorption in sepsis and other inflammatory states such as coronavirus disease 2019 (COVID-19) (Table 3).

With selective HP being an alluring approach for removing circulating endotoxin and theoretically preventing the biological cascade in sepsis, research had focused on the
use of polymyxin-bound membranes. Besides, antiendo-
toxin drug therapies and intravenous polymyxin B have
failed to prove a clinical benefit [48]. Therefore, direct HP
with a polymyxin device (Toraymyxin; Today Industries
Ltd.) was initially introduced and approved in Japan as an
adjuvant sepsis therapy [49]. Later on, its use was expand-
ed to other countries. Several randomized trials have pro-
vided conflicting results on the clinical benefit of polymyx-
in B in terms of mortality, hemodynamic parameters, and
respiratory function of patients with septic shock due to an
abdominal cause compared to conventional care [50–52].
Currently, the TIGRIS study (NCT03901807), a prospective,
multicenter, randomized open-label trial, is investigating
the effects of standard medical care plus polymyxin-based
HP versus the standard care of treatment.

Regarding nonselective HP, extracorporeal cytokine ad-
sorption with the CytoSorb cartridge (CytoSorbents Cor-
poration) has been investigated in case series and small
comparative studies [53–55]. CytoSorb consists of specially
designed polymers with large surfaces, high flow, and low
resistance. It is indicated for clinical situations with high
plasma concentrations of cytokines. CytoSorb binds cy-
tokines 10 to 50 kDa in size, with a removal rate of >90%
to 95% [56]. However, in a multicenter randomized trial
comparing conventional care with CytoSorb in ventilated
patients with sepsis and either acute lung injury (ALI) or
acute respiratory distress syndrome (ARDS), no significant
differences in interleukin (IL)-6 concentration were ob-
served [57]. In a recent randomized controlled trial (RCT;
the REMOVE trial), the authors failed to demonstrate a
reduction in postoperative organ dysfunction or 30-day
mortality with intraoperative use of CytoSorb in patients
undergoing cardiac surgery for infective endocarditis. Even
though CytoSorb achieved a lower level of plasma key cy-

### Table 2. List of drugs and the recommended extracorporeal therapy in case of acute poisoning

<table>
<thead>
<tr>
<th>Drug</th>
<th>The first choice of extracorporeal modality</th>
<th>Acceptable alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D), CRRT (3D)</td>
</tr>
<tr>
<td>Baclofen</td>
<td>Not recommended (1D)</td>
<td>HP (1D) or CRRT (3D)</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Intermittent HD (1D)</td>
<td></td>
</tr>
<tr>
<td>B-blockers</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Atenolol</td>
<td>Intermittent HD (1D) only in severe poisoning with kidney impairment</td>
<td></td>
</tr>
<tr>
<td>Sotalol</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D), CRRT (3D)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Gabapentin/pregabalin</td>
<td>Intermittent HD (1D) only in severe poisoning with kidney impairment</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Not recommended (2D), consider extracorporeal therapy where pyri-doxyne cannot be administrated (2D)</td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>Intermittent HD (1D)</td>
<td>CRRT (1D)</td>
</tr>
<tr>
<td>Metformin</td>
<td>Intermittent HD (1D)</td>
<td>CRRT (2D)</td>
</tr>
<tr>
<td>Methanol</td>
<td>Intermittent HD (1D)</td>
<td>CRRT (1D)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Not recommended (2D) when glucarpidase is not administrated, not recommended (1D) when glucarpidase is administrated, not recommended (1D) instead of administrating glucarpidase</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D)</td>
</tr>
<tr>
<td>Quinine/chloroquine</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Salicylates</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D), CRRT (3D)</td>
</tr>
<tr>
<td>Thallium</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D), CRRT (1D)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Intermittent HD (1C)</td>
<td>Intermittent HP (1C), CRRT (3D)</td>
</tr>
<tr>
<td>Tricyclic antidepressants</td>
<td>Not recommended (1D)</td>
<td></td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Intermittent HD (1D)</td>
<td>Intermittent HP (1D), CRRT (2D)</td>
</tr>
</tbody>
</table>

CRRT, continuous renal-replacement therapies; HD, hemodialysis; HP, hemoperfusion.
toxins, no clinical benefit was obtained [58].

HP with the Jafron HA cartridges (Jafron Biomedical Company) has also been tested in acute respiratory failure caused by sepsis, with promising results concerning hemodynamic parameters, respiratory function, and mortality within 28 days of hospitalization [59]. The HA 330 cartridge has an electrically porous resin that specifically removes cytokines, complements, and other endotoxins with MWs of 10 to 60 kDa. HA 330-based HP was studied in multiple cohorts in the context of inflammatory conditions such as sepsis, ALI, hepatitis, and pancreatitis [2]. In a small nonrandomized study, intensive care unit (ICU) mortality and length of ICU stay were found to be better in septic patients receiving HA 330-based HP compared to those given standard therapy, albeit with no effect on mortality [60]. Encouraging results come from a case series of children with sepsis and underlying hematological disorders receiving HA 330-based HP as an adjunctive treatment to counterbalance the cytokine storm [61]. In another study, patients with ALI induced by extrapulmonary sepsis were randomized to HA 330-based HP or standard therapy. In the HP group, significant reductions in the duration of mechanical ventilation and ICU stay and the ICU mortality rate were observed. Improved respiration parameters were also observed and correlated with the significant removal of inflammatory cytokines (tumor necrosis factor [TNF] and IL-1). In a recent study by Chu et al. [62], the combination of the same cartridge with pulse high-volume HP in

Table 3. Summary of characteristics and main results of the most frequently used HP filters in the field of sepsis and COVID-19 infection

<table>
<thead>
<tr>
<th>Filter</th>
<th>Selectivity</th>
<th>Targets</th>
<th>Indications</th>
<th>Combined treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toraymyxin (Today Industries Ltd.) [49–52,69]</td>
<td>+</td>
<td>Endotoxins, inflammatory mediators, cytokines</td>
<td>Sepsis with positive culture for Gram-negative bacteria/high endotoxin activity assay level, severe sepsis or septic shock from an abdominal cause, COVID-19 infection with septic shock</td>
<td>Higher hemodynamic and ventilation parameters</td>
<td>Unclear results for mortality</td>
</tr>
<tr>
<td>CytoSorb (CytoSorbents corporation) [53–58,69–78]</td>
<td>-</td>
<td>Inflammatory mediators, cytokines, albumin-bound substances and pathogenic toxins but not effective removal of endotoxin</td>
<td>Severe sepsis or septic shock (cytokine storm) and ARDS, COVID-19 infection</td>
<td>HD SCUF CRRT ECMO</td>
<td>Higher hemodynamic parameters and improved respiratory distress Unclear results for mortality and for reduction of IL-6</td>
</tr>
<tr>
<td>HA series (Jafron Biomedical Company) [59–62,69]</td>
<td>-</td>
<td>Inflammatory mediators, cytokines, complement, free hemoglobin and myoglobin</td>
<td>Severe sepsis or septic shock +/− acute lung injury, COVID-19 infection</td>
<td>CRRT ECMO</td>
<td>Reduction of inflammatory cytokine levels and improved hemodynamic parameters Unclear results for mortality</td>
</tr>
<tr>
<td>oXiris (Baxter Inc.) [64–68,70,83–85]</td>
<td>-</td>
<td>Endotoxins, inflammatory mediators and cytokines with potential antithrombogenic properties</td>
<td>COVID-19 infection a, sepsis with AKI</td>
<td>Stand-alone filter for SCUF and CRRT</td>
<td>Reduction in inflammatory markers and improved hemodynamics Limited experience on mortality</td>
</tr>
<tr>
<td>Seraph 100 Microbind Blood Affinity Filter (ExThera Medical Corporation) [70,79–82]</td>
<td>-</td>
<td>Pathogens and proinflammatory cytokines</td>
<td>COVID-19 a</td>
<td>HD CRRT</td>
<td>Improvement in circulatory dysfunction and in inflammatory markers (CRP and IL-6), initial results showing lower mortality</td>
</tr>
<tr>
<td>Spectra Optia Apheresis and Depuro D2000 Adsorption Cartridge (Terumo BCT) [70,86]</td>
<td>-</td>
<td>Endotoxins, inflammatory mediators and cytokines</td>
<td>COVID-19 a</td>
<td>Therapeutic apheresis</td>
<td>Limited experience</td>
</tr>
</tbody>
</table>

Data reproduced from references.

AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CRRT, continuous renal-replacement therapy; ECMO, extracorporeal membrane oxygenation; HD, hemodialysis; IL, interleukin; SCUF, slow-continuous ultrafiltration.

aDevices approved by U.S. Food and Drug Administration for the treatment of severe COVID-19 infection.
patients experiencing septic shock led to beneficial effects on cardiovascular physiology and greater decreases in IL-6, IL-10, and TNF-α concentration when compared to patients who received continuous venous-venous HF.

Finally, the AN69-based oXiris membrane (Baxter Inc.), which is a heparin-grafted membrane specifically designed for cytokine and endotoxin adsorption, alongside RRT, presents three layers: 1) AN69 copolymer hydrogel structure that adsorbs cytokines and removes solutes via convection through membrane pores, 2) a multilayer structure of polyethyleneimine that adsorbs endotoxin and offers better biocompatibility, and 3) a heparin graft that reduces local thrombogenicity [63]. In vitro comparison of oXiris with Toraymyxin and CytoSorb revealed similar efficacies in lipopolysaccharide clearance and inflammatory mediator clearance, respectively [64]. However, there are a limited number of studies to support its action in septic shock compared to the above-mentioned products [65–67].

Viral infections, including severe acute respiratory syndrome coronavirus 2

Uncontrolled cytokine response was considered the hallmark of severe COVID-19 during the first months of the pandemic [68]. Several extracorporeal blood-purification techniques have been used in COVID-19 patients to restore “immune homeostasis” by removing inflammatory molecules [69].

Recently, experts’ recommendations state that, in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and cytokine release syndrome, cytokine-removal strategies should be reserved for COVID-19 patients with evidence of high levels of circulating cytokines like IL-6 and IL-8, a biochemically determined inflammatory status, a high SOFA (Sequential Organ Failure Assessment) score, clinical symptoms of hemodynamic instability requiring vasopressors, and initial signs of immune dysregulation or coagulation disorders [69]. Polymyxin-based HP is indicated in the early phase for suspected sepsis (indicated by a high procalcitonin level and/or positive bacterial culture) or an elevated endotoxin level proven by activity assay. If HP is indicated for cytokine removal, sessions with CytoSorb or HA 380 might follow.

In fact, the U.S. Food and Drug Administration (FDA) has approved four blood-purification devices to treat COVID-19, including 1) CytoSorb, 2) the Seraph 100 Microbind Affinity Blood Filter (ExThera Medical Corporation), 3) the oXiris Filter, and 4) the Spectra Optia Apheresis System (Terumo BCT) [70].

The first case of CytoSorb use in conjunction with CRRT in a critically ill patient with COVID-19 was reported by Rizvi et al. [71], underlining a plausible contribution to early improvement in inflammatory markers. Other case-control and retrospective studies followed, highlighting a potentially beneficial role of adjuvant HP with CytoSorb in the early phase of COVID-19 in terms of cytokine reductions (mainly IL-6 levels), a better clinical course with less need for vasoactive agents, and the improvement of respiratory distress. However, data on mortality rates were inconsistent [72–77]. Indeed, in a recent prospective, randomized pilot study with 50 COVID-19 patients receiving CytoSorb for 3 to 7 days or standard therapy, HP did not improve the resolution of vasoplegic shock (primary outcome) or the predefined secondary endpoints, which included mortality, IL-6 concentration, and catecholamine requirement [78]. Ongoing randomized trials and a large registry of CytoSorb therapy in COVID-19 ICU patients (NCT04391920) aim to enrich the current literature regarding the role of CytoSorb as a potential therapy in severe COVID-19 [79].

HP with the Seraph 100 Microbind Affinity Blood Filter, a biomimetic adsorber that has been shown to bind pathogens, including SARS-CoV-2, from the blood using ultra-high MW adsorptive beads [79], received Emergency Use Authorization for severe COVID-19 from the FDA. Olson et al. [80] were the first to report its use in COVID-19 patients with ARDS and septic shock who required mechanical ventilation. Rapid improvement in vasopressor needs, overall circulatory dysfunction as well as C-reactive protein and IL-6 levels were noticed following the initiation of HP. Similar results were documented by Sandoval et al. [81] who used Seraph 100 in four elderly, multimorbid ESRD patients on HD with severe COVID-19. Data from the COSA (COVID-19 patients treated with the Seraph 100 Microbind Affinity filter) registry support that Seraph 100 treatment is easy to deploy either as a stand-alone HP treatment or in combination with RRT. The observed mortality rate was lower than that calculated by established scores, but the data are limited due to the lack of a control group [82]. Initial data from an observational retrospective study (PURIFY-OBS-1; NCT04606498) suggest improvements in
the survival of severely ill COVID-19 patients treated with Seraph 100.

Evidence of significant reductions in inflammatory markers and improved hemodynamics, organ function, and clinical outcomes with oXiris comes mostly from case series, the oXirisNet registry, and small observational studies [83–85]. An RCT (oXAKI-COV Study; NCT04597034) is ongoing and aims to demonstrate the clinical efficacy of AN69-oXiris compared to the AN69 standard membrane in decreasing vasopressor requirements to sustain a stable mean arterial pressure in critically ill patients with COVID-19 and AKI requiring CRRT.

Finally, the Spectra Optia Apheresis System provides therapeutic apheresis in combination with HP with the Depuro D2000 adsorption cartridge. The Depuro D2000 cartridge is composed of activated uncoated coconut shell charcoal and the non-ionic resins Amberlite XAD-7HP and Amberchrom GC300C, and its placement downstream in the apheresis circuit allows for cytokine removal with subsequent return of the treated plasma to the patient. Its use as a rescue therapy for cardiogenic shock due to stress-cardiomyopathy in severe COVID-19 has been reported only in a single patient by Faqihi et al. [86]. An ongoing large multicenter single-arm clinical trial (Plasma Adsorption in Patients With Confirmed COVID-19; NCT04358003) of the United States is expected to provide information about the effects of the D2000 cartridge with the Optia protocol on morbidity and mortality rates of COVID-19 patients admitted to the ICU.

Maximizing toxin removal and clinical benefits in patients with end-stage renal disease

ESRD has been increasingly recognized as an inflammatory state with protein-bound uremic toxins (PBUTs) and middle molecules like B2M being key factors and inducing various cardiovascular complications. Therefore there is a rationale for the increasing research on synergic approaches that combine HP with other dialytic techniques to achieve complementary elimination of metabolites and effectively prevent and treat complications and improve clinical outcomes [6,87].

Regarding overall survival, a systematic review and meta-analysis showed that the combination of HD with HP improves survival rates [88].

Important ameliorations of blood pressure—even in dialysis patients with refractory hypertension—and left ventricular mass index, reduced dosages of epoetin, and higher hemoglobin levels, have been reported when HD with HP are combined compared to HD alone [89–91]. Considering the more pronounced decrease in levels of myocardial enzymes associated with the combination of HP and HD, it was speculated that their concurrent use can lighten the cardiovascular burden and protect the myocardium [92]. Besides, the improvement of microinflammatory indicators associated with the combination of these therapies could partially explain the lower incidence of cardiocerebrovascular events and the improvement of anemia in patients who had received both HP and HD treatment [93].

Along the same lines, in a study by Raine et al. [94], apart from the greater reduction in inflammation markers, an important improvement in the indices of nutritional status occurred in the HD plus HP group.

Greater benefits in terms of B2M and PTH reductions have been shown by several studies when HP and HD were combined.

Hence, there are potential to improve secondary hyperparathyroidism, pruritus, and dialysis-related amyloidosis [95–97].

Interestingly, based on the reported relationship between the intestinal environment and renal disease, HP combined with dialysis showed encouraging results with respect to the potential improvement of microbiota disorders. Indeed, significantly higher levels of beneficial bacteria like Lactobacillus acidophilus and lower levels of harmful bacteria such as Escherichia coli were reported in colony distributions of patients receiving HP combined with HD plus hemodiafiltration compared to patients receiving HD plus HF [98]. Research is now focusing on promising sorbent materials—such as a divinylbenzene sorbent coated with polyvinylpyrrolidone (DVB-PVP) and cellulose with hexadecyl chains—which show a high adsorption ability of PBUTs or hydrophobic cytokines. A synergistic effect on the reduction of PBUTs was recently demonstrated during HD therapy combined with DVB-PVP resins and symbiotic formulation [99].

Moreover, improved sleep disturbance and sleep efficiency accompanied by an increase in nocturnal melatonin levels were reported with HP therapy (1–2 times/wk) for 2 years [100].
Finally, there is some evidence that the combination of HP with HD can improve the life quality of ESRD patients [97,101]. Symptoms like skin itching, fatigue, sleep quality, and sexual function were significantly improved by adding HP, probably due to the greater clearance of middle and large molecular toxins such as PTH and B2M [88].

**Experimental indications of sorbent use in systemic diseases with kidney involvement**

Some interesting results have arisen from case series of patients with systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and vasculitis with and without renal involvement [102,103]. Improvements in renal function and dialysis independence following HP sessions in combination with chemotherapy have also been reported in a patient with cast nephropathy [104]. Finally, AKI can occur as a side effect of medications used in autoimmune disease; thus, HP could also be of value in this context. A recent small case series of patients with high-dose methotrexate-induced AKI showed a possibly positive effect of using charcoal HP as a rescue therapy until glucarpidase is available [105].

**Conclusion**

Whereas HP was once only indicated for treating poisoning from certain substances, emerging evidence suggests that other indications might be also considered. Advances in the biocompatibility of new cartridges and the selective removal of key molecules in different clinical settings and diseases like sepsis, hepatitis, and SARS-CoV-2 infection have been considered as the triggering force in that direction. With the increasing research interest in the removal of PBUTs and their involvement in CKD-related systemic complications, HP is also regaining its place as a vital accessory to dialysis treatment.

Despite this progress, current clinical use of HP remains limited, with possible reasons including the cost of performance, local practice or physician preference, a lack of consensus clinical guidelines and established indications for HP, and the absence of consistent data derived from RCTs.

In conclusion, the role of HP remains a point of discussion until its clinical effectiveness can be verified by further positive RCTs. Although in this era of disease-targeting treatments new indications are being investigated, efforts to better evaluate the applicability of HP and to shed light on the role of HP in current clinical practice are needed.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: All authors
Investigation: AD, ES, ZA
Supervision: AD, DP
Writing–original draft: AD, ES, ZA
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Shortening of primary cilia length is associated with urine concentration in the kidneys

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Background: The primary cilium, a microtubule-based cellular organelle present in certain kidney cells, functions as a mechano-sensor to monitor fluid flow in addition to various other biological functions. In kidneys, the primary cilia protrude into the tubular lumen and are directly exposed to pro-urine flow and components. However, their effects on urine concentration remain to be defined. Here, we investigated the association between primary cilia and urine concentration.

Methods: Mice either had free access to water (normal water intake, NWI) or were not allowed access to water (water deprivation, WD). Some mice received tubastatin, an inhibitor of histone deacetylase 6 (HDAC6), which regulates the acetylation of α-tubulin, a core protein of microtubules.

Results: WD decreased urine output and increased urine osmolality, concomitant with apical plasma membrane localization of aquaporin 2 (AQP2) in the kidney. After WD, compared with after NWI, the lengths of primary cilia in renal tubular epithelial cells were shortened and HDAC6 activity increased. WD induced deacetylation of α-tubulin without altering α-tubulin levels in the kidney. Tubastatin prevented the shortening of cilia through increasing HDAC6 activity and consequently increasing acetylated α-tubulin expression. Furthermore, tubastatin prevented the WD-induced reduction of urine output, urine osmolality increase, and apical plasma membrane localization of AQP2.

Conclusions: WD shortens primary cilia length through HDAC6 activation and α-tubulin deacetylation, while HDAC6 inhibition blocks the WD-induced changes in cilia length and urine output. This suggests that cilia length alterations are involved, at least in part, in the regulation of body water balance and urine concentration.

Keywords: Primary cilia, Aquaporin 2, Histone deacetylase 6, Osmolality, Water deprivation

Introduction

Primary cilia are nonmotile antenna-like cellular organelles that function as mechano- and chemo-sensors. The core of the primary cilium consists of microtubules with a 9 + 0 configuration anchored to the basal body. The length of the primary cilium changes dynamically in response to physiological and pathological stimuli. Defects in primary
cilia cause a loss of flow-sensing ability and an osmotic stress response [1]. Recent findings have demonstrated that alterations in primary cilia length affect the functions of cells and organs and that aberrant primary cilia structure and function are associated with various diseases such as obesity, hypertension, diabetes, and polycystic kidney disease [2–5].

In the kidneys, primary cilia project into the tubular lumen and are directly exposed to urinary fluid flow, osmolality, and electrolytes such as sodium. Several studies have demonstrated that primary cilia length is associated with flow-sensing abilities [6,7]. We and others recently found that unilateral ureteral obstruction and unilateral nephrectomy change primary cilia length in renal tubule epithelial cells, both in the contralateral kidney and the remaining kidney [8–11]. Furthermore, we found that primary cilia length in the kidney varies during the injury and repair process [9–12]. Based on a clinical study, Verghese et al. [13] reported that cilia length was correlated with the function of the transplanted kidney. Similarly, evidence is accumulating that the length and composition of the primary cilia are associated with renal function [8–11]. However, how primary cilia respond to the intrarenal environment remains unclear.

Elongation and shortening of primary cilia depend on the assembly and disassembly of microtubules, which are composed of tubulin [14]. Acetylation of α-tubulin induces the assembly of microtubules in primary cilia, whereas deacetylation induces the disassembly of these microtubules. Studies have demonstrated that microtubule assembly and disassembly are associated with histone deacetylase 6 (HDAC6, a member of the class II histone deacetylases), which regulates tubulin acetylation [15–17]. Smith et al. [18] recently reported that inhibition of HDAC6 stimulates ciliogenesis in nonciliary pluripotent stem cell-derived endothelial cells, which then enables mechanosensation by these cells. Furthermore, it has been reported that localization of aquaporin 2 (AQP2), a channel protein that plays an important role in concentrating urine, is regulated by microtubules [19,20], which are central organelles in the regulation of cilia length. Together, these data suggest that primary cilia are associated with kidney function. Supporting this, it has been reported that changes in HDAC6 expression and activity are associated with autosomal dominant polycystic kidney disease (ADPKD), a disease characterized by primary cilia defects [21].

Therefore, we hypothesized that primary cilia length is associated with urine concentration, including the conservation of water and electrolytes. To test this hypothesis, we investigated whether WD affected cilia length in kidney tubule epithelial cells and translocation of AQP2, and if so, whether blockage of tubulin assembly by HDAC6 inhibition affected cilia length and urine output. We found that water deprivation (WD) shortened cilia length in kidney tubule cells with concomitant concentration of urine, while HDAC6 inhibition blocked these WD-induced changes.

**Methods**

**Animal**

Ten-week-old C57BL/6 male mice (Koatech) were used in this study. All experiments were approved and performed in accordance with the approved guidelines of the Institutional Animal Care and Use Committee of Kyungpook National University, Republic of Korea (No. KNU-2022-0335). Mice either had free access to water (normal water intake, NWI) or were not allowed access to water (WD) for 24 or 48 hours. During WD, mice were given free access to standard mouse chow. Tubastatin A (a specific inhibitor of HDAC6 activation, 10 mg/kg body weight; Selleckchem) or 2% DMSO/saline (vehicle) was injected intraperitoneally each day starting from 2 days before WD until sacrifice. The dose of tubastatin was determined based on previous studies [22]. Kidney samples were obtained for biochemical and histological studies as described previously [23].

**Scanning electron microscopy**

Scanning electron microscopy (SEM) images were obtained using a scanning electron microscope (H-2500; Hitachi) as described previously [24]. Briefly, kidneys were perfusion-fixed with a fixing agent (0.5% glutaraldehyde and 0.5% paraformaldehyde) and then immersed in the fixing agent for 12 hours. Kidneys were post-fixed with 1% osmium tetroxide for 1 hour at 4 °C. After rinsing with 0.1-M phosphate buffer, kidneys were immersed serially in 25% and 50% DMSO for 30 minutes each. Kidneys were frozen rapidly on a metal plate by chilling with liquid nitrogen and then cracked using a scalpel and hammer. Cracked kidneys were freeze-dried and then cracked using a scalpel and hammer. Cracked kidneys

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were thawed with 50% DMSO, washed thrice with 0.1-M phosphate buffer, and placed in 1% osmium tetroxide for 1 hour at 4 °C. Kidneys were transferred to 25% tannic acid for 2 hours at room temperature and placed in 1% osmium tetroxide for 1 hour at 4 °C. Kidneys were dehydrated using an ethanol series and isoamyl acetate. Dehydrated kidneys were subjected to critical point drying and then mounted.

**Immunochemical and immunofluorescence staining**

Kidney sections and fixed cells were immunostained as described previously [10]. For immunochemical staining, sections were stained with anti-AQP2 (Cat. No. AQP-002; Alomone Laboratories) antibody. Hematoxylin was used for counter-staining. For immunofluorescence, the following antibodies were used: anti–acetylated (ac)-α-tubulin (Cat. No. T7451; Sigma-Aldrich), anti–Na/K-ATPase (Cat. No. ab76020; Abcam), anti-AQP1 (Cat. No. AQP-001; Alomone Laboratories), and anti-AQP2 (Cat. No. AQP-002). To detect cell nuclei, DAPI was applied to samples. Images were captured using a Leica microscope (DM2500; Wetzlar).

**Measurement of primary cilium length**

Five to 10 images per kidney (five animals) were randomly captured under 400 magnification using a Leica microscope (DM2500), and then primary cilium length was measured in more than 100 cells using i-Solution software (IMT i-Solution Inc.). Cilium length was measured by tracing the cilium curvilinear line with several straight lines as instructed by the user’s guide for i-Solution. Primary cilium length in cultured cells (3–5 experiments) was measured in more than 50 cells. Cilium length was measured blindly by a person unaware of the grouping of the samples.

**Bromodeoxyuridine incorporation assay**

To assess cell proliferation, bromodeoxyuridine (BrdU, 50 mg/kg body weight; Sigma-Aldrich) was administered to mice every other day until sacrifice starting from 1 day before WD. BrdU was determined by immunohistochemical staining using anti-BrdU (Cat. No. MCA2060; Serotec) antibody.

**Measurement of histone deacetylase 6 activity**

HDAC6 activity was measured using an HDAC6 activity assay kit (Biovision Inc.) according to the manufacturer’s instructions.

**Western blot analyses**

Western blot analyses were performed as described previously [24]. Antibodies used were as follows: anti-proliferating cell nuclear antigen (anti-PCNA, Cat. No. m879; DAKO), anti–ac-α-tubulin (Cat. No. T7451; Sigma-Aldrich), anti–α-tubulin (Cat. No. T7451; Sigma-Aldrich), anti-AQP2 (Cat. No. ab3274; Merck Millipore), anti–E-cadherin (Cat. No. 610181; BD Bioscience), anti-GAPDH (Cat. No. NB300-221; NOVUS), and anti–β-actin (Cat. No. A2228; Sigma-Aldrich).

**Membrane and cytoplasmic protein extraction**

Membrane and cytoplasmic protein extraction in the kidneys was performed using the ExKine membrane and cytoplasmic protein extraction kit (Abbkine) according to the manufacturer’s instructions. Fractions were confirmed by western blot analysis using anti-E-cadherin (BD Bioscience) and anti-GAPDH (NOVUS) antibodies as markers of the cell membrane and cytosol, respectively.

**Blood and urine biochemistry**

Urine was collected using metabolic cages for 42 to 48 hours before mice were sacrificed. Blood was collected during sacrifice of mice. Urine and blood were subjected to biochemistry analyses. Hematocrit was measured using a hematology analyzer (scil Vet abc Plus, Allied Analytic, LLC.). Urine and blood osmolalities were measured using a cryoscopic osmometer (Osmomat 030-D; Gonotec). Urinary proteins were determined by Coomassie brilliant blue assay according to the manufacturer’s instructions.

**Statistical analyses**

All data were analyzed using GraphPad Prism 6 software (GraphPad Software Inc.). Results are expressed as means ± standard errors of the mean. The statistical significance
of differences among groups was assessed using Student t test for comparison between two groups (Fig. 1–3) or two-way analysis of variance with repeated measures followed by Tukey multiple comparisons post hoc test for more than three groups (Table 1, Fig. 4, 5). Differences were considered statistically significant at p < 0.05.

Results

Water deprivation decreases the length of primary cilia in kidney tubule cells

First, we determined whether WD affected the length of primary cilia in the kidney tubule epithelial cells of mice. When cells were observed using SEM, primary cilia were seen protruding into the tubular lumen of both WD and NWI mice (Fig. 1). Lengths of the primary cilia in WD mice were shorter than those in NWI mice (Fig. 1). When primary cilia were visualized by immunofluorescence using antibody to anti-ac-α-tubulin, primary cilia were observed in all renal tubule epithelial cells, with the exception of intercalated cells, which are known to not possess primary cilia [25]. Consistent with the SEM data, the lengths of primary cilia in kidney tubule cells in WD mice were shorter than those in the kidney tubule cells of NWI mice; the frequencies of short primary cilia increased gradually after WD. Primary cilia length of proximal tubular cells was the longest among tubules in NWI mice; primary cilia shortening after WD was greater in proximal tubules than distal tubules and collecting ducts (Fig. 2). These data indicate that WD induced the shortening of primary cilia.

To evaluate whether the decrease in primary cilia length was associated with cell proliferation (cells undergo deciliation before dividing) and/or cilia damage [11–13,26], we performed a BrdU-incorporation assay and examined PCNA expression to investigate proliferation, and also performed a urinary protein determination assay to look for cilia damage [10,12]. BrdU-positive cells were rarely observed, indicating that cell proliferation was not the cause of the decreased length of primary cilia (Fig. 3A, B). In addition, WD did not induce a change in PCNA expression in kidneys compared to NWI (Fig. 3C, D). Next, we evaluated whether WD-induced cilia shortening was due to the disruption of primary cilia and release of cellular contents into urine by determining urinary protein levels and ac-α-tubulin expression in the urine samples of both WD and NWI mice. The urine of WD mice contained higher amounts of proteins compared with the urine of NWI mice (Fig. 3E). Ac-α-tubulin in the urine of both WD and NWI mice was virtually undetectable by western blot analysis (Fig. 3F;
Figure 2. Shortening of primary cilia in the renal tubule cells of water-deprived mice. Mice were either allowed free access to water (normal water intake, NWI) or not allowed access to water (water deprivation, WD) for 24 or 48 hours. Forty-eight hours after either NWI or WD, kidneys were harvested. Kidneys were sectioned at 5-μm thickness using a microtome. Kidney sections were stained with anti–acetylated-α-tubulin (ac-α-tubulin, a marker of primary cilia; green), anti–aquaporin-1 (AQP1, a marker of principal cells of the collecting duct [CD]; red), or anti–Na⁺-K⁺-ATPase (a basolateral protein and a marker of distal tubule [DT] cells; red) antibodies. DAPI (blue) was used to visualize nuclei. Pictures were taken from the cortex (A–D), outer medulla (E–H), and inner medulla (I, J). Averages and percentages of primary cilia length were determined in S1-2 segments of PT cells, DT cells, principal cells in the CD, cortex (A–D), S3 segments of PT cells (S3 PT), medullary thick ascending limb cells (mTAL), principal cells in the CD in the outer medulla (E–H), and principal cells in the CD in the inner medulla (I, J). Primary cilia length was measured in more than 100 cells in tubules per time point from five independent animals. Arrowheads indicate primary cilia. Results are expressed as mean ± standard error of the mean. NS, no significant difference. ∗p < 0.05 vs. NWI.
Figure 3. Proliferation of tubular cells and primary ciliary protein expression in the urine after water deprivation (WD). Mice were either allowed free access to water (normal water intake, NWI) or not allowed access to water (WD) for 48 hours. Some mice were administered bromodeoxyuridine (BrdU) intraperitoneally every alternate day, beginning 24 hours before WD until the end of the experiment. Kidneys were harvested and then frozen with liquid nitrogen or fixed with periodate-lysine-paraformaldehyde fixative. (A) Kidney sections of 3 μm were immunohistochemically stained using anti-BrdU antibody (brown). (B) BrdU-positive (BrdU+) cells were counted in the cortex (arrowhead indicates BrdU cell). (C) Kidney samples were subjected to western blotting analysis using anti-proliferating cell nuclear antigen (PCNA) antibody. β-actin was used as the loading control. (D) Densities of PCNA blots were determined using ImageJ software (n = 4). (E, F) Mouse urine was collected 48 hours after WD. As a positive control, urine from ischemia/reperfusion-injured mice was used. (E) Twenty microliters of urine from each sample was subjected to Coomassie brilliant blue staining. (F) Same amount of urine from each sample was subjected to western blotting analysis using anti–acetylated-α-tubulin (ac-α-tubulin) antibody. Results are expressed as mean ± standard error of the mean (n = 4).

NS, no significant difference.

Table 1. Effect of tubastatin on WD-induced changes in water intake volume, body weight, urine output, osmolality, plasma osmolality, and hematocrit

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle NWI (n = 8)</th>
<th>Vehicle WD (n = 10)</th>
<th>Tubastatin NWI (n = 8)</th>
<th>Tubastatin WD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (mL/day)</td>
<td>6.3 ± 0.2</td>
<td>-</td>
<td>6.5 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>22.5 ± 0.3</td>
<td>22.01 ± 0.4</td>
<td>23.3 ± 0.4</td>
<td>22.71 ± 0.4</td>
</tr>
<tr>
<td>Urine volume (μL)</td>
<td>543.3 ± 44.8</td>
<td>50.0 ± 18.4</td>
<td>423.3 ± 97.0</td>
<td>342.5 ± 49.7</td>
</tr>
<tr>
<td>Urine osmolality (mosmol/kg H2O)</td>
<td>2,250.3 ± 395.0</td>
<td>4,085.0 ± 157.3</td>
<td>2,380.0 ± 260.0</td>
<td>2,685.0 ± 106.6</td>
</tr>
<tr>
<td>Plasma osmolality (mosmol/kg H2O)</td>
<td>268.2 ± 3.8</td>
<td>274.5 ± 3.0</td>
<td>268.2 ± 5.0</td>
<td>274.0 ± 7.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.3 ± 5.3</td>
<td>45.3 ± 3.3</td>
<td>43.4 ± 4.4</td>
<td>44.1 ± 6.2</td>
</tr>
</tbody>
</table>

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

Mice were either allowed free access to water (normal water intake, NWI) or not allowed access to water (water deprivation, WD) for 48 hours. Some mice were administered either tubastatin A (10 mg/kg body weight) or saline (vehicle) intraperitoneally each day, starting 48 hours before WD until the end of the experiment.

*p < 0.05 vs. respective NWI; **p < 0.05 vs. vehicle WD.
the last lane in Fig. 3F represents urine collected from the kidneys with ischemia/reperfusion injury as a positive control. These data indicate that the WD-induced decrease in cilia length was not associated with either cell proliferation or cilia disruption.

**Water deprivation increases histone deacetylase 6 activity and deacetylates α-tubulin in the kidney**

Deacetylation of α-tubulin causes the disassembly of the microtubule axoneme, a central structure of primary cilia, leading to a decrease in the length of primary cilia, and α-tubulin is a substrate of HDAC6 [16,27]. Therefore, we investigated whether the WD-induced decrease in primary cilia length was associated with HDAC6. First, we investigated HDAC6 activity in the kidneys. HDAC6 activity was significantly higher in the kidneys of WD mice than NWI mice (Fig. 4A). Ac-α-tubulin expression was significantly lower in the kidneys of WD mice than in NWI mice, but level of total α-tubulin expression was similar in these two groups of mice (Fig. 4B–D), indicating that HDAC6 deacetylates α-tubulin.

Next, we investigated if tubastatin prevented WD-induced primary cilia shortening. Primarily, we investigated if tubastatin blocked HDAC6 activation. Tubastatin A was administered 2 days before WD on a daily basis until sacrifice. Tubastatin treatment significantly blocked the WD-induced increase in HDAC6 activity (Fig. 4A) and decrease in ac-α-tubulin levels (Fig. 4B–D). In NWI mice, tubastatin slightly reduced HDAC6 activity with an increase in ac-α-tubulin levels (Fig. 4B–D). Secondarily, we measured primary cilia length. Tubastatin almost completely blocked the decrease in primary cilia length induced by WD (Fig. 4E–K). However, tubastatin in NWI mice did not induce significant changes in primary cilia length (Fig. 4E–K). These results indicated that the WD-induced shortening of primary cilia was associated with HDAC6 activation.

**Water deprivation-induced urine production is regulated by histone deacetylase 6**

Since AQP2 is a critical player in the regulation of urine output and urine osmolality, we investigated if the localization and level of expression of AQP2 was associated with HDAC6 by quantitative and histological analysis, respectively. First, we determined AQP2 expression in whole kidney lysates. WD significantly increased AQP2 expression compared to NWI (Fig. 5A, B). Tubastatin treatment inhibited the WD-induced increase in AQP2 expression (Fig. 5A, B). In NWI mice, tubastatin slightly, but not significantly, increased AQP2 expression (Fig. 5A, B).

Second, we assessed AQP2 expression in membrane and cytosolic fractions from the kidney. WD increased AQP2 expression in the membrane fraction and reduced it in the cytosolic fraction (Fig. 5C–F). Tubastatin treatment significantly inhibited the WD-induced increase in AQP2 expression in the apical membrane fraction (Fig. 5C–F). In NWI mice, tubastatin slightly increased AQP2 expression (Fig. 5C–F).

Third, we determined the localization of AQP2 in the principal cells by immunohistochemical staining. Consistent with the western blot results, WD led to increased AQP2 expression at the apical plasma membrane with reduced cytosolic expression in principal cells (Fig. 5G). Tubastatin blocked the WD-induced membrane localization and increase in AQP2 protein expression (Fig. 5G).

Lastly, we investigated whether primary cilia length was associated with urine output and osmolality. Urine output from WD mice was significantly less than that from NWI mice and urine osmolality was higher in WD mice than in NWI mice (Table 1). However, there were no significant differences in plasma osmolality or hematocrit between WD and NWI mice (Table 1). Tubastatin injection in WD mice significantly increased urine output and decreased urine osmolality (Table 1). Tubastatin injection in NWI mice induced slight, but not statistically significant changes in urine output and osmolality compared with vehicle injection (Table 1). Plasma osmolality was not significantly affected by WD or tubastatin administration (Table 1). Tubastatin did not induce significant changes in the amount of water intake in NWI mice (Table 1). These data indicate that WD facilitates the apical localization of AQP2 and an increase in AQP2 expression through HDAC6 activation, consequently leading to concentrated urine production. Furthermore, tubastatin inhibits WD-induced apical localization of AQP2.

**Discussion**

To the best of our knowledge, this is the first study to
Figure 4. Blockage of water deprivation (WD)-induced primary cilia shortening by administration of tubastatin 

A. Mice were either allowed free access to water (normal water intake, NWI) or not allowed access to water (WD) for 48 hours. Some mice were administered either tubastatin A (10 mg/kg body weight) or saline (vehicle) intraperitoneally each day starting 48 hours before WD until the end of the experiment. Kidneys were harvested, fixed with the periodate-lysine-paraformaldehyde fixative, and sectioned to 5-μm thickness using a microtome. (A) Histone deacetylase 6 (HDAC6) activity was determined in whole kidneys (n = 5–8). (B–D) Kidney samples were subjected to western blotting analysis using anti-α-acetylated-α-tubulin (ac-α-tubulin) and anti-α-tubulin antibodies. GAPDH was used as the loading control. Densities of blots were determined using ImageJ software (n = 4). (E) Kidney sections were stained with anti-ac-α-tubulin (green), anti-aquaporin-1 (AQP1, a marker of proximal tubule [PT] cell; red), anti-AQP2 (a marker of principal cell of collecting duct [CD], red), and anti-Na⁺K⁺-ATPase (a basolateral protein, red) antibodies. DAPI (blue) was used to visualize nuclei. Pictures were taken from the cortex. Averages (F, H, J) and percentages (G, I, K) of primary cilia length were determined in the proximal tubular cells of the S1–S2 segments of the PT (E, F, G), distal tubular cells of the distal tubule (DT; E, H, I), and principal cells in the CD (E, J, K). Arrowheads indicate primary cilia. Primary cilia length was measured in each segment from five independent animals. Results are expressed as mean ± standard error of the mean. NS, no significant difference. *p < 0.05.

demonstrate that WD shortens primary cilia length in kidney tubule cells and that this is mediated by HDAC6 activity; HDAC6 inhibition impaired the water intake-associated changes in urinary output and osmolality as well as AQP2 localization. These data suggest that shortening of primary cilia length after WD is associated with urine concentration, and that, since HDAC6 inhibition impairs these kidney responses to water intake changes, HDAC6 is involved in this process. Our data provide insights into how primary cilia length is altered dynamically in response to physio-
are directly exposed to changes in fluid flow and osmolality and/or channels in kidney tubular epithelial cells. Nephrons excrete concentrated urine via regulation of transmembrane transporters. When water intake decreases, kidney function, and how kidney tubule cells regulate primary cilia length. Unlike urine osmolality and urine output, hematocrit and plasma osmolality after WD tended to increase but not by statistically significant amounts. This indicates that plasma osmolality and hematocrit are tightly maintained within certain ranges under water-deprived conditions, with relatively minimal changes compared with urine volume. For these reasons, 48 hours of WD did not cause a significant imbalance in body water balance, and the body water balancing system compensated for this level of WD. Similarly, Brazzuna et al. [28] reported that 6 days of dehy-

Figure 5. Increased apical expression of aquaporin-2 (AQP2) after water deprivation (WD) and blockage of this increase by tubastatin. Mice were either allowed free access to water (normal water intake, NWI) or not allowed access to water (WD) for 48 hours. Tubastatin A (10 mg/kg body weight) or saline (vehicle) was administered intraperitoneally each day, starting 48 hours before WD until the end of the experiment. (A) Whole kidney lysates were subjected to western blotting analysis using anti-AQP2 antibody. GAPDH was used as the loading control. (B) Densities of blots were determined using ImageJ software (n = 4). (C–F) Expression of AQP2 was assessed in the membrane (C) and cytosolic (E) fractions from whole kidneys. E-cadherin and GAPDH were used as loading controls for the membrane and cytosolic fractions, respectively. Densities of blots were determined using ImageJ software (n = 3). (G) Kidney sections of 3 um were immunohistochemically stained using anti-AQP2 antibody (brown). Higher magnification is indicated by the lined rectangles (Scale bar: 12.5 μm). Results are expressed as means ± standard error of the mean. *p < 0.05.
hydration did not change hematocrit levels due to restriction of plasma water loss. de Fost et al. [29] reported that WD caused the release of arginine vasopressin into the circulation to preserve water and restore normal plasma osmolality. However, contrary to our results, Bekkevold et al. [30] reported that 48 hours of WD increased plasma osmolality by about 4% in mice.

In the present study, we observed that WD caused a decrease in the length of primary cilia, that the cilia length shortening induced by WD was associated with increased HDAC6 activity, and that this shortening was blocked by HDAC6 inhibition. Furthermore, tubastatin blocked WD-induced shortening of cilia, increased urine output, and decreased urine osmolality. In addition, we found that the length of primary cilia in proximal tubular cells was the longest among the tubules of NWI mice and that WD-induced primary cilia shortening in proximal tubules was greater than in distal tubules or collecting ducts. We speculate that the greater WD-induced shortening of cilia in proximal tubular cells may be due to the greater reduction in ultrafiltrate volume and flow compared to collecting ducts (or distal tubules) and the longer basal cilia length before WD in proximal tubular cells. Analysis of HDAC6 activity in each tubular segment may provide more insight into the link between HDAC6 activity and cilia length.

In previous studies, we found that the cessation of urine flow induced by ureteral obstruction resulted in a decrease in cilia length in renal tubule cells [8], whereas an increase in urine flow by unilateral nephrectomy led to elongated primary cilia in the remaining kidney [9]. In addition, we found that kidney tubule cells contained primary cilia of various lengths following a kidney insult such as ischemia/reperfusion injury, which induces dramatic changes in glomerular filtration rate and fractional excretion of electrolytes [23,31]. In addition, studies have reported that primary cilia are functionally involved in flow sensing [32,33]. Gilmer et al. [34] reported that the flow rate in proximal tubules is the greatest among renal tubules. Furthermore, it has been reported that osmolality-sensing proteins are localized on the membrane of cilia and that cilia are required for the response to osmotic stress [35]. Therefore, our data indicate that fluid flow and/or urine osmolality may cause alterations in cilia length and, conversely, that alterations in cilia length may contribute to urine output and osmolality. Therefore, we speculate that the cilia length changes and HDAC6 activation observed in WD-mouse kidneys may be normal responses of kidney cells and critical for the adaptation of kidneys to kidney burdens such as excessive hydration, leading to maintenance of body water balance.

We recently found that a decrease in the length of primary cilia was associated with deciliation and resorption of cilia [10-12,36]. Therefore, to define the involvement of cell proliferation and deciliation in dynamic changes in cilia length, we performed a BrdU incorporation assay and PCNA expression assay in kidney tissue and investigated the presence of ciliary proteins in urine. In previous studies, we reported that disrupted cilia in the kidney tubular epithelial cells were excreted into the urine [10-12,36]. In the current study, we found that WD-mouse urine contained greater amounts of protein than NWI mouse urine, but α-tubulin was undetectable in the urine samples of both WD and NWI mice by western blot analysis. This indicates that primary cilia were not disrupted by WD. Furthermore, WD did not induce significant changes in the number of BrdU-positive cells or PCNA expression. Taken together, these data indicate that changes in WD-induced primary cilia length are not caused by either cell proliferation or deciliation.

The assembly and disassembly of microtubules by α-tubulin acetylation and deacetylation are associated with an increase and decrease in cilia length, respectively [10-12,26]. Recent studies have shown that HDAC6 serves as a regulator of primary cilia length; HDAC6 activation and overexpression induce deacetylation of α-tubulin and a subsequent decrease in primary cilia length [18,27,37]. Similarly, in the present study, we found that WD increased HDAC6 activity and led to α-tubulin deacetylation without a significant change in the total amount of α-tubulin expression. These data suggest that the shortening of primary cilia length induced by WD is associated with disassembly of microtubules and that this change may be an adaptive response to WD. Supporting this, inhibition of HDAC6 activation via tubastatin in water-restricted mice restored the ratio of ac-α-tubulin to total α-tubulin to that seen in NWI mice.

Microtubules are known to play an important role in the cellular localization and expression level of AQP2, a water channel that is essential for urine concentration [19,20].
Therefore, to define the functional association between cilia length changes and HDAC6, we evaluated WD-induced AQP2 localization, because the apical localization of AQP2 is critical for the generation of concentrated urine formation [38]. As expected, WD resulted in the movement of cytosolic AQP2 to the apical plasma membrane, and tubastatin blocked the apical localization of AQP2 following WD. Furthermore, WD increased AQP2 expression in the kidneys. Furthermore, tubastatin increased the WD-induced changes in urine output, osmolality, and cilia length to levels similar to those observed in control mice. These data indicate that WD regulates urine concentration through the apical membrane localization and de novo production of AQP2 and that this is at least partly regulated by HDAC6 activation. Smith et al. [18] recently reported that inhibition of HDAC6 activates cilia formation in human-induced pluripotent stem cell-derived endothelial cells lacking cilia and restores their mechano-sensing ability. Rymut et al. [39] reported that HDAC6 inhibition reversed the cystic fibrotic phenotype, with lowering of α-α-tubulin levels. Ke et al. [21] reported that HDAC6 expression and activity were low in kidney tubule cells in ADPKD, a disease associated with defects in the primary cilia of kidney tubule cells. Furthermore, in a previous study, we found that inhibition of microtubule dynamics by taxol treatment during recovery delayed the restoration of kidney functional damage [40]. Therefore, we speculate that alteration of primary cilia length regulated by HDAC6 is associated with urine concentration.

In summary, although several limitations, including determining if there is a causal relationship between primary cilia and urine concentration remain, our data show for the first time that changes in primary cilia length are, at least in part, associated with urine concentration, suggesting that primary cilia length regulated by HDAC6 is involved in urine concentration and the maintenance of body water balance.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization, Methodology: MJK, KHH, JHL, KMP
Formal analysis, Investigation: MJK, SJH, SYS
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Writing–original draft: MJK, KHH, JHL, KMP
Writing–review & editing: All authors
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The flavonoid fisetin ameliorates renal fibrosis by inhibiting SMAD3 phosphorylation, oxidative damage, and inflammation in ureteral obstructed kidney in mice

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Background: Renal fibrosis is characterized by the accumulation of extracellular matrix and inflammatory cells and kidney dysfunction, which is a major pathway in the progression of chronic kidney disease (CKD). Accumulating evidence indicates that oxidative stress plays a critical role in the initiation and progression of CKD via proinflammatory and profibrotic signaling pathways. Fisetin (3,3′,4′,7-tetrahydroxyflavone) has biological activities including antioxidant, anti-inflammatory, and anti-aging effects. Therefore, we evaluated the antifibrotic effects of fisetin on unilateral ureteral obstruction (UUO)-induced kidneys.

Methods: C57BL/6 female mice were subjected to right UUO and intraperitoneally injected every other day with fisetin (25 mg/kg/day) or vehicle from 1 hour before surgery to 7 days after surgery. Kidney samples were analyzed for renal fibrosis (α-smooth muscle actin [α-SMA] expression, collagen deposition, and transforming growth factor [TGF] β1/SMAD3 signaling pathway), oxidative damage (4-HNE and 8-OHdG expression), inflammation (proinflammatory cytokine/chemokine, macrophage, and neutrophil infiltration), and apoptosis (TUNEL staining). Cultured human proximal tubule cells were treated with fisetin before TGF-β to confirm the TGF-β downstream pathway (SMAD2/3 phosphorylation).

Results: We found that fisetin treatment protected against renal fibrosis by inhibiting the phosphorylation of SMAD3, oxidative damage, inflammation, apoptotic cell death, and accumulation of profibrotic M2 macrophages in the obstructed kidneys. In cultured human proximal tubular cells, fisetin treatment inhibited TGF-β1-induced phosphorylation of SMAD3 and SMAD2.

Conclusion: Fisetin alleviates kidney fibrosis to protect against UUO-induced renal fibrosis, and could be a novel therapeutic drug for obstructive nephropathy.

Keywords: Antioxidants, Chronic kidney diseases, Fisetin, Inflammation, Kidney fibrosis, Ureteral obstruction
Introduction

Renal fibrosis is a prominent feature of chronic kidney disease (CKD) progression. CKD is a critical kidney disease that can lead to end-stage renal disease (ESRD), which is defined as the complete loss of kidney function. Patients with ESRD are currently treated with therapies such as kidney transplants and hemodialysis [1]. Although many studies have been conducted on renal fibrosis, an effective treatment for renal fibrosis has not yet been established. Therefore, there is an urgent need to develop antifibrotic strategies to prevent renal fibrosis and treat CKD.

Fibrosis is characterized by the marked accumulation of extracellular matrix (ECM) in the tubulointerstitial space. This phenomenon occurs from various events, including fibroblast activation, interstitial macrophage infiltration, and activation of signaling factors such as transforming growth factor β (TGF-β) [1]. As a pathogenic factor, TGF-β1 is a major mediator of fibrosis development. TGF-β1/SMAD3 signaling is a key pathway regulating the initiation and progression of renal fibrosis. Upon exposure to stimuli such as reactive oxygen species (ROS), TGF-β1 phosphorylates intercellular signaling factors such as SMAD2/3; the activated SMAD complex enters the nucleus to transcribe genes involved in myofibroblast activation and matrix deposition. Various studies have suggested that myofibroblasts that produce ECM in the kidney are derived from several sources, such as epithelial cells, macrophages, endothelial cells, and resident fibroblasts through the TGF-β1/SMAD3 pathway [2,3].

Although there are various etiologies of renal damage in obstructive nephropathy, accumulating evidence indicates that oxidative stress caused by ROS plays a critical role. Increased ROS causes tubular epithelial cell death and damage to cellular macromolecules, including DNA, proteins, and lipids. In addition, ROS promotes the infiltration of inflammatory cells that release proinflammatory cytokines and chemokines [4]. Infiltrating inflammatory cells contribute to the maintenance and enhancement of the inflammatory response, as well as the stimulation of fibrogenic, apoptotic, and gene regulatory signaling pathways such as TGF-β, nuclear factor-kappa B (NF-κB), and the mitogen-activated protein kinase pathways [5].

Flavonoids have received substantial attention as medications and health food supplements because of their prospective therapeutic pharmacological and nutritional properties. Fisetin (3,3′,4′,7 tetrahydroxyflavone) is a flavonoid that is isolated from various seaweeds, fruits, and vegetables, including strawberries, apples, persimmons, and onions [6]. Studies reported that fisetin has biological activities including antioxidant [7], anti-inflammatory [8], and anticancer [9] effects. Sahu et al. [10] demonstrated that fisetin exhibited renoprotective effects by alleviating oxidative stress and apoptosis in renal tubular cells and regulating NF-κB activation in a cisplatin-induced nephrotoxicity model. Ren et al. [11] also demonstrated that fisetin protects against hyperuricemic nephropathy by modulating the STAT3 and β1/SMAD3 signaling pathway. However, the effect of fisetin on the progression of renal fibrosis and the underlying pathogenic mechanisms, with potential involvement of TGF-β1/SMAD3, ROS, inflammation, and renal tubular cell death, remain to be elucidated.

In this study, we investigated whether fisetin protects against renal fibrosis by regulating the TGF-β1/SMAD3 signaling pathway and by attenuating oxidative stress, inflammation, and cell death in mice with unilateral ureteral obstruction (UUO), a representative model of CKD.

Methods

Animals and establishment of the unilateral ureteral obstruction model

Female C57BL6 mice (8–12 weeks old) weighing 18–21 g were used for experiments. The mice were provided free access to water and standard chow. All animal surgeries were approved by the Institutional Animal Care and Use Committee of Pukyong National University (No. PKNUI-ACUC-2021-49) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 2011).

All mice were anesthetized with pentobarbital sodium (50 mg/kg or to effect; Hanlim Pharma Co.) intraperitoneally before the operation. The mice were subjected to right UUO, as previously described [12]. Briefly, to induce ureteral obstruction, the right kidney was exposed via flank incision, the right ureter was completely tied with a 6-0 silk thread, and the incision was sutured. The left kidney was used as the control. The body temperature was maintained...
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at 36.5–37 °C during surgery and before and after anesthesia using a surgical heating pad (FHC, Inc.). The animals were divided into two groups: one group was intraperitoneally injected with fisetin (25 mg/kg; Pytolab; n = 5) 1 hour before surgery and on days 2, 4, and 6 after surgery and the second group received vehicle (n = 5) at the same time points. The concentration of fisetin was selected based on previous studies [13-15]. All mice were sacrificed 7 days after the surgery. Harvested kidneys were frozen in liquid nitrogen or fixed in 4% paraformaldehyde for subsequent analysis.

Human proximal tubule cell culture

HK-2 cells, a human proximal tubular cell line, were purchased from the Korean Cell Line Bank. The cells were cultured in Dulbecco’s modified Eagle’s medium (Corning)-Ham’s F12 (Welgene, Inc.) supplemented with 10% fetal bovine serum (MP Biomedicals) at 37 °C in a humidified 5% CO₂ incubator. After 16 hours of serum deprivation, cells were treated with 10 ng/mL human recombinant TGF-β for 30 minutes. In some experiments, cells were pretreated with 40 μM fisetin (n = 3) or vehicle (n = 3) 1 hour before TGF-β treatment.

Western blotting

Kidney tissues were lysed and homogenized using the radioimmunoprecipitation assay lysis buffer (50 mM Tris-HCl, pH 8.0, 1% Triton-X 100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 M NaF plus protease inhibitor cocktail [Sigma-Aldrich] and phosphatase inhibitor cocktail [Sigma-Aldrich]). Extracted protein samples were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (GVS S.p.A.). After blocking with 5% bovine serum albumin or skim milk for 30 minutes, the membranes were incubated with antibodies against α-smooth muscle actin (α-SMA, 1:20,000; Sigma-Aldrich), phosphorylated SMAD3 (p-SMAD3, 1:2,000; Abcam), p-SMAD2 (1:2,000; Abcam), t-SMAD 2/3 (1:2,000; Abcam), 4-hydroxynonenal (4-HNE, 1:2,000; Abcam), Ly6G (1:2,000; Fisher Scientific), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:10,000; Bioworld Technology) overnight at 4 °C. The membranes were then treated for 1 hour at room temperature with horseradish peroxidase (HRP)-labeled goat anti-mouse immunoglobulin G (IgG, 1:3,000; Bethyl-Laboratories) or HRP-labeled goat anti-rabbit IgG (1:3,000; Bethyl-Laboratories). Total protein expression levels were normalized to GAPDH. Band intensities were analyzed using ImageJ software.

Quantitative real-time polymerase chain reaction

Quantitative real-time (qRT) polymerase chain reaction (PCR) was performed to measure the messenger RNA (mRNA) expressions of markers of inflammation (monocyte chemoattractant protein-1 [MCP-1], macrophage inflammatory protein-2 [MIP-2], and interleukin-1β [IL-1β]), TGF-β1, and inducible nitric oxide synthase (iNOS) in the kidney after surgery. Total RNA was extracted from kidney tissues using TRIzol (Ambion) and synthesized as complementary DNA (cDNA) with random primers using reverse-transcription PCR. cDNA was measured by qRT-PCR (Bio-Rad) with the FastStart Universal SYBR Green Master Mix (Sigma-Aldrich) and primers (Table 1). mRNA levels were normalized to GAPDH mRNA. Relative expression was calculated using the cycle threshold method. Specificity was confirmed using melting curve analysis.

Periodic acid-Schiff staining

The kidneys were fixed with 4% paraformaldehyde. Paraffin-embedded kidney tissue sections were stained with the periodic acid-Schiff (PAS) staining kit (Abcam) according to the manufacturer’s protocol. To evaluate morphological damage to tubular cells, damage in a PAS-stained kidney section was scored in five fields in cortical areas per kidney using the following scoring method: 0, no damage; 1, mild damage with dilated tubular lumen; 2, moderate damage with flattened epithelial cells, dilated lumen, and congested lumen; and 3, severe damage with flat epithelial cells lacking nuclear staining and congested lumen [12].

Masson’s trichrome staining and Sirius red staining

Paraffin-embedded kidney tissue sections were stained using the Picro Sirius Red Stain Kit (Abcam) and Masson’s trichrome stain. For picrosirius red staining, deparaffinized sections were covered completely with picrosirius red solution for 1 hour. The samples were then rinsed twice
with acetic acid solution (Sigma-Aldrich). For Masson’s trichrome staining, deparaffinized sections were refixed in Bouin’s solution for 1 hour at 56 °C, and sections were stained in Weigert’s iron hematoxylin working solution for 10 minutes. After washing, the sections were stained in Biebrich scarlet-acid fuchsin solution for 10 to 15 minutes, followed by staining in phosphomolybdic-phosphotungstic acid solution for 15 minutes. The picrosirius red or Masson’s trichrome-stained sections were then continuously dehydrated in different concentrations of alcohol solutions. Finally, the sections were mounted on coverslips with Permount mounting medium (Fisher Scientific). Micrographs were taken randomly in 200× microscope image fields in cortical areas using a microscope (Leica DM2500; Leica Microsystems GmbH). Areas of collagen accumulation in the stained kidney tissues were analyzed using the ImageJ Fiji program.

### Table 1. Primer sequences for the quantitative polymerase chain reaction analysis in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (sense/antisense)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse TGF-β1</td>
<td>5'-TTGTACGGCCAGTGCTGAA-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>5'-GGGGCTATCCCGTTGATTTC-3'</td>
<td></td>
</tr>
<tr>
<td>Mouse MCP-1</td>
<td>5'-ACCTGCTGCTACTCATTACAC-3'</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>5'-TTGAGGTTGTGGTGAGAAAGG-3'</td>
<td></td>
</tr>
<tr>
<td>Mouse MIP-2</td>
<td>5'-CCAAGGTGTAGCTCAGAAC-3'</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>5'-AGCGAGGGCAGATCGAGATCG-3'</td>
<td></td>
</tr>
<tr>
<td>Mouse IL-1β</td>
<td>5'-CTGAAAGCTCTACACCTG-3'</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>5'-TGCTGATGACAGATCAGGG-3'</td>
<td></td>
</tr>
<tr>
<td>Mouse iNOS</td>
<td>5'-GCCGTTGACCGTTTGTGTGCTC-3'</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>5'-CCGCTGAGCTTCTACTCTG-3'</td>
<td></td>
</tr>
<tr>
<td>Mouse GAPDH</td>
<td>5'-ACCAACATGTCATGCCCATTAC-3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>5'-CACCACCTGTGCTGAGCC-3'</td>
<td></td>
</tr>
</tbody>
</table>

Annealing temperatures used for each primer are also provided.

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; TGF, transforming growth factor.

**Immunohistochemical staining**

Immunohistochemical (IHC) staining was performed to confirm the expression of α-SMA, which is a marker of fibrosis, infiltration of macrophages, and generation of 8-hydroxy-2′-deoxyguanosine (8-OhdG), an oxidized nucleoside of DNA. Paraffin-embedded kidney tissue sections were rehydrated, followed by antigen retrieval, peroxide quenching, and blocking. Sections were then incubated with primary antibodies in a humid chamber overnight at 4 °C. Primary antibodies against the following proteins were used for staining: mannose receptor (CD206, 1:200; Abcam), F4/80 (1:100; Bio-Rad), α-SMA (1:400; Sigma-Aldrich), and 8-OhdG (1:1,000; Abcam). Sections were then stained with HRP-conjugated goat anti-rat IgG or HRP-conjugated goat anti-mouse IgG (Bethyl-Laboratories). Hematoxylin was used to stain nuclei. The sections were observed using a Leica DM2500 microscope. Micrographs were taken randomly in 200× and 400× microscope image fields in the cortical areas. The α-SMA-, F4/80-, and 8-OhdG-positive cells were counted and recorded using the counting tool.

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

We performed terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining using the DeadEnd Fluorometric TUNEL System Kit (Promega) according to the manufacturer’s protocol. Briefly, kidney sections were deparaffinized and rehydrated. Next, the sections were incubated with the TUNEL reagent mixture for 1 hour at 37 °C and then washed with phosphate-buffered saline. Sections were mounted on coverslips with an antifade mounting medium. Images were obtained randomly at 200× microscope image fields in cortical areas under a Leica DM2500 microscope. TUNEL-positive cells were counted and recorded in five fields per kidney.
Statistical analysis

Data were analyzed using the Student t test, one-way analysis of variance, and Tukey post hoc multiple comparison test. Results are expressed as the mean ± standard error of the mean. Statistical significance was set at p < 0.05.

Results

Fisetin alleviates unilateral ureteral obstruction-induced fibrosis in the kidney

First, we assessed the effect of fisetin on kidney fibrosis in obstructed kidneys in mice. Compared with controls, UUO-induced myofibroblast accumulation, which was characterized by increased expression of α-SMA (an activated fibrotic cell marker); western blotting also showed that treatment with fisetin significantly reduced the elevation of α-SMA (p = 0.02) (Fig. 1A, B). Moreover, the number of α-SMA-positive cells, as assessed by IHC staining, was upregulated in obstructed kidneys. Fisetin suppressed the upregulation of α-SMA-positive cells in renal tissues of UUO mice compared with that of vehicle-treated UUO mice (Fig. 1E, F). These results demonstrate that fisetin inhibits myofibroblast expansion in the fibrous kidney.

To further investigate the underlying mechanism, we examined whether fisetin affected activation of the TGF-β1/SMAD3 signaling pathways in UUO mice. A previous study shows that TGF-β1 served as a key mediator of fibrosis development and progression in CKD through SMAD3 phosphorylation [16]. Western blot analysis showed that p- SMAD3 expression was increased in the kidneys of mice subjected to UUO compared with that of vehicle-treated UUO mice (Fig. 1A, C). However, there was no significant difference in TGF-β1 mRNA expression between vehicle-treated mice and fisetin-treated mice after UUO (Fig. 1D). Together, these results indicate that fisetin treatment may alleviate kidney fibrosis by inhibiting SMAD3 phosphorylation after UUO. To further examine the effect of fisetin on the TGF-β1/SMAD pathway, HK-2 cells, a human kidney epithelial cell line, were pretreated with 40 μM fisetin or vehicle 1 hour before treatment with 10 ng/mL TGF-β1. Western blot analysis showed that fisetin pretreatment significantly reduced the TGF-β1-induced phosphorylation of SMAD2 (p = 0.03) and SMAD3 (p = 0.02) in HK-2 cells compared with vehicle-treated cells (Fig. 1G–K).

Fisetin reduces unilateral ureteral obstruction-induced collagen deposition in renal interstitial area

To examine the effects of fisetin on UUO-induced ECM accumulation, we analyzed collagen deposition using Sirius red staining and Masson’s trichrome staining. Collagen deposition was increased in the interstitium after UUO, and this increase was significantly lower in the kidneys of fisetin-treated mice than in the kidneys of vehicle-treated mice (p = 0.005) (Fig. 2A). The quantified results indicated that the deposition of collagen in the interstitial area was notably elevated in mice subjected to UUO, while it was partially restored in mice treated with fisetin (Fig. 2B, C).

Fisetin protects against renal damage and tubule cell apoptosis after unilateral ureteral obstruction

We analyzed the effect of fisetin on renal tubular injury after UUO using PAS staining. The obstructed kidneys of UUO mice exhibited severe structural disorders characterized by tubular dilation and atrophy, as well as luminal congestion. In contrast, obstructed kidneys of mice treated with fisetin showed significantly less luminal congestion and tubular dilation and atrophy compared with the obstructed kidneys of vehicle-treated mice, indicating that fisetin treatment ameliorated the UUO-induced renal morphological damage (Fig. 3A). The renal damage score (scale, 0–3) for histological grading was used to measure the extent of renal tubular damage after UUO. Fisetin-treated mice subjected to UUO had significantly lower renal damage scores than vehicle-treated mice after UUO (p < 0.001) (Fig. 3B). We then investigated whether fisetin treatment showed an effect on renal tubular apoptosis after UUO. Fisetin-treated mice subjected to UUO had significantly lower renal damage scores than vehicle-treated mice after UUO (p < 0.001) (Fig. 3B). We then investigated whether fisetin treatment showed an effect on renal tubular apoptosis after UUO. UUO increased the number of TUNEL-positive tubule cells, and this increase was lower in fisetin-treated mice than in vehicle-treated mice (Fig. 3A, C). These results demonstrate that fisetin treatment protects against UUO-induced renal tubular damage and apoptosis of tubule cells.
Figure 1. Effect of fisetin on myofibroblast expansion and the TGF-β/SMAD3 signaling pathway in ureteral obstructed kidneys and TGF-β1-treated HK-2 cells. Mice were subjected to right UUO and treated with fisetin or vehicle. Seven days after surgery, kidney samples were harvested for western blotting using α-SMA and p-SMAD3 antibodies (A), which are markers of fibrosis. GAPDH was used as a loading control. (B, C) Band densities of α-SMA and p-SMAD3 were measured using ImageJ software. (D) TGF-β1 mRNA expression was measured using quantitative real-time polymerase chain reaction. (E) Representative images of kidney sections subjected to immunohistochemical staining using an α-SMA antibody. Hematoxylin was used to visualize the nuclei of cells. (F) The number of α-SMA in the interstitial area is shown. HK-2 cells were pretreated with 40-μM fisetin or vehicle for 1 hour, followed by TGF-β treatment for 30 minutes. (G) HK-2 cells (n = 3) were subjected to western blotting using p-SMAD2, p-SMAD3, and t-SMAD2/3 antibodies. GAPDH was used as a loading control. (H, I) Band densities of p-SMAD3 and p-SMAD2 were measured using ImageJ software. (J, K) The ratio of p-SMAD3 and p-SMAD2 to t-SMAD2/3 was calculated. Results were expressed as the mean ± standard error of the mean (vehicle or fisetin control, n = 4; vehicle or fisetin UUO, n = 5). One-way analysis of variance plus Tukey post hoc multiple comparison test was used to detect significant changes.

α-SMA, α-smooth muscle actin; Con, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemical; mRNA, messenger RNA; p-SMAD, phosphorylated SMAD; TGF, transforming growth factor; t-SMAD, total-SMAD; UUO, unilateral ureteral obstruction.

*p < 0.05 vs. vehicle control kidneys or vehicle-treated HK-2 cells, *p < 0.05 vs. vehicle UUO kidneys or TGF-β–treated HK-2 cells.
Fisetin attenuates inflammation in obstructed kidneys after unilateral ureteral obstruction.

Fig. 4A shows representative images of IHC staining for macrophages (dark brown) in the kidneys of each group of mice. Macrophage infiltration was significantly increased in the renal cortex of mice subjected to UUO. Fisetin-treated mice showed decreased macrophage infiltration in obstructed kidneys compared with that in vehicle-treated mice (Fig. 4A, B). Western blotting also showed that neutrophil expression was increased in vehicle-treated mice subjected to UUO. In addition, fisetin treatment significantly attenuated the levels of neutrophils in obstructed kidneys compared with vehicle treatment (p = 0.03) (Fig. 4C, D). Next, we examined the mRNA expression of proinflammatory cytokine and chemokines (MCP-1, MIP-2, and IL-1β) in the kidneys 7 days after UUO by qRT-PCR. MCP-1, MIP-2, and IL-1β mRNA expressions were remarkably increased in vehicle-treated mice subjected to UUO, and this induction was attenuated by fisetin treatment (Fig. 4E–G).
Figure 3. Effects of fisetin on UUO-induced tubular damages and apoptotic tubular cell death. Mice were subjected to right UUO and treated with fisetin or vehicle. Seven days after surgery, kidney sections were subjected to PAS staining and TUNEL staining. Images were obtained from the cortex. Representative images of kidney sections subjected to PAS staining (A: top panel, magnification ×200; middle panel, magnification ×400) are presented. (B) Kidney damage was scored as described in the Methods section. Representative images of kidney sections subjected to TUNEL assay (A, bottom panel) are presented. Images were obtained from the cortex. (C) TUNEL-positive cells were counted. Results were expressed as the mean ± standard error of the mean (vehicle or fisetin control, n = 3; vehicle or fisetin UUO, n = 5). The Mann-Whitney nonparametric test was used to detect significant changes in histological damage score and the one-way analysis of variance plus Tukey post hoc multiple comparison test was used to detect significant changes in TUNEL-positive cells.

ND, not detected; PAS, periodic acid-Schiff; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; UUO, unilateral ureteral obstruction.

*p < 0.05 vs. vehicle control, #p < 0.05 vs. vehicle UUO.
Figure 4. Effect of fisetin on inflammatory cell infiltration and proinflammatory cytokines and chemokines synthesis in ureteral obstructed kidneys. Mice were subjected to right UUO and treated with fisetin or vehicle. Seven days after surgery, kidney sections were subjected to immunohistochecmetry staining using F4/80 antibody, which is a macrophage marker. Hematoxylin was used to visualize the nuclei of cells. (A) The representative images of F4/80 IHC staining and number of macrophages (B) are shown. (C) Kidney samples were subjected to western blotting using the Ly6G (a marker of neutrophil) antibody. GAPDH was used as a loading control. (D) Band densities were measured using the ImageJ software. The mRNA expressions of MIP-2 (E), MCP-1 (F), and IL-1β (G) were measured with quantitative real-time polymerase chain reaction. Each mRNA expression was normalized to GAPDH expression. Results were expressed as the mean ± standard error of the mean (vehicle or fisetin control, n = 3; vehicle or fisetin UUO, n = 5). One-way analysis of variance plus Tukey post hoc multiple comparison test was used to detect significant changes.

Con, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemical; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; mRNA, messenger RNA; UUO, unilateral ureteral obstruction.

Fisetin attenuates the accumulation of M2 macrophage in the obstructed kidneys after unilateral ureteral obstruction

M1 macrophages initiate an inflammatory response at the initial stage of injury and differentiate into profibrotic M2 macrophages as the injury progresses [17]. We demonstrated that fisetin attenuated renal inflammation in mice with UUO nephropathy (Fig. 4). Therefore, we examined the infiltration of M2 macrophage using CD206, a marker...
of M2, and the mRNA expression of iNOS, a marker of M1. **Fig. 5A** shows representative images of IHC for M2 macrophages (dark brown) in the kidneys of each group of mice. M2 macrophage accumulation was significantly increased in the renal cortex of mice subjected to UUO. However, fisetin treatment decreased M2 macrophage accumulation in interstitial area of kidney compared with vehicle treatment in UUO group (**Fig. 5A, B**). In contrast, there was no difference in iNOS mRNA expression levels between the vehicle-treated groups and the fisetin-treated groups after UUO (**Fig. 5C**).

**Figure 5. Effect of fisetin on M1 and M2 macrophage expressions in ureteral obstructed kidneys.** Mice were subjected to right UUO and treated with fisetin or vehicle. Seven days after surgery, kidney sections were subjected to immunohistochemical staining using anti-CD206 antibody, which is an M2 macrophage marker. Hematoxylin was used to visualize the nuclei of cells. (A) The representative images of CD206 immunohistochemical staining and number of CD206-positive cells (**B**) are shown. (C) The mRNA expressions of iNOS, an M1 macrophage marker, were analyzed by quantitative real-time polymerase chain reaction. Each mRNA expression was normalized to GAPDH expression. Results were expressed as the mean ± standard error of the mean (vehicle or fisetin control, n = 3; vehicle or fisetin UUO, n = 5). One-way analysis of variance plus Tukey post hoc multiple comparison test was used to detect significant changes.

Con, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; iNOS, inducible nitric oxide synthase; mRNA, messenger RNA; UUO, unilateral ureteral obstruction.

* p < 0.05 vs. vehicle control, †p < 0.05 vs. vehicle UUO.
Fisetin attenuates oxidative damage caused by oxidative stress after unilateral ureteral obstruction

We next assessed the effect of fisetin on oxidative damage in UUO mice. Fig. 6A shows representative images of IHC for 8-OHdG (dark brown) in the kidneys of each group of mice. The results showed that 8-OHdG expression was significantly increased in the renal cortex of mice subjected to UUO. In addition, fisetin treatment reduced the expression of 8-OHdG in obstructed kidneys compared to that in vehicle-treated mice (Fig. 6A–C). Western blotting also showed that the expression of 4-HNE, an indicator of lipid peroxidation, was significantly increased in kidneys after UUO, and this increase was lower in mice treated with fisetin compared to mice treated with vehicle (Fig. 6D, E).

Discussion

In this study, we demonstrated, for the first time, that fisetin treatment effectively protects against UUO-induced renal fibrosis by decreasing ECM accumulation, oxidative damage, inflammation, and cell apoptosis. Obstructed kidneys show typical features of obstructive nephropathy, such as increased collagen deposition, tubular damage, infiltration of inflammatory cells, and tubular cell death [18]. In addition, the expression of α-SMA, a marker for activated myofibroblasts, was increased, which was also accompanied by an increase in SMAD3 phosphorylation after UUO.

TGF-β1/SMAD3 signaling is a major signaling pathway in the pathogenesis of tubulointerstitial fibrosis. TGF-β1 binds to TβRII, which activates TβRI, resulting in the activation of TGF-β1 downstream effectors [19]. The activated forms, p-SMAD2 and SMAD3 complexes with SMAD4, translocate into the nucleus and regulate the transcription of target fibrogenesis genes [20]. p-SMAD3 is a key factor in the transcription of fibrogenesis genes mediated by TGF-β1, leading to UUO-induced renal fibrosis [21]. As a result of confirming the expression level of p-SMAD3 and TGF-β1, we demonstrated that fisetin alleviates fibrosis by regulating the downstream effectors of TGF-β1 signaling based on the result that there was no significant difference in TGF-β1 mRNA expression between vehicle-treated mice and fisetin-treated mice after UUO. Fisetin treatment also dramatically suppressed SMAD3 phosphorylation in the UUO model. Furthermore, we also confirmed that fisetin significantly suppressed TGF-β-induced SMAD2/3 phosphorylation in cultured human kidney tubular cells. Based on these results, we speculate that fisetin treatment inhibited the phosphorylation of SMAD3, resulting in suppression of nuclear translocation of the SMAD complex and the expression of SMAD-mediated genes, resulting in reductions in α-SMA and collagen production rather than direct regulation of TGF-β1 expression. Another study reported that GQ5, a small molecular phenolic compound, attenuated renal fibrosis by selectively inhibiting TGF-β1 mediated SMAD3 phosphorylation [22]. MAF, a renin-angiotensin system inhibitor, attenuates epithelial-to-mesenchymal transition and interstitial fibrosis by selectively suppressing the TGF-β1-induced SMAD3 phosphorylation [23]. Moreover, fisetin has been reported to significantly inhibit SMAD3 phosphorylation in myocardial infarction-induced adverse atrial fibrosis [24] and bleomycin-induced pulmonary fibrosis [15] animal models. However, the underlying molecular mechanism of fisetin-regulated SMAD3 phosphorylation in a UUO model has not yet been defined.

Oxidative stress may be exacerbated by increased ROS production after UUO, and this oxidative stress contributes significantly to the pathogenesis of UUO [25]. In addition, ROS act as central molecules of inflammatory and apoptotic signaling, ultimately leading to cell death [26]. Modification of DNA, proteins, and lipids by oxidative stress has been shown to play important roles in many biological pathways, such as cell apoptosis and ECM expansion [27]. 8-OHdG is a product of oxidative damage to 2’-deoxyguanosine and is a ubiquitous marker for measuring oxidative DNA damage [28]. Meanwhile, reactive aldehyde 4-HNE is a major bioactive product of polyunsaturated fatty acids under oxidative stress and is used as an indicator of lipid oxidation [29]. We confirmed that fisetin significantly reduced HNE and 8-OHdG expression as well as tubular cell apoptosis in UUO mice. This indicates that fisetin treatment has a protective effect against renal damage by alleviating oxidative stress produced during UUO.

Interstitial myofibroblast accumulation and macrophage recruitment are associated with progression of renal injury in mice with obstructive nephropathy [30]. Macrophages secrete proinflammatory cytokines and chemokines, as well as growth factors such as TGF-β and fibroblast growth factor, leading to tissue injury and development of renal fibrosis.
Figure 6. Effect of fisetin on oxidative damage in ureteral obstructed kidneys. Mice were subjected to right UUO and treated with fisetin or vehicle. Seven days after surgery, kidney sections were subjected to IHC staining using 8-OHdG (marker of damaged DNA) antibody. Hematoxylin was used to visualize the nuclei of cells. (A) Representative images of 8-OHdG IHC staining and number (B) and intensity (C) of 8-OHdG are shown. (D) Kidney samples were subjected to western blotting using an antibody against 4-HNE, which is an α, β-unsaturated hydroxyalkenal produced by lipid peroxidation. GAPDH was used as a loading control. (E) Band densities were measured using the ImageJ software. Results were expressed as the mean ± standard error of the mean (vehicle or fisetin control, n = 3; vehicle or fisetin UUO, n = 5). One-way analysis of variance plus Tukey post hoc multiple comparison test was used to detect significant changes.

Con, control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IHC, immunohistochemical; UUO, unilateral ureteral obstruction. 4-HNE, 4-hydroxynonenal; 8-OhdG, 8-hydroxy-2'-deoxyguanosine.

*p < 0.05 vs. vehicle control, **p < 0.05 vs. vehicle UUO.

In this study, we found that proinflammatory cytokines and chemokines (MCP-1, MIP-2, and IL-1β) were increased in obstructed kidneys after UUO. MCP-1 and IL-1β promote the migration and infiltration of monocytes and macrophages, as well as the differentiation of monocytes into macrophages, whereas MIP-2 is produced by various
cell types such as macrophages, monocytes, and epithelial cells to recruit and activate neutrophils, thereby playing a key role in the inflammatory response [32–34]. In the present study, decreased expression of macrophage-recruiting cytokines (MCP-1, MIP-2, and IL-1β) and neutrophil expression in fisetin-treated mice are consistent with our finding that fisetin treatment significantly attenuated macrophage infiltration including profibrotic M2 macrophages after UUO compared to vehicle-treated mice. Furthermore, SMAD3 is a critical factor in macrophage/monocyte chemotaxis [21,35]. Inazaki et al. [21] also demonstrated that accumulation of renal interstitial inflammatory cells, such as macrophages, were remarkably suppressed by SMAD3 deficiency. M1 macrophages initiate an inflammatory response at the initial stage of injury and differentiate into M2 macrophages as injury progresses in obstructed kidneys [36]; in addition, M1 macrophage-producing or -recruiting chemokine/cytokine mRNA levels were higher in the vehicle-treated group than in fisetin-treated group after UUO. Consequently, we speculate that M1 macrophages showed more infiltration in the initial stage of injury in vehicle-treated UUO kidneys than in fisetin-treated UUO kidneys and that more M1 macrophages were differentiated into M2 macrophages in the vehicle-treated group than in the fisetin-treated group, so the amount of undifferentiated M1 macrophages in obstructed kidneys as evaluated by iNOS mRNA expression was similar the two groups at the end point of our experiments at the 7th day after UUO (the end point of our experiments).

In summary, we demonstrated that fisetin protects against obstructive nephropathy. We found that fisetin exhibits powerful antifibrotic effects in obstructed kidneys by inhibiting SMAD3 phosphorylation. However, previous studies showed that SMAD3 as well as other SMADs such as SMAD2 and SMAD4 are activated in the TGF-β1-mediated signaling pathway and regulate the transcription of fibrosis genes by interacting with each other. To confirm that fisetin alleviates fibrosis through SMAD3 phosphorylation, it is necessary to demonstrate that fisetin selectively and specifically inhibits SMAD3 by investigating the expression levels of other factors in the TGF-β1/SMAD3 signaling pathway. In addition, SMAD3 and SMAD2 are recruited to and phosphorylated by TβRI by adapter proteins such as SMAD anchor for receptor activation (SARA) upon TGF-β1 stimulation [37]. To elucidate the mechanism by which fisetin inhibits SMAD3 phosphorylation, further studies on these factors will be needed. Nonetheless, we clearly showed that fisetin treatment remarkably attenuated renal fibrosis, tubular damage, oxidative damage, inflammation, and apoptosis induced by UUO, suggesting that fisetin may be a potent inhibitor of TGF-β1/SMAD3 signaling, a major pathway in fibrosis, and may be a novel therapeutic drug for obstructive nephropathy.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization, Data curation: HYJ, SJH
Formal analysis, Investigation, Methodology: All authors
Resources, Supervision: SJH
 Visualization: JK
Funding acquisition: SJH
Writing–original draft: All authors
Writing–review & editing: All authors
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References


Body water percentage from childhood to old age

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Background: Total body water (TBW) increases with growth, but the body water percentage (TBW%) decreases with aging. The objective of our study was to delineate TBW% in males and females by bioelectrical impedance analysis (BIA) from early childhood to old age.

Methods: We enrolled 545 participants aged 3 to 98 years (258 male, 287 female). Among the participants, 256 had a normal weight and 289 were overweight. The TBW was measured by BIA, and TBW% was derived by dividing the TBW (L) value by body weight (kg). For analysis, we divided participants into the four age groups of 3–10, 11–20, 21–60, and ≥61 years.

Results: In normal-weight subjects, the TBW% was similar at 62% between males and females in the 3–10-year group. It remained unchanged in males until and through adult life, then decreased to 57% in the ≥61-year group. In normal-weight female subjects, the TBW% decreased to 55% in the 11–20-year group, remained relatively unaltered in the 21–60-year group, then decreased to 50% in the ≥61-year group. In overweight subjects, the TBW% values in males, as well as females, were significantly lower as compared to those with normal weight.

Conclusion: Our study showed that the TBW% in normal-weight males changes very little from early childhood to adult life compared to that of females, who showed a decrease in TBW% during the pubertal years. In normal-weight subjects of both sexes, the TBW% decreased after the age of 60 years. Overweight subjects had significantly lower TBW% as compared to those with normal weight.

Keywords: Aged, All ages, Bioelectrical impedance analysis, Body water, Child, Obesity

Introduction

Body water is critical for maintaining normal physiological functions. The amount of total body water (TBW) in an individual varies according to age, sex, and body fat content. Assessing body water is important in clinical practice for diagnosing dehydration or fluid overload. Many of the medications are administered with their dosage based on their presumed volume of distribution, i.e., water as a percentage of total body weight. The same is true in calculations of body water and solute deficits.

There are several methods for measuring body water content, including the isotope dilution technique, nuclear magnetic resonance imaging, and bioelectrical impedance analysis (BIA) [1,2]. In the absence of any gold-standard method, the deuterium dilution method is considered the reference method for body water measurement. This method is based on the principle of water distribution in all parts of the body except fat. It requires the administration of an isotope into the body by ingestion or intravenously.
and the collection of a blood, saliva, or urine sample after a period of equilibration. The major limitations inherent with the dilution method include that it is time-consuming and requires ingestion of an isotope and collection of a biological specimen. As a result, this method cannot be used consistently in routine ambulatory clinic settings, particularly in younger children and elderly people.

BIA is a noninvasive method for body water measurement that measures the resistance and reactance of body tissue to an imperceptible low-intensity alternating current as it traverses through the body. Resistance to the electric current comes from body water (extracellular and intracellular), and the reactance is a result of a brief delay caused by cell membranes, which is also called capacitance [3,4]. A low-frequency current is used for measuring extracellular water, whereas a high-frequency current is used for measuring the TBW (intracellular and extracellular). The amount of intracellular water is calculated by subtracting the extracellular water content from the TBW. In comparison to the previously used single-frequency BIA method, the currently used multifrequency BIA method is believed to yield more accurate results [5]. Studies have shown that BIA results are comparable to those attained by the dilution method in both children and adults [3,4].

BIA provides a reliable estimate of TBW in most conditions and is an easy, inexpensive, safe, and portable method to deploy [6]. BIA works well in healthy participants and in patients with stable water and electrolyte balances [7,8]. Many studies have confirmed a good overall agreement between the results of dilution techniques and BIA in healthy children as well as adults, hospitalized elderly patients, and children with obesity [9–14]. Prior studies on body water content have grouped patients on the basis of their weight (normal, obese, and extreme or morbid obesity as well as weight loss after gastric bypass surgery for extreme obesity) [15]. In our previous study, we reported the feasibility of data collection by BIA in ambulatory clinic settings and the impact of excessive weight on TBW in children and young adults [16].

The main objective of this study was to delineate TBW% values in normal-weight and overweight male and female participants by BIA from early childhood to old age, which, to the best of our knowledge, has not been done before.

Methods

This prospective study included 335 children and young adults recruited from the Nephrology Clinic at the Children’s Hospital of Michigan or the Pediatric Clinic of the Wayne State University Physician Group and 210 adults recruited from the Wayne State University Physician Group clinic, Geriatric Clinic of University Health Center, or the St. Patrick Senior Center. The study protocol was approved by the Institutional Review Board of the Wayne State University (No. #092314MP2E). Depending on age, consent/assent forms were signed by participants or their legal guardians.

Normal-weight or overweight (including obese) participants aged ≥3 years were included in the study. We excluded patients with diabetes; dehydration as assessed by history, physical examination, or blood pressure measurement; the presence of an internal defibrillator or pacemaker; missing limbs; menstruation or pregnancy; or chronic kidney disease or another known comorbid condition as well as those taking medications that affect the body water content, such as glucocorticoids. Children with hypertension were also excluded from this study, whereas adults with hypertension on antihypertensive medications only were included in the study. Children on diuretics were excluded from this study, whereas adults on a low-dose diuretic (12.5 mg/day) or chlorthalidone (15 mg/day) for hypertension only were included in the study. Patients with a recent history of moderate exercise or consumption of a big meal within 2 hours before the procedure were also excluded from data collection. Any formal exercise or sports activity was considered moderate exercise. Any meal larger than breakfast or a light lunch was considered a big meal.

Height and weight were measured. Body weight was defined on the basis of body mass index (BMI) as normal weight (BMI of <85th percentile in children or <25 kg/m² in adults), overweight (BMI of ≥85th–95th percentile in children or 25–29 kg/m² in adults), and obesity (BMI of ≥95th percentile in children or ≥30 kg/m² in adults) according to the U.S. Centers for Disease Control and Prevention guidelines (https://www.cdc.gov/growthcharts/). Overweight participants in our study included both overweight and obese individuals. TBW% was derived by dividing the TBW (L) value by body weight (kg).

The InBody s10 direct segmental multifrequency BIA device (InBody Co., Ltd.) was used in this study. Measure-
ments were gathered in temperature-controlled offices while the patient sat on an examination table with their legs hanging and arms and legs abducted. Before measurement, study participants were asked to void and sit down for 10 to 15 minutes on the examination table. Touch-type electrodes were placed on participants’ feet near the ankles and hands near the wrists, and current frequencies of 50, 100, 500, and 1,000 kHz at five segments (right arm, left arm, right leg, left leg, and trunk) were applied for a total period of about 1.5 minutes until completion of the recording, which was indicated on the screen and with a beeping sound. The same BIA machine was used for data collection from all study participants.

We evaluated our cohort by broadly classifying them into four groups partly based on the ages during which the TBW is known to change [6,8]. As such, the study groups included a prepubertal group (3–10 years), pubertal group (11–20 years), adult group (21–60 years), and elderly group (≥61 years). Study results were analyzed using the NCSS version 11.0 (NCSS Statistical Software; NCSS, LLC, Kaysville, UT, USA). Student t test or the Mann-Whitney U test was used to study the quantitative variables between two groups, and Pearson product-moment correlation was used to study the correlation between BIA parameters.

**Results**

The study included 545 participants aged 3 to 98 years. The mean ages for various age groups were 6.6 ± 2.17 years (3–10-year age group), 14.4 ± 2.39 years (11–20-year age group), 35.3 ± 14.4 years (21–60-year age group) and 74.8 ± 9.1 years (≥61-year age group), respectively. Male and female participants totaled 258 and 287, respectively, while numbers of normal-weight and overweight participants totaled 256 and 289, respectively. There were significant differences in weight and BMI between the normal-weight and overweight participants across all age groups (all p < 0.001). Further details of the demographic data are shown in Tables 1 and 2.

The mean TBW (L) in normal-weight females was significantly lower than that in normal-weight males across all age groups (p < 0.01) except the 3–10-year age group. Similarly, the mean TBW (L) was also significantly lower in overweight females compared to overweight males across all age groups (p < 0.01) except in the 3–10-year age group (Table 3).

As shown in Table 4 and Fig. 1, in normal-weight study participants, the TBW% was very similar at 62% between males and females in the 3–10-year age group. In males, it remained unchanged thereafter before decreasing to 57% in the ≥61-year age group. Conversely, in normal-weight females, the TBW% decreased significantly to 55% in the 11–20-year age group, remained relatively the same in the 21–60-year age group, then decreased to 50% in the ≥61-year age group.

In overweight study participants, the TBW% was lower for males as well as females in all age groups. In the 3–10-year age group, the TBW% was slightly higher for males

**Table 1. Demographic details for various age groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>3–10</th>
<th>11–20</th>
<th>21–60</th>
<th>≥61</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>160</td>
<td>186</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>6.6 ± 2.2</td>
<td>14.4 ± 2.4</td>
<td>35.3 ± 14.4</td>
<td>74.8 ± 9.1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81</td>
<td>96</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>79</td>
<td>90</td>
<td>52</td>
<td>66</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>30.0 ± 13.7</td>
<td>66.0 ± 27.2</td>
<td>87.4 ± 28.7</td>
<td>81.7 ± 19.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>124.4 ± 15.6</td>
<td>162.8 ± 12.2</td>
<td>168.7 ± 10.2</td>
<td>166.7 ± 10.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>18.5 ± 4.5</td>
<td>24.5 ± 8.7</td>
<td>30.5 ± 8.5</td>
<td>29.3 ± 6.1</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or median (interquartile range).
Table 3. Total body water (L) in males and females with normal and excessive weight in each age group

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>No. of patients</th>
<th>Total body water (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>3–10</td>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>6.5 (5.0–9.0)</td>
<td>23.4 (18.4–28.8)</td>
</tr>
<tr>
<td></td>
<td>Excessive</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>7.7 (6.0–9.0)</td>
<td>36.1 (28.8–46.4)</td>
</tr>
<tr>
<td>11–20</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>14.5 (12.6–17.0)</td>
<td>51.9 (43.0–61.0)</td>
</tr>
<tr>
<td></td>
<td>Excessive</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>15.0 (12.6–17.5)</td>
<td>81.9 (63.0–98.0)</td>
</tr>
<tr>
<td>21–60</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>22.5 (20.0–38.0)</td>
<td>59.1 (53.0–67.0)</td>
</tr>
<tr>
<td></td>
<td>Excessive</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>38.5 (24.0–50.0)</td>
<td>94.6 (82.0–110.0)</td>
</tr>
<tr>
<td>≥61</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>81.0 (72.0–88.0)</td>
<td>56.1 (52.0–67.0)</td>
</tr>
<tr>
<td></td>
<td>Excessive</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>73 (67–80)</td>
<td>82.8 (74–96.0)</td>
</tr>
</tbody>
</table>

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

Normal weight, body mass index (BMI) of 85th percentile in children and <25 kg/m² in adults. Excessive weight includes individuals with overweight as well as obesity (BMI of ≥85th percentile in children and ≥25 kg/m² in adults).

All body weights in the excessive weight group are significantly higher than body weight in normal weight (p < 0.01). The body weights in the male groups are significantly higher than that in the female group by each age group (p < 0.01) except in the 3–10-year age group.

Discussion

It is well-known that TBW increases with growth and that the TBW% is reduced from about 80% at birth [17] to about 60% in adult men and 50% in adult women [18]. Very little data are available about the TBW% in the elderly population, although one study suggests it may be as low as 46% (53%) compared to females (49%). Thereafter, it decreased in males in the 11–20-year age group (49%) before remaining fairly similar in the 21–60-year (49%) and ≥61-year (50%) age groups. In overweight females, it decreased slightly in the 11–20-year (45%) and 21–60-year (41%) age groups, with not much change thereafter (Table 4, Fig. 1).
Table 4. Mean TBW (L)/BW (kg) in each group

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>No. of patients</th>
<th>Normal weight</th>
<th>Excessive weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>3–10</td>
<td>160</td>
<td>0.63 ± 0.05</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.7 ± 1.3</td>
<td>0.63 (0.62–0.65)</td>
<td>0.63 (0.59–0.65)</td>
</tr>
<tr>
<td>11–20</td>
<td>186</td>
<td>0.63 ± 0.05</td>
<td>0.64 (0.59–0.67)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.7 ± 2.6</td>
<td>0.56 ± 0.06</td>
<td>0.56 (0.52–0.60)</td>
</tr>
<tr>
<td>21–60</td>
<td>100</td>
<td>0.62 ± 0.03</td>
<td>0.64 (0.60–0.65)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 ± 2.2</td>
<td>0.54 ± 0.05</td>
<td>0.53 (0.50–0.58)</td>
</tr>
<tr>
<td>≥61</td>
<td>99</td>
<td>0.57 ± 0.05</td>
<td>0.58 (0.54–0.60)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 1.3</td>
<td>0.50 ± 0.05</td>
<td>0.50 ± 0.05</td>
</tr>
</tbody>
</table>

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

Normal weight, BMI of 85th percentile in children and <25 kg/m² in adults. Excessive weight includes individuals with overweight as well as obesity (BMI of ≥85th percentile in children and ≥25 kg/m² in adults).

BMI, body mass index; BW, body weight; TBW, total body water.

*p < 0.01 by comparing TBW% in normal weight in female between the groups aged 11–20 years and 3–10 years. The TBW% significantly reduced in the group aged 11–20 years.

*p < 0.01 by comparing TBW% in normal weight in female between the groups aged 21–60 years and 3–10 years. The TBW% significantly reduced in the group aged 21–60 years.

*p < 0.05 by comparing TBW% in excessive weight in male or female in different age groups. The TBW% significantly reduced in the group aged 21–60 years.

*p < 0.01 by comparing TBW% in normal weight in male or female in different age groups. The TBW% significantly reduced in the group aged ≥61 years.

Our results are similar to those of studies that used dilution methods to measure TBW% values in different age groups [20–22]. Pierson and Lin reported a dramatic drop in TBW% from >0.75 at birth to <0.65 at around the age of 1 year in both boys and girls [23]. We did not study this age group because BIA is designed for application to children aged ≥3 years only. The same study also noted a steady decrease in TBW% to <0.6 in (both male and female) adolescents. In another study conducted by dilution method, Haschke [24] reported that the TBW% did not change significantly in males between 10 and 14 years of age, who presented an average TBW% of 0.64, which is similar to our result measured in normal-weight males of this age group. Hume and Weyers [25] studied TBW% in adolescent boys and girls by dilution method, which showed an increase in boys from 0.621 (12–13 years) to 0.659 (17–18 years) but a reduction in girls, as seen in our study also, from 0.603 to 0.536 in the same age groups. Among normal-weight adults, a dilution study by Hume and Weyers [25] reported TBW% values of 0.602 ± 0.04 in males aged 40–69 years.

in males and 43% in females [19]. The changes in TBW% that occur with aging have been documented by previous studies, including those that used the dilution method [14]. However, no study to date has evaluated TBW% by any method across the full age spectrum of childhood to old age. This is likely because of the logistical limitations in studying all age groups and the complexity of methods that are not feasible to deploy in ambulatory clinic settings, particularly in young children and elderly people.

BIA made it possible for us to study TBW in ambulatory settings in participants ranging from 3 to 98 years of age. Our study revealed that the TBW% in normal-weight males changes very little from the age of 3 years to adult life (21–60 years), while normal-weight females showed a significant decrease of the TBW% in the 11–20-year age group. This is because of a relatively greater increase in body fat during pubertal growth in girls compared to boys [20]. Both sexes showed a steady decline of the TBW% in the ≥61-year age group to 57% in males and 50% in females, which is due to a reduction in muscle mass.

Our results are similar to those of studies that used dilution methods to measure TBW% values in different age groups [20–22]. Pierson and Lin reported a dramatic drop in TBW% from >0.75 at birth to <0.65 at around the age of 1 year in both boys and girls [23]. We did not study this age group because BIA is designed for application to children aged ≥3 years only. The same study also noted a steady decrease in TBW% to <0.6 in (both male and female) adolescents. In another study conducted by dilution method, Haschke [24] reported that the TBW% did not change significantly in males between 10 and 14 years of age, who presented an average TBW% of 0.64, which is similar to our result measured in normal-weight males of this age group. Hume and Weyers [25] studied TBW% in adolescent boys and girls by dilution method, which showed an increase in boys from 0.621 (12–13 years) to 0.659 (17–18 years) but a reduction in girls, as seen in our study also, from 0.603 to 0.536 in the same age groups. Among normal-weight adults, a dilution study by Hume and Weyers [25] reported TBW% values of 0.602 ± 0.04 in males aged 40–69 years.
Their study also recorded TBW% values in overweight adults, which were 0.524 ± 0.04 in males aged 35–71 years (n = 11) and 0.42 ± 0.05 in females aged 33–68 years (n = 13), respectively [25]. In another study conducted by dilution method, the TBW% in normal-weight males aged 32–67 years (n = 5) and females aged 30–67 years (n = 4) were 0.582 ± 0.04 and 0.494 ± 0.036, respectively [26]. The sex difference in TBW values among normal-weight individuals of various ages is well-known. Males have greater TBW values compared to females mainly because of their typically higher body weight and greater muscle mass. Similar differences exist in TBW% values between normal-weight males and females at all ages beyond early childhood. A higher TBW% in males compared to females is also attributed to the relatively increased fat-free mass, encompassing muscles and bones, found in men [27]. Our study also revealed that the TBW% was lower in all age groups in overweight (and obese) males and females compared to normal-weight participants. This is because excess body weight is the result of a relatively high percentage of body fat with a net decrease in fat-free mass and TBW for weight. Only 20% to 30% of body fat is water, while about 72% of the fat-free body mass is water [28].

As seen in our study, previous studies of adults have also revealed that overweight individuals have lower TBW% values and hence are hypohydrated compared to normal-weight individuals [15,29–31]. In our previous study of children and young adults, overweight individuals had a 16.5% higher mean TBW value for height and age and a 7.4% lower TBW value for weight and body surface area compared to normal-weight individuals [16]. Unlike in study subjects with normal weights, no further decrease in TBW% was noted in the present study in overweight males as well as females aged ≥61 years. Lower TBW% values in overweight and obese people are particularly important because obesity is increasing worldwide. More research is needed to study the impact of lower TBW% in overweight individuals and elderly people who have many other risk factors for dehydration.

BIA is not a reference method for measuring TBW and its use could be seen as a study limitation. However, as mentioned in the introduction section of this paper, the reliability of data collected by BIA has been validated by an increasing number of studies [32–34]. Furthermore, our study showed that TBW% measurement by BIA correlates with previously published studies of various age groups that used reference methods. BMIs in overweight study subjects aged ≥61 years of either sex were lower than those of younger age groups, which may have resulted in slightly higher TBW% values in this age group.

To our knowledge, this is the only study that offers a full

![Figure 1. TBW (L) per BW (kg) at various age groups.](image-url)

BW, body weight; NW, normal weight; EW, excessive weight including overweight and obesity; TBW, total body water.
profile on TBW% values from early childhood to old age, and we believe it provides a better perspective on age-related changes in TBW% and the potential risk of dehydration with aging. We were unable to recruit more normal-weight elderly participants because of a greater prevalence of obesity in the community and our study exclusion criteria, i.e., no diabetes or the use of medications (other than antihypertensives) in particular.

The accuracy of BIA in obese subjects has been questioned in the past [35]. In 2004, the European Society of Clinical Nutrition and Metabolism (ESPEN) recommended using BMI for TBW measurement only for individuals with BMIs of 16–34 kg/m² [6]. However, studies published after the publication of ESPEN guidelines have reported that BIA accurately estimates TBW in overweight and obese participants, and this may be due to the advent of multifrequency BIA methods [36]. Nonetheless, TBW% may not be an ideal method for measuring body water in the presence of obesity. Most studies using the dilution method did not study TBW% in terms of body weight, so no data are available on TBW% in obesity across all age groups. Lastly, a relatively higher mean age among subjects ≥61 years old in our study could indicate an inadvertent patient selection bias.

In conclusion, our study showed that TBW% in normal-weight males changes very little from early childhood to adult life compared to that in females, who showed a reduction in TBW% during the pubertal years. In normal-weight individuals of either sex, the TBW% decreases after the age of 60 years. In overweight individuals, the TBW% was lower in all age groups when compared to that of normal-weight individuals. Unlike in normal-weight study participants, however, no further reduction in TBW% was noted in either overweight males or females aged ≥61 years.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization: HL, TKM
Data curation, Formal analysis, Methodology, Validation: HL
Funding acquisition: TKM
Investigation: All authors
Resources: EA, PP, TKM
Writing–original draft: HL, TKM
Writing–review & editing: TKM
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References


Clinical characteristics of acute kidney injury in patients with glyphosate surfactant herbicide poisoning

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Background: In this study, we investigated the clinical characteristics of acute kidney injury (AKI) in patients with glyphosate surfactant herbicide (GSH) poisoning.

Methods: This study was performed between 2008 and 2021 and included 184 patients categorized into the AKI (n = 82) and non-AKI (n = 102) groups. The incidence, clinical characteristics, and severity of AKI were compared between the groups based on the Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease (RIFLE) classification.

Results: The incidence of AKI was 44.5%, of which 25.0%, 6.5%, and 13.0% of patients were classified into the Risk, Injury, and Failure categories, respectively. Patients in the AKI group were older (63.3 ± 16.2 years vs. 57.4 ± 17.5 years, p = 0.02) than those in the non-AKI group. The length of hospitalization was longer (10.7 ± 12.1 days vs. 6.5 ± 8.1 days, p = 0.004) and hypotensive episodes occurred more frequently in the AKI group (45.1% vs. 8.8%, p < 0.001). Electrocardiographic (ECG) abnormalities on admission were more frequently observed in the AKI group than in the non-AKI group (80.5% vs. 47.1%, p < 0.001). Patients in the AKI group had poorer renal function (estimated glomerular filtration rate at the time of admission, 62.2 ± 22.9 mL/min/1.73 m² vs. 88.9 ± 26.1 mL/min/1.73 m², p < 0.001) on admission. The mortality rate was higher in the AKI group than in the non-AKI group (18.3% vs. 1.0%, p < 0.001). Multiple logistic regression analysis showed that hypotension and ECG abnormalities upon admission were significant predictors of AKI in patients with GSH poisoning.

Conclusion: The presence of hypotension on admission may be a useful predictor of AKI in patients with GSH intoxication.

Keywords: Acute kidney injury, Glyphosate, Hypotension, Poisoning

Introduction

Glyphosate is currently the most common post-emergent, nonselective herbicide used in worldwide agriculture [1]. The 2012 paraquat ban in South Korea was followed by an increase in the annual number of suicide attempts using glyphosate surfactant herbicides (GSH) [2] with approximately 1,000 cases of GSH toxicity occurring annually in South Korea [2,3]. GSH poisoning is known to cause gastrointestinal dysfunction, acute respiratory failure, cardiovascular instability, central nervous system complications, and acute kidney injury (AKI) [4,5], all of which are associated with GSH-mediated toxicity including mitochondrial dysfunction, lipid peroxidation, oxidative stress, and DNA
injury [6-9].

Previous studies have reported GSH-induced AKI; direct GSH toxicity or renal ischemia secondary to circulatory failure has been implicated as a possible pathophysiological mechanism underlying AKI [6,10,11]. Moderate-to-severe GSH intoxication typically presents with renal dysfunction, which is also an important predictor of poor outcomes [6,12]. However, few studies have investigated the incidence and clinical characteristics of AKI in patients with GSH intoxication [13]. A variety of definitions are used in clinical practice; therefore, the incidence of GSH poisoning-induced AKI remains unclear [14,15]. The Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease (RIFLE) criteria, which were originally validated for ischemic AKI [16], are used to define and classify AKI [17].

In this study, we investigated the incidence and clinical characteristics of GSH poisoning-induced AKI using the RIFLE criteria.

Methods

Patient selection

We enrolled 202 patients with a history of GSH ingestion who visited our hospital between 2008 and 2021. Exclusion criteria were as follows: unclear history of exposure, co-exposure with non-pharmaceutical agents including other pesticides, non-oral exposure, discharge against medical advice, and transfer to another hospital. Eventually, 184 patients were included in this study and were categorized into the AKI or non-AKI group. This study was approved by the Institutional Review Board of the Presbyterian Medical Center, Jeonju, Republic of Korea (No. 2020-06-029). Written informed consent was waived due to its retrospective nature.

Clinical and laboratory data

All data were obtained through retrospective chart review. Acute GSH intoxication was defined based on a history of exposure, container labels, or product information provided by the patient or family. Following detailed clinical history taking, all patients underwent thorough physical and biochemical evaluation including complete blood counts, liver and renal function tests, arterial blood gas analysis, urinalysis, and chest radiography. Electrocardiographic (ECG) recordings obtained upon arrival at the emergency department were interpreted by a cardiologist. The corrected QT interval (QTc) was calculated using Bazett’s formula (QTc = QT/√RR) [18]. QTc interval of >470 ms was defined as QTc interval prolongation [19]. Hypotension was defined as a systolic blood pressure (BP) of <90 mmHg. The estimated amount of GSH ingestion was defined as follows: a spoonful (5 mL), a mouthful (25 mL), a cupful (100 mL), and a bottleful (300 mL) [20].

AKI was defined based on the RIFLE criteria, and patients were categorized into the Risk (R), Injury (I), and Failure (F) categories [17]. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation [21]. In patients for whom the baseline serum creatinine value was unavailable, it was calculated using the standard four-variable MDRD formula considering an eGFR of 75 mL/min/1.73 m². The RIFLE class was determined based on the worst serum creatinine value, eGFR, and urine output criteria. RIFLE classes I and F were defined as severe AKI in this study. Renal replacement therapy was initiated based on standard indications. All data are presented as the mean ± standard deviation unless otherwise specified. Baseline characteristics of patients in the non-AKI and AKI groups were compared using t test, chi-square test, or Fisher exact test. Using paired t test, the lowest eGFR during AKI was compared following recovery eGFR. Fisher exact test was used to compare frequencies between AKI and ECG findings.

Clinically, the variables that were significantly associated with AKI on univariate analysis were subjected to multivariate analysis using binary logistic regression analysis. Survival curves for mortality were calculated using the Kaplan-Meier method and compared using the log-rank test. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using the IBM SPSS version 22.0 (IBM Corp.).

Results

Comparison of clinical characteristics between the acute kidney injury and non-acute kidney injury groups

Compared with those in the non-AKI group, patients in the
AKI group were older (63.3 ± 16.2 years vs. 57.4 ± 17.5 years, p = 0.02) and had ≥1 comorbidities such as hypertension or diabetes (52.4% vs. 32.7%, p = 0.005) (Table 1). The length of hospitalization was longer (10.7 ± 12.1 days vs. 6.5 ± 8.1 days, p = 0.004), and hypotensive episodes upon admission were more frequent in the AKI group (45.1% vs. 8.8%, p < 0.001). Moreover, ECG abnormalities were observed more frequently in the AKI group than in the non-AKI group (80.5% vs. 20.6%, p < 0.001) and mechanical ventilatory support (36.6% vs. 4.9%, p < 0.001) were more frequently required in the AKI group. The mean amount of GSH ingested was higher in the AKI group than in the non-AKI group (236 ± 150 mL vs. 174 ± 122 mL, p = 0.004). The mortality rate was higher in the AKI group than in the non-AKI group (18.3% vs. 1.0%, p < 0.001).

Clinical course of acute kidney injury in patients with glyphosate surfactant herbicide poisoning

Based on the RIFLE criteria, 46 (56.1%), 12 (14.6%), and 24 patients (29.3%) were categorized into the R, I, and F categories, respectively (Table 2). Among patients with AKI, 14 (17.1%) underwent renal replacement therapy. Of the total 82 patients with AKI, renal function returned to baseline values within 72 hours in 59 patients (72.0%). Posttreatment renal function (indicated by eGFR measurements) improved significantly from the lowest renal function (89.3 ± 42.9 mL/min/1.73 m² vs. 44.3±22.1 mL/min/1.73 m², p < 0.001). Of 82 AKI patients, only 23 patients (28.0%) could be followed-up beyond 3 months.

We found no significant change in the renal function of these 23 patients at the time of discharge and 3 months later (94.2 ± 25.5 mL/min/1.73 m² vs. 88.4 ± 29.1 mL/min/1.73 m², p = 0.08). Univariate analysis revealed that age, comorbidities, hypotension, ECG abnormalities on admission, serum bicarbonate concentration, and amount of GSH ingested were significant predictors of AKI. Multivariate analysis confirmed that these factors remained significant predictors of AKI in the multivariate model.
logistic regression analysis performed after adjustment for these factors showed that hypotensive episodes and ECG abnormalities, such as ST-T abnormality on admission, remained significant predictors of AKI (Table 3). The most common ECG abnormality in patients with AKI was QTc interval prolongation followed by sinus tachycardia (Table 4). However, compared to the non-AKI group, only the ST-T abnormality was observed to be more prevalent in the AKI group. Furthermore, multiple logistic regression analysis for prediction of mortality showed that severe AKI was an important prognostic factor in patients with GSH intoxication (Table 5). Fig. 1 shows the Kaplan-Meier curves comparing the in-hospital mortality rates between the groups. The cumulative survival rate was lower in patients with AKI than in those without it.

Discussion

Hypotension and ECG abnormalities on admission were more frequently observed in patients with AKI than in

Table 2. Clinical characteristics of 82 patients with acute kidney injury

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIFLE category</td>
<td></td>
</tr>
<tr>
<td>Risk</td>
<td>46 (56.1)</td>
</tr>
<tr>
<td>Injury</td>
<td>12 (14.6)</td>
</tr>
<tr>
<td>Failure</td>
<td>24 (29.3)</td>
</tr>
<tr>
<td>FENa &lt; 1%a</td>
<td>20 (49.0)</td>
</tr>
<tr>
<td>Recovery of renal function within 72 hr</td>
<td>59 (72.0)</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>14 (17.1)</td>
</tr>
<tr>
<td>Renal function, eGFR (mL/min/1.73 m²)</td>
<td></td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;adm&lt;/sub&gt;</td>
<td>62.2 ± 22.9</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;low&lt;/sub&gt;</td>
<td>44.3 ± 22.1</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;rec&lt;/sub&gt;</td>
<td>89.3 ± 42.9</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;3m&lt;/sub&gt;</td>
<td>88.4 ± 29.1</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or mean ± standard deviation. eGFR, estimated glomerular filtration rate; eGFR<sub>adm</sub>, eGFR at the time of admission; eGFR<sub>low</sub>, the lowest eGFR during hospitalization; eGFR<sub>rec</sub>, eGFR at the time of recovery; eGFR<sub>3m</sub>, eGFR at the time of 3 months later; FENa, fractional excretion of sodium; RIFLE, Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease criteria.

Table 3. Univariate and multivariate analysis of predictors of acute kidney injury

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (1.00–1.04)</td>
<td>0.02</td>
<td>1.01 (0.98–1.03)</td>
</tr>
<tr>
<td>ECG change</td>
<td>4.64 (2.37–9.07)</td>
<td>&lt;0.001</td>
<td>3.40 (1.49–7.74)</td>
</tr>
<tr>
<td>ST-T change</td>
<td>26.40 (3.43–203.20)</td>
<td>&lt;0.001</td>
<td>22.10 (2.61–187.20)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>2.27 (1.25–4.14)</td>
<td>0.007</td>
<td>1.19 (0.50–2.84)</td>
</tr>
<tr>
<td>HCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.16 (1.08–1.24)</td>
<td>&lt;0.001</td>
<td>1.03 (0.95–1.12)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>8.22 (3.66–18.48)</td>
<td>&lt;0.001</td>
<td>5.38 (2.14–13.63)</td>
</tr>
<tr>
<td>Amount of GSH ingested</td>
<td>1.00 (1.00–1.01)</td>
<td>0.01</td>
<td>1.00 (0.995–1.00)</td>
</tr>
</tbody>
</table>

CI, confidence interval; ECG, electrocardiographic; HCO<sub>3</sub>, bicarbonate; GSH, glyphosate surfactant herbicide; HR, hazard ratio.

Table 4. Initial electrocardiographic findings in patients with glyphosate surfactant herbicide intoxication

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 184)</th>
<th>AKI (n = 82)</th>
<th>Non-AKI (n = 102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>114 (62.0)</td>
<td>66 (80.5)</td>
<td>48 (47.1)</td>
<td></td>
</tr>
<tr>
<td>Prolonged QTc</td>
<td>49 (43.0/26.6)</td>
<td>23 (34.8/28.0)</td>
<td>26 (52.2/25.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>32 (28.1/17.4)</td>
<td>17 (25.8/20.7)</td>
<td>15 (32.6/14.7)</td>
<td>0.33</td>
</tr>
<tr>
<td>1 AV block</td>
<td>5 (4.4/2.7)</td>
<td>3 (4.5/3.7)</td>
<td>2 (4.3/2.0)</td>
<td>0.66</td>
</tr>
<tr>
<td>ST-T abnormality</td>
<td>16 (14.0/8.7)</td>
<td>15 (22.7/18.3)</td>
<td>1 (2.2/1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sinus bradycardia</td>
<td>4 (3.5/2.2)</td>
<td>2 (3.0/2.4)</td>
<td>2 (4.3/2.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>PSVT</td>
<td>1 (0.9/0.5)</td>
<td>1 (1.5/1.2)</td>
<td>0 (0/0)</td>
<td>0.45</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>4 (3.5/2.2)</td>
<td>2 (3.0/2.4)</td>
<td>2 (6.5/2.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Wide QRS tachycardia</td>
<td>3 (2.6/1.6)</td>
<td>3 (4.5/3.7)</td>
<td>0 (0/0)</td>
<td>0.09</td>
</tr>
<tr>
<td>NSR</td>
<td>70 (61.4/38.0)</td>
<td>16 (24.2/19.5)</td>
<td>54 (112.5/52.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) or number (%) for GSH patients/% for total patients. AKI, acute kidney injury; AV, atrioventricular; PSVT, paroxysmal supraventricular tachycardia; QTc, corrected QT interval; NSR, normal sinus rhythm.
those without AKI, and these variables recorded on admission were significant predictors of AKI in patients with GSH poisoning. Therefore, our findings provide a strong rationale for close monitoring of BP and ECG findings such as ST-T change on admission as useful predictors of AKI in patients with GSH intoxication.

The percentage of suicide attempts using GSH increased from 10.0% in 2010 to 29.5% in 2014 following the paraquat ban in Korea in 2012 [2]. GSH poisoning may precipitate multi-organ dysfunction and death [6]. GSH intoxication-induced mortality rates were shown to range from 2.0% to 30.0% [5,6,12,22,23]. The fatality rate of 8.6% observed in our study was similar to that reported in previous studies [5,12]. The incidence of AKI in patients with GSH intoxication varied from 10.0% to 51.0% [12,13,22,24]. In our view, the wide variation in incidence rates may be attributable to differences in AKI definitions and cohort characteristics such as age or disease severity. Based on the RIFLE criteria, we identified a 44.5% incidence of AKI. AKI is known to be associated with poor clinical outcomes [22,24], which is consistent with our observations in this study. Mild AKI was not associated with poor clinical outcomes in our study (data not shown); however, severe AKI was a significant predictor of mortality in patients with GSH poisoning, which is similar to the findings reported by Mohamed et al. [13]. Therefore, aggressive supportive therapy is important for reducing mortality in patients with GSH intoxication and severe AKI.

The definition of AKI evolved rapidly following the 2004 introduction of the RIFLE, Acute Kidney Injury Network (AKIN), and Kidney Disease Improving Global Outcome (KDIGO) classifications. AKIN published their AKI classification for adults by incorporating the absolute increase in the serum creatinine level among the defining criteria [25–27]. The KDIGO’s guidelines merged the RIFLE and AKIN classifications. However, AKIN criteria are intended to exclude transient changes in creatinine or urine output due to volume depletion [28]. In addition, changes in eGFR are not included in the AKIN or KDIGO AKI classification systems in cases in which steady-state serum creatinine concentrations are unavailable for eGFR measurement [25–27]. In contrast, the RIFLE criteria consider changes in any measure of renal function from baseline values, which

### Table 5. Univariate and multivariate analysis of predictors of mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate HR (95% CI)</th>
<th>p-value</th>
<th>Multivariate HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe AKI</td>
<td>46.45 (9.88–218.40)</td>
<td>&lt;0.001</td>
<td>9.99 (1.64–58.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>Amount of GSH ingested</td>
<td>1.01 (1.00–1.01)</td>
<td>0.002</td>
<td>1.00 (0.997–1.01)</td>
<td>0.43</td>
</tr>
<tr>
<td>Age</td>
<td>1.05 (1.01–1.09)</td>
<td>0.01</td>
<td>1.07 (1.00–1.15)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypotension</td>
<td>29.75 (6.44–137.50)</td>
<td>&lt;0.001</td>
<td>10.05 (1.37–73.95)</td>
<td>0.02</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>1.29 (1.15–1.46)</td>
<td>&lt;0.001</td>
<td>1.09 (0.92–1.28)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; CI, confidence interval; GSH, glyphosate surfactant herbicide; HCO₃⁻, bicarbonate; HR, hazard ratio.

### Figure 1. Survival outcomes in patients with glyphosate surfactant herbicide intoxication according to AKI.

Patients without AKI had longer overall survival than patients with AKI (p < 0.001). AKI, acute kidney injury.
serves as a major strength of this classification system [25]. In this study, baseline renal function data were available in only 36 patients (19.6%), and 72.0% of AKI patients had pre-renal AKI. Therefore, we defined AKI based on the RIFLE classification system instead of the AKIN classification or the KDIGO AKI guidelines. Furthermore, the diagnostic criteria for AKI based on the AKIN and KDIGO systems necessitate measurement of at least two serum creatinine values within 48 hours. However, 41 patients (50.0%) with GSH poisoning-induced AKI presented with poor renal function on admission. Considering the clinical characteristics of AKI in GSH intoxication, our data may be more reliable and useful for estimating AKI incidence in patients with GSH.

To the best of our knowledge, only one study to date has described AKI severity in patients with GSH poisoning. The authors reported that acute renal dysfunction was common following GSH intoxication, which led to mild AKI [13]. However, in our study, 54 of 82 patients (65.9%) with AKI were classified into the I and F categories of AKI based on the RIFLE criteria. Furthermore, 14.0% of patients with AKI received renal replacement therapy in the present study. This discrepancy may be attributable to differences in the characteristics of patients enrolled in these studies. The patients in our study were older and had ingested larger amounts of GSH than those included in the study by Mohamed et al. [13]. Notably, appropriate therapy led to significant improvement in renal function (from its lowest level) in the AKI group. Therefore, we emphasize the role of aggressive supportive therapy for effective management of GSH intoxication in patients with AKI. Recent studies have reported that AKI can lead to chronic kidney disease (CKD) and eventually end-stage kidney disease in the long term [29,30]. In this study, of the 82 patients with AKI, only 23 (28.0%) could be followed-up beyond 3 months. No significant changes were observed in the renal parameters during the follow-up period in these patients. Future prospective studies are needed to investigate the incidence of AKI-to-CKD transition.

In addition to GSH-induced mitochondrial toxicity, GSH causes renal injury secondary to circulatory failure following dehydration or myocardial suppression [6,10,11]. Here, we found that hypotension and ECG abnormalities recorded on admission were significant predictors of AKI. Notably, 75.0% of patients with AKI either had fractional sodium excretion of <1.0% or showed a return of renal function to baseline levels within 72 hours after proper replacement of volume depletion, both of which are suggestive of prerenal failure [31-33]. Additionally, 66 patients (80.5%) with AKI showed ECG abnormalities upon admission. Volume depletion and cardiovascular abnormalities may contribute to hypotension observed in patients with GSH intoxication with consequent development of AKI. Therefore, close and cautious monitoring of initial BP and ECG findings is important for optimal management of patients with GSH poisoning.

ECG abnormalities associated with GSH intoxication have been reported frequently in previous literature [20,24,34]. Various ECG findings have been reported in patients with GSH intoxication including QTc prolongation, sinus tachycardia, and ST-T abnormalities; these were also found in our study. Although the exact mechanism underlying the ECG changes induced by glyphosate herbicides in humans is unknown, the associated hypoxemia, acidosis, and electrolyte abnormalities may cause cardiac complications in glyphosate-poisoning patients [35]. In addition, sympathetic activation, which is related to hemodynamic alterations, has been shown to cause myocardial damage [36,37]. In this study, patients with AKI had lower serum bicarbonate levels and received ventilator care more frequently than those without AKI. We believe that such clinical presentations, including acidosis and hypoxemia, induce hypotension during GSH intoxication resulting in subsequent myocardial damage. Furthermore, surfactants in glyphosate herbicides have been suggested to contribute to hypotension through myocardial depression [38]. Therefore, such mixed pathomechanisms may cause cardiac toxicity during GSH intoxication.

Previous studies have reported that prolonged QTc is not only the most common ECG finding but also predicts mortality in patients with GSH intoxication [20,24,34]. However, there are few studies on the clinical implications of ECG findings in patients with AKI after GSH ingestion. In this study, ECG abnormalities on admission were observed more frequently in the AKI group than in the non-AKI group. However, with the exception of the ST-T change, comparing individual ECG changes between the two groups showed no significant differences in individual ECG findings. In our study, out of the ECG abnormalities, the ST-T change was the only finding that was useful in predict-
ing AKI in patients with GSH intoxication. The ST-T change was also detected during episodes of hypotension [39], which is an important predictor of AKI. Therefore, larger prospective studies are needed to clarify the relationship between ECG changes and GSH intoxication.

The epidemiologic characteristics of toxic agent-related AKI, including herbicides, differ by country, socioeconomic status, and healthcare facility [40]. The incidence of GSH-poisoning–associated AKI was 44.5% in this study, which is higher than that (10%–15%) of general patients admitted to the hospital [41]. The rate of renal replacement therapy initiation in our cohort was 17.1%, which was similar to that of patients admitted to intensive care units reported by Hwang et al. [42]. However, Vilay et al. [43] reported that poisoned patients with renal impairment had a higher rate of renal replacement therapy (27.7%). In addition, the mortality rate identified in the present study was 8.7%, which is significantly lower than that (56%–81%) of herbicide poisoning, including paraquat [44–46]. Therefore, out of toxic agent-related AKI, renal dysfunction after GSH poisoning is considered as a mild type of AKI despite its high incidence. Furthermore, initial hypotension, a risk factor for AKI in previous studies [47,48], was also a predictor of AKI in patients with GSH poisoning. However, further studies are needed to clarify the characteristics of GSH-poisoning–associated AKI.

Our study did have some limitations. First, it was a retrospective single-center design. Second, we did not obtain patient medication history; therefore, data regarding the use of medications that may be associated with ECG abnormalities were unavailable in this study. Large-scale prospective randomized controlled studies are warranted to investigate the clinical characteristics of patients with GSH toxicity.

In this study, the incidence of AKI in patients with GSH intoxication was 44.5%. Hypotension and ECG abnormalities on admission were predictors of AKI in patients with GSH poisoning. Additionally, hypotension and severe AKI were significant predictors of mortality. Therefore, close monitoring of BP is important for optimal management of patients with GSH intoxication.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

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

Authors’ contributions

Conceptualization: IOS
Investigation: AYC, JHO, KYL, IOS
Data curation: AYC, JHO, SSO, IOS
Formal analysis: AYC, IOS
Writing–original draft: AYC, IOS
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Background: This study compares the incidence of post-contrast acute kidney injury (PC-AKI) in patients who received a single administration of iodine-based contrast medium (ICM) with that in patients who received a sequential administration of ICM and gadolinium-based contrast agents (GBCA) in a single visit to an emergency department (ED) to determine the risk factors for PC-AKI.

Methods: Patients who received one or more contrast media in the ED from 2016 to 2021 were included in this retrospective study. They were divided into the ICM alone and ICM + GBCA groups, and the incidence of PC-AKI was compared between the groups. The risk factors were assessed using a multivariable analysis after propensity score matching (PSM).

Results: Overall, 6,318 patients were analyzed, of whom 139 were in the ICM + GBCA group. The incidence of PC-AKI was significantly higher in the ICM + GBCA group than in the ICM alone group (10.9% vs. 27.3%, p < 0.001). In the multivariable analysis, sequential administration was a risk factor for PC-AKI, and single administration was not (adjusted odds ratio [95% confidence interval] in the 1:1, 2:1, and 3:1 PSM cohorts: 2.38 [1.25–4.55], 2.13 [1.26–3.60], and 2.28 [1.39–3.72], respectively). In subgroup analyses of the ICM + GBCA group, osmolality (1.05 [1.01–1.10]) and estimated glomerular filtration rate (eGFR, 0.93 [0.88–0.98]) were associated with PC-AKI.

Conclusion: Compared with a single administration of ICM alone, sequential administration of ICM and GBCA during a single ED visit might be a risk factor for PC-AKI. Osmolality and eGFR might be associated with PC-AKI after sequential administration.

Keywords: Acute kidney injury, Contrast media, Magnetic resonance imaging, X-ray computed tomography
Introduction

Contrast media are indispensable for enhanced imaging examinations, such as computed tomography (CT) and magnetic resonance imaging (MRI), because they allow physicians to gain essential information. Despite their advantages, intravenous iodine-based contrast medium (ICM) and gadolinium-based contrast agents (GBCA) have been identified as causes of post-contrast acute kidney injury (PC-AKI) [1–3], though well-designed meta-analysis studies have reported that the nephrotoxicity associated with these contrast media has been overestimated [4–7]. Nonetheless, multiple administrations of contrast medium in a short period are still proposed as a risk factor for PC-AKI [8]. Because the association between sequential administrations of ICM and GBCA on the same day and the development of PC-AKI is not yet clear, the current guideline from the European Society of Urogenital Radiology recommends that patients with normal or moderately reduced renal function (estimated glomerular filtration rate [eGFR] of >30 mL/min/1.73 m²) should have an interval of at least 4 hours between administrations of ICM and GBCA, based on their half-lives for excretion from the body [9]. On the other hand, the American College of Radiology (ACR) guideline regards that as ambiguous and does not endorse a specific time interval for sequential administrations of two contrast media [10,11].

The currently available evidence about risk factors for PC-AKI, particularly in patients who sequentially receive ICM and GBCA on a single visit to an emergency department (ED), is still limited because this issue is uncommon in a general clinical environment. However, urgent or emergency medical issues can lead to this clinical situation, especially in an ED. Therefore, we examined the incidence of PC-AKI in patients with a baseline eGFR of >30 mL/min/1.73 m² and compared those who received a single administration of ICM alone with those who received sequential administrations of ICM and GBCA during a single ED visit. We also investigated the risk factors for developing PC-AKI among patients who received sequential administrations of ICM and GBCA during a single ED visit.

Methods

Study design and population

This was a single-center, retrospective cohort study conducted by reviewing data extracted from electronic medical records of patients who visited the ED of Chungnam National University Hospital; approximately 56,000 patients visit the ED annually. This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Chungnam National University Hospital (No. 2021-11-024). The extracted data included only clinical data; no personally identifiable information was collected. Therefore, the need for informed consent was waived.

Patients who were admitted from the ED after either a single administration of ICM or sequential administration of both contrast media were included in this study. Among them, pediatric patients (aged <18 years) and patients who had an eGFR of <30 mL/min/1.73 m², as measured in the ED; had a medical history of kidney transplantation; or were missing data for creatinine or eGFR measured before the administration of contrast medium were excluded. The enrolled patients were divided into two groups: ICM alone (single administration of ICM in the ED) and ICM + GBCA (sequential administration of ICM and GBCA).

Data from July 2016 to July 2021 were extracted by an experienced research assistant who underwent rigorous training on our explicit protocol, including clearly defined variables and standardized coding methods. The systematic data abstraction was performed by abstractors who were blinded to the overall goals of the research to ensure unbiased chart reviews. Any conflicting or ambiguous charts were flagged by the abstractors for additional review by two board-certified emergency physicians and nephrologists. We recorded the following data from the index visit: baseline demographics (age, body mass index [BMI], sex), Charlson comorbidity index (CCI) calculated from preexisting illness, ED chief complaint, ED disposition, ED vital signs, laboratory results in ED, type and interval of each enhanced image examination in the cohort (both CT and MRI) performed in the ED on the same day, and mortality rates.
Interventions (process of radiologic examinations)

The enhanced CT and MRI procedures included a chest CT, abdominal CT, three-phase CT, brain CT angiography, head and neck CT, neck CT angiography, extremity CT angiography, head MRI with magnetic resonance angiography, spine MRI, and perfusion brain MRI. All CT and MRI scans were performed using a 64-channel system (Somatom Sensation 64; Siemens Healthineers) and a 3T scanner (Achieva 3T; Philips Healthcare), respectively. According to our institutional policy, preventive hydration with a fixed volume (500 mL) of normal saline was infused into patients at high risk of developing PC-AKI after the administration of contrast medium (serum creatinine level of >1.7 mg/dL or eGFR of <45 mL/min/1.73 m²) at the discretion of the attending physicians.

All administrations of contrast medium (Supplementary Table 1, available online) were performed according to institutional protocols (available online at https://www.ctius.com/protocols). The ICM used in the ED is a low-osmolality and non-ionic contrast agent that was administered intravenously according to image examination-specific protocols at a volume of 80 to 120 mL. Similarly, GBCA was administered at 0.1 mL/kg according to examination-specific protocols.

Outcomes

The primary outcome of this study was the development of PC-AKI. Traditionally, PC-AKI has been defined as a significant increase in serum creatinine from baseline within 72 hours after the last administration of contrast medium [9]. Recently, however, eGFR has gained attention as a potentially better marker of PC-AKI risk [12,13] because it predicts the true GFR more accurately than serum creatinine [14]. Therefore, we added an eGFR-based criterion and defined PC-AKI in this study as an increase in serum creatinine of ≥25% or 0.5 mg/dL or eGFR of <45 mL/min/1.73 m²) at the discretion of the attending physicians.

Statistical analysis

Categorical and continuous variables had a non-normal distribution in this study, so differences between the groups were analyzed using the chi-square test with continuity correction in 2 x 2 tables or Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables, with the results expressed as a frequency with the percentile and median values with interquartile ranges (IQRs), respectively.

We performed propensity score matching (PSM) between the ICM alone and ICM + GBCA groups to balance potential covariables. A binary logistic regression model was used to determine the propensity scores for the ICM + GBCA group using baseline characteristics and clinical status in the ED. For the PSM analysis, each patient in the ICM + GBCA group was matched to one patient in the ICM alone group to the nearest fifth decimal point using a nearest-neighbor algorithm. A caliper setting of 0.2 was used. Standardized differences (SDs) were used to confirm a balanced matching result. The matching result was considered balanced when the SD was <0.1. There was no overlapping of non-exposure cases in the final models. Many subjects received ICM alone; therefore, we also used many-to-one PSM (1:1, 2:1, and 3:1) to minimize the standard error between the groups. After PSM, multivariable logistic regression analyses were performed for each PSM cohort. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) for the exposure variable were calculated in each analysis. Backward selection was used to develop the final adjusted model. The goodness of fit of the final model was evaluated using the Hosmer-Lemeshow test. The results of the logistic regression analysis are expressed as aORs with 95% CIs.

A subgroup multivariable logistic regression analysis was performed to identify the independent risk factors for PC-AKI in the ICM + GBCA group. All variables with a p-value of <0.1 in the univariable analyses were included using the same multivariable logistic regression method just described. Potential multicollinearity was assessed using tolerance and the variance inflation factor (VIF) to verify that multicollinearity did not significantly influence the model’s coefficients. Multicollinearity between variables was defined as a tolerance of <0.1 or a VIF of >10 [18]. Receiver-operating characteristic (ROC) curves were constructed
to determine the predictive value of factors independently associated with the development of PC-AKI, and the area under the ROC curve (AUROC) was obtained for single effective variables and their combination. The combination was divided into two steps: first, a probability value was obtained using a binary logistic regression analysis; second, an ROC curve analysis was performed using this probability value as a test variable. The analysis was performed using R software (version 4.1.0; R Foundation for Statistical Computing) and MedCalc version 15.2.2 (MedCalc Software). Results were considered statistically significant at a p-value of <0.05.

**Results**

**Study population**

Of the 13,839 patients who received ICM for enhanced CT in the ED, 349 pediatric patients, 64 patients for whom the data for estimating renal function before the use of contrast medium were unavailable, 6,974 patients whose eGFR was <30 mL/min/1.73 m$^2$ at baseline, and 134 patients who had undergone kidney transplantation were excluded (Fig. 1). Of the remaining 6,318 patients, 6,179 (97.8%) and 139 (2.2%) were in the ICM alone and ICM + GBCA groups, respectively.

The demographic and baseline characteristics of the total cohort in this study are provided in Table 1. The groups differed significantly in liver disease (335 [5.4%] vs. 18 [12.9%], p < 0.001) and ischemic heart disease (417 [6.7%] vs. 22 [15.8%], p < 0.001) as past medical history; however, the CCI did not differ between the groups (3 [IQR, 1–4] vs. 3 [IQR, 1–5], p = 0.14) (Table 1). Neurologic symptoms (675 [10.9%] vs. 47 [33.8%], p < 0.001) and cardiac arrest (54 [0.9%] vs. 7 [5.0%], p < 0.001) as the chief complaint during the ED visit were significantly higher in the ICM + GBCA group than in the ICM alone group (Table 1). After performing PSM to adjust the balance between the groups, the baseline characteristics and clinical status of the two groups were as displayed in Table 2, and the adjustment status after PSM that we estimated using the standard mean differences between the groups is shown in Supplementary Fig. 1 (available online). None of the baseline characteristics or clinical status in the ED differed significantly between the groups after PSM (Table 2).
### Table 1. Baseline demographics, characteristics, and outcomes of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cohort</th>
<th>ICM alone group</th>
<th>ICM + GBCA group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>6,318</td>
<td>6,179</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>65 (49–77)</td>
<td>65 (49–77)</td>
<td>63 (49–73)</td>
<td>0.16</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>23.5 (21.0–25.9)</td>
<td>23.5 (21.0–26.0)</td>
<td>23.0 (21.1–24.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>Male sex</td>
<td>3,574 (56.6)</td>
<td>3,484 (56.4)</td>
<td>90 (64.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Preexisting illnesses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2,460 (38.9)</td>
<td>2,405 (38.9)</td>
<td>55 (39.6)</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1,539 (24.4)</td>
<td>1,503 (24.3)</td>
<td>36 (25.9)</td>
<td>0.55</td>
</tr>
<tr>
<td>Liver disease</td>
<td>353 (5.6)</td>
<td>335 (5.4)</td>
<td>18 (12.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>179 (2.8)</td>
<td>171 (2.8)</td>
<td>8 (5.6)</td>
<td>0.004</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>230 (3.6)</td>
<td>223 (3.6)</td>
<td>7 (5.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>214 (3.4)</td>
<td>207 (3.4)</td>
<td>7 (5.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>439 (6.9)</td>
<td>417 (6.7)</td>
<td>22 (15.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>750 (11.9)</td>
<td>733 (11.9)</td>
<td>17 (12.2)</td>
<td>0.79</td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>3 (1–4)</td>
<td>3 (1–4)</td>
<td>3 (1–5)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Chief complaint at ED visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>2,355 (37.3)</td>
<td>2,310 (37.4)</td>
<td>45 (32.4)</td>
<td>0.25</td>
</tr>
<tr>
<td>Neurologic</td>
<td>722 (11.4)</td>
<td>675 (10.9)</td>
<td>47 (33.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>61 (1.0)</td>
<td>54 (0.9)</td>
<td>7 (5.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vital sign at ED visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>95.0 (83.0–107.0)</td>
<td>95.0 (83.0–107.0)</td>
<td>91.0 (77.0–107.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>93.0 (80.0–108.0)</td>
<td>93.0 (80.0–108.0)</td>
<td>87.0 (72.0–104.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>Respiratory rate (beats/min)</td>
<td>20.0 (20.0–22.0)</td>
<td>20.0 (20.0–22.0)</td>
<td>20.0 (20.0–22.0)</td>
<td>0.64</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>37.0 (36.5–37.7)</td>
<td>37.0 (36.5–37.7)</td>
<td>36.7 (36.2–37.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>SpO$_2$ (%)</td>
<td>97.0 (96.0–98.0)</td>
<td>97.0 (96.0–98.0)</td>
<td>97.0 (96.0–98.0)</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Laboratory data at ED visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell (×10$^3$/μL)</td>
<td>10.0 (7.5–14.5)</td>
<td>10.1 (7.5–14.5)</td>
<td>9.5 (7.3–14.2)</td>
<td>0.67</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.0 (11.2–14.4)</td>
<td>13.0 (11.2–14.4)</td>
<td>13.1 (11.0–14.4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>38.2 (33.6–42.1)</td>
<td>38.2 (33.6–42.2)</td>
<td>37.9 (33.1–42.0)</td>
<td>0.90</td>
</tr>
<tr>
<td>Platelet (*10$^3$/μL)</td>
<td>222.0 (168.0–281.0)</td>
<td>222.0 (168.0–281.0)</td>
<td>217.0 (146.0–264.0)</td>
<td>0.097</td>
</tr>
<tr>
<td>Osmolality (mOsmol/kg)</td>
<td>292.0 (283.0–301.0)</td>
<td>292.0 (283.0–301.0)</td>
<td>296.2 (285.0–308.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>137.1 (134.4–139.5)</td>
<td>137.0 (134.4–139.5)</td>
<td>139.2 (136.6–142.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.0 (3.7–4.4)</td>
<td>4.0 (3.7–4.4)</td>
<td>4.0 (3.7–4.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>Cl (mEq/L)</td>
<td>102.6 (98.5–105.9)</td>
<td>102.6 (98.5–105.8)</td>
<td>104.1 (99.0–109.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>127.0 (106.0–165.0)</td>
<td>128.0 (106.0–165.0)</td>
<td>119.0 (94.0–170.0)</td>
<td>0.046</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7 (3.2–4.1)</td>
<td>3.7 (3.2–4.1)</td>
<td>3.8 (3.3–4.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>16.0 (11.6–22.0)</td>
<td>16.0 (11.6–22.0)</td>
<td>17.7 (14.0–23.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.8 (0.7–1.1)</td>
<td>0.8 (0.7–1.1)</td>
<td>0.9 (0.7–1.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m$^2$)</td>
<td>86.6 (67.7–103.6)</td>
<td>86.6 (67.6–103.6)</td>
<td>93.1 (71.2–107.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>4.6 (0.8–11.1)</td>
<td>4.7 (0.8–11.1)</td>
<td>2.3 (0.6–8.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>2.1 (1.5–3.2)</td>
<td>2.1 (1.5–3.2)</td>
<td>2.5 (1.5–4.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Development of PC-AKI</td>
<td>714 (11.3)</td>
<td>676 (10.9)</td>
<td>38 (27.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal recovery from PC-AKI</td>
<td>531 (74.4)</td>
<td>501 (74.1)</td>
<td>30 (78.9)</td>
<td>0.57</td>
</tr>
<tr>
<td>Survival discharge</td>
<td>6,050 (95.8)</td>
<td>5,919 (95.8)</td>
<td>131 (94.2)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

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

BUN, blood urea nitrogen; CRP, C-reactive protein; ED, emergency department; eGFR, estimated glomerular filtration rate; GBCA, gadolinium-based contrast agents; ICM, iodine-based contrast media; PC-AKI, post-contrast acute kidney injury; SpO$_2$, oxygen saturation.
Table 2. Comparison of baseline characteristics and clinical status between two groups in each propensity score matched cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>ICM + GBCA group (n = 139)</th>
<th>1:1 PSM cohort (n = 139) p-value</th>
<th>ICM alone group (n = 417)</th>
<th>p-value</th>
<th>ICMB</th>
<th>GBCA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>63 (49–73)</td>
<td>79 (61–103)</td>
<td>0.78</td>
<td>0.86</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 (21.1–24.9)</td>
<td>23.3 (20.8–25.1)</td>
<td>0.13</td>
<td>0.25</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>90 (64.7)</td>
<td>86 (61.9)</td>
<td>0.69</td>
<td>0.73</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preexisting illnesses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>55 (39.6)</td>
<td>55 (39.6)</td>
<td>0.15</td>
<td>&gt;0.99</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>36 (25.9)</td>
<td>36 (25.9)</td>
<td>0.88</td>
<td>0.90</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td>18 (12.9)</td>
<td>19 (13.7)</td>
<td>&gt;0.99</td>
<td>0.86</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>8 (5.6)</td>
<td>8 (5.8)</td>
<td>&gt;0.99</td>
<td>0.99</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>7 (5.0)</td>
<td>7 (5.0)</td>
<td>0.68</td>
<td>&gt;0.99</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>7 (5.0)</td>
<td>6 (4.3)</td>
<td>&gt;0.99</td>
<td>0.82</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>22 (15.8)</td>
<td>21 (15.1)</td>
<td>0.78</td>
<td>0.64</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>17 (12.2)</td>
<td>18 (12.9)</td>
<td>&gt;0.99</td>
<td>0.74</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>3 (1–5)</td>
<td>4 (2–6)</td>
<td>0.49</td>
<td>0.18</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chief complaint at ED visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>45 (32.4)</td>
<td>44 (31.7)</td>
<td>0.40</td>
<td>&gt;0.99</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurologic</td>
<td>47 (33.8)</td>
<td>47 (33.8)</td>
<td>&gt;0.99</td>
<td>0.99</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>7 (5.0)</td>
<td>7 (5.0)</td>
<td>&gt;0.99</td>
<td>0.99</td>
<td>0.99</td>
<td></td>
<td></td>
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<tr>
<td>Vital sign at ED visit</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>91.0 (77.0–107.0)</td>
<td>92.0 (80.0–99.8)</td>
<td>0.43</td>
<td>0.25</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>87.0 (72.0–104.0)</td>
<td>91.5 (75.5–106.8)</td>
<td>0.60</td>
<td>0.07</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory rate (beats/min)</td>
<td>20.0 (20.0–22.0)</td>
<td>20.0 (19.5–22.0)</td>
<td>0.49</td>
<td>0.73</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>36.7 (36.2–37.4)</td>
<td>36.9 (36.4–37.6)</td>
<td>0.88</td>
<td>0.59</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>97.0 (96.0–98.0)</td>
<td>97.0 (96.0–98.0)</td>
<td>0.80</td>
<td>0.64</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory data at ED visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cell (×10³/µL)</td>
<td>9.5 (7.3–14.2)</td>
<td>9.3 (7.2–14.4)</td>
<td>0.65</td>
<td>0.76</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.1 (11.0–14.4)</td>
<td>12.9 (10.5–14.1)</td>
<td>0.39</td>
<td>0.62</td>
<td>0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.9 (33.1–42.0)</td>
<td>37.9 (31.6–41.6)</td>
<td>0.499</td>
<td>0.80</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet (×10³/µL)</td>
<td>217.0 (146.0–264.0)</td>
<td>188.0 (136.3–272.0)</td>
<td>0.75</td>
<td>0.97</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolality (mOsmol/kg)</td>
<td>296.2 (285.0–308.0)</td>
<td>295.6 (287.0–306.9)</td>
<td>0.61</td>
<td>0.49</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>139.2 (136.6–142.0)</td>
<td>138.5 (136.2–140.7)</td>
<td>0.38</td>
<td>0.36</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.0 (3.7–4.4)</td>
<td>4.0 (3.7–4.4)</td>
<td>0.90</td>
<td>0.76</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (mEq/L)</td>
<td>104.1 (99.0–109.2)</td>
<td>104.2 (99.8–107.1)</td>
<td>0.70</td>
<td>0.69</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>119.0 (94.0–170.0)</td>
<td>119.5 (102.3–158.0)</td>
<td>0.30</td>
<td>0.27</td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.8 (3.3–4.1)</td>
<td>3.7 (3.0–4.1)</td>
<td>0.28</td>
<td>0.39</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>17.7 (14.0–23.0)</td>
<td>17.3 (13.0–25.5)</td>
<td>0.46</td>
<td>0.51</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 (0.7–1.1)</td>
<td>0.9 (0.6–1.1)</td>
<td>0.93</td>
<td>0.89</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>93.1 (71.2–107.1)</td>
<td>88.7 (60.7–102.9)</td>
<td>0.99</td>
<td>0.92</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>2.3 (0.6–8.6)</td>
<td>3.1 (0.6–9.6)</td>
<td>0.498</td>
<td>0.37</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>2.5 (1.5–4.1)</td>
<td>2.5 (1.7–3.6)</td>
<td>0.66</td>
<td>0.71</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development of PC-AKI</td>
<td>38 (27.3)</td>
<td>38 (12.9)</td>
<td>0.008</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival discharge</td>
<td>131 (94.2)</td>
<td>128 (92.1)</td>
<td>0.46</td>
<td>0.41</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%). BUN, blood urea nitrogen; CRP, C-reactive protein; ED, emergency department; eGFR, estimated glomerular filtration rate; GBCA, gadolinium-based contrast agents; ICM, iodine-based contrast media; PC-AKI, post-contrast acute kidney injury; SpO₂, oxygen saturation. p-value was calculated using Mann-Whitney test between ICM + GBCA and matched ICM alone groups, respectively.
Incidence of post-contrast acute kidney injury and the renal recovery rate within 7 days after the emergency department visit

The ICM + GBCA group developed PC-AKI more frequently than the ICM alone group (676 [10.9%] vs. 49 [35.3%], p < 0.001) (Table 1), but the rate of renal recovery did not differ significantly between the groups (501 [74.1%] vs. 30 [78.9%], p = 0.57) (Table 1). The incidence of PC-AKI remained significantly higher in the ICM + GBCA group than the ICM alone group in all PSM cohorts (Table 2).

Risk factors for developing post-contrast acute kidney injury in all propensity-matched cohorts

The multivariable logistic regression analysis for each PSM cohort is provided in Table 3. Sequential administration of ICM and GBCA, compared with a single administration of ICM, was independently associated with the development of PC-AKI in all PSM cohorts (Table 3).

Subgroup analyses in the iodine contrast medium + gadolinium-based contrast medium group to investigate the risk factors for developing post-contrast acute kidney injury

The demographic and baseline characteristics of subgroups of patients who received sequential administrations of ICM and GBCA are provided in Supplementary Table 2 (available online). The PC-AKI group showed significantly higher osmolality (303.0 mOsmol/kg [IQR, 295.0–316.5] vs. 291.0 mOsmol/kg [IQR, 283.0–305.1], p < 0.01), sodium (142.1 mEq/L [IQR, 138.9–146.3] vs. 138.8 mEq/L [IQR, 136.2–140.8], p < 0.01), chloride (106.2 mEq/L [IQR, 98.0–110.5] vs. 103.3 mEq/L [IQR, 99.0–108.1], p = 0.04), and glucose (152.0 mg/dL [IQR, 110.8–227.0] vs. 114.0 mg/dL [IQR, 92.0–154.5], p < 0.01) and lower platelet counts (180.0 × 103/μL [IQR, 130.8–247.5] vs. 226.0 × 103/μL [IQR, 167.5–281.5], p = 0.03) than the non-PC-AKI group. In addition, the time interval between the administrations of ICM and GBCA (<4 or ≥4 hours) in the PC-AKI group was significantly shorter than in the non-PC-AKI group (4.6 hours [IQR, 2.2–8.0] vs. 1.8 hours [IQR, 1.0–3.4], p < 0.01).

In our assessment for potential multicollinearity, sodium and glucose did not show significant collinearity with serum osmolality (sodium [tolerance, 0.818; VIF , 1.223] and glucose [tolerance, 0.919; VIF, 1.089]); however, they were excluded from the multivariable analysis because unrecognized collinearity was strongly expected between them and serum osmolality. In the multivariable analysis, the following variables were adjusted: serum osmolality, whether or not the time between the administration of the contrast media was >4 hours, platelet count, creatinine level, eGFR, and chloride level. The multivariable analysis revealed that osmolality (aOR, 1.05 [95% CI, 1.01–1.10]; p = 0.02) and eGFR (aOR, 0.93 [95% CI, 0.88–0.98]; p = 0.01) were independently associated with the development of PC-AKI, whereas the time interval between contrast administrations was not (Table 4).

Subgroup analysis to find the predictive performance of osmolality and estimated glomerular filtration rate for post-contrast acute kidney injury development

Fig. 2 shows the predictive performance of serum osmolality and eGFR when examined independently and in combination. The AUROC values for serum osmolality and

Table 3. Multivariable logistic regression analyses for development of post-contrast acute kidney injury in each propensity score matched cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariable analysis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single administration of ICM</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Sequential administration of ICM and GBCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In 1:1 propensity score matched cohort*</td>
<td>2.60 (1.35–5.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>In 2:1 propensity score matched cohort*</td>
<td>2.44 (1.43–4.18)</td>
<td>0.001</td>
</tr>
<tr>
<td>In 3:1 propensity score matched cohort*</td>
<td>2.77 (1.67–4.59)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; GBCA, gadolinium-based contrast agents; ICM, iodine-based contrast media.
*Those analyses were adjusted with covariables, generally accepted as the risk factors for acute kidney injury: age, body mass index, estimated glomerular filtration rate, creatinine, and past medical history of diabetes or chronic kidney disease.
Table 4. Multivariable analysis for development of PC-AKI in the patients sequentially administered ICM and GBCA

<table>
<thead>
<tr>
<th>Variable</th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality, per mOsmol/kg</td>
<td>1.05 (1.01–1.10)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Chloride, per mEq/L</td>
<td>1.06 (0.99–1.12)</td>
<td>0.08</td>
</tr>
<tr>
<td>Platelet, per (\times 10^3)/μL</td>
<td>1.00 (0.98–1.11)</td>
<td>0.20</td>
</tr>
<tr>
<td>eGFR, per mL/min/1.73 m²</td>
<td>0.93 (0.88–0.98)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Creatinine, per mg/dL</td>
<td>0.06 (0.00–1.42)</td>
<td>0.09</td>
</tr>
<tr>
<td>&lt;4 hr between each administration of ICM and GBCA</td>
<td>0.33 (0.07–1.62)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; GBCA, gadolinium-based contrast agents; ICM, iodine-based contrast media; PC-AKI, post-contrast acute kidney injury.

*Variables showed p-value of <0.1 in univariate analysis were included in this multivariate logistic analysis; serum osmolality, eGFR, the period between the administration of each contrast media (<4 or >4 hours), platelet count, creatinine, and chloride.

*p < 0.05.

Figure 2. Predictive performance of serum osmolality (A), eGFR (B), and their combination (C) on the development of PC-AKI in patients who received sequential administration of ICM and GBCA.

AUROC, area under the receiver-operating characteristic curve; CI, confidence interval; eGFR, estimated glomerular filtration rate; GBCA, gadolinium-based contrast agents; ICM, iodine-based contrast media; NA, not applicable; PC-AKI, post-contrast acute kidney injury.

eGFR were 0.701 (95% CI, 0.592–0.796) and 0.613 (95% CI, 0.503–0.716), respectively (Fig. 2). Their corresponding cut-off values, sensitivity, and specificity were as follows: 292 mOsmol/kg, 87.1% (95% CI, 70.2–96.4), and 50.0% (95% CI, 36.1–63.9), respectively, for osmolality; 78 mL/min/1.73 m², 58.3% (95% CI, 36.6–77.9), and 66.7% (95% CI, 53.7–78.0), respectively, for eGFR (Fig. 2). The AUROC values for the development of PC-AKI were numerically higher when the two variables (osmolality and eGFR) were used in combination than when they were used alone (AUROC, 0.714 [95% CI, 0.578–0.825]) (Fig. 2).

Discussion

In this study, the incidence rates for PC-AKI after the administration of contrast media were 10.9% and 27.3% in the ICM alone and ICM + GBCA groups, respectively. This finding is in line with the results of previous studies that the rate of overall contrast-induced nephropathy was 10.6% in patients who received ICM alone [19,20], and the incidence of AKI was higher after combined use of ICM and GBCA than after the use of a single agent [21]. Furthermore, we found that the sequential administration of ICM and GBCA during a single ED visit for sequential radiologic examinations increased the risk of PC-AKI compared with a single administration of ICM alone. We investigated the risk factors for the development of PC-AKI using subgroup analyses of the ICM + GBCA group. Notably, a time interval of 4 hours between contrast administrations, as suggested in a current guideline [9], was not independently asso-
associated with the development of PC-AKI, whereas serum osmolality and eGFR were independently associated with the development of PC-AKI. Although urgent medical issues that might require sequential administrations of ICM and GBCA are generally uncommon, they do occur, and ED physicians in charge of resuscitation occasionally encounter this circumstance. Therefore, we suggest that ED physicians consider the effects of serum osmolality and reduced kidney function in urgent medical circumstances that require them to perform sequential administration of ICM and GBCA in the ED.

The findings of this study are contrary to those of a previous study that examined the association between sequential administration of contrast media and the development of PC-AKI [22]. The discrepancy can be attributed to our use of eGFR as a marker of PC-AKI in this study, whereas the other study evaluated the development of PC-AKI using only the serum creatinine level [22]. Several studies have shown that GBCA induces kidney injury in several hours [23,24], in addition to causing a significant decrease in the eGFR [25,26]. These factors could explain why we found a higher incidence of PC-AKI after sequential administration of ICM and GBCA than in the previous study [22]. Although the equations for calculating eGFR can induce measurement errors and augment the clearance rate, often resulting in overestimation, it is commonly used in AKI screening to predict the risk status [27–29]. Furthermore, one previous study stated that the serum creatinine level increases immediately after a decrease in the eGFR in the presence of AKI, making creatinine a suboptimal indicator of renal function in patients with AKI [30]. Consequently, a recent randomized prospective study for the development of PC-AKI after the administration of contrast media used eGFR as an additional marker of AKI [15]. In addition, GBCA, which is a high-osmolar contrast medium, can induce intense and prolonged vasoconstriction at the corticomedullary junction of the kidney and directly impair the autoregulatory ability of the kidney by causing a decline in nitric oxide production [31,32]. In line with that pathophysiologic pathway, our subgroup analysis showed that high serum osmolality and low eGFR at baseline were independently associated with the development of PC-AKI. It appears that ICM and GBCA synergistically interact with osmolar and renal function status, causing a rapid decline in eGFR and the development of PC-AKI.

We found that low eGFR was independently associated with the development of PC-AKI in patients who sequentially received ICM and GBCA during the same ED visit. Low eGFR is widely accepted as an independent risk factor for the development of PC-AKI induced by ICM or GBCA [33,34]. McDonald et al. [34] found that eGFR of ≤45 mL/min/1.73 m² before an administration of ICM increased the risk of renal replacement therapy. However, the cut-off value for eGFR associated with the development of PC-AKI in patients who sequentially received ICM and GBCA within a short interval remains unclear. In our results, the eGFR cut-off value for predicting the development of PC-AKI was 78 mL/min/1.73 m², which suggests that the optimal safe eGFR in patients who sequentially receive ICM and GBCA within a short interval could be higher than that in patients who receive a single administration of contrast medium. In other words, PC-AKI can develop even in patients with normal kidney function (eGFR of >60 mL/min/1.73 m²) after sequential administrations of two contrast media. However, the clinical relevance of that finding is limited by our study design. Therefore, we emphasize that a further study is needed to investigate an optimal eGFR cut-off value for patients who receive sequential administrations of ICM and GBCA and develop preventive strategies for patients whose eGFR is lower than that in an ED setting.

High serum osmolality was independently associated with the development of PC-AKI in patients who sequentially received ICM and GBCA on the same ED visit. This finding is in line with previous studies that commonly revealed that high serum osmolality was associated with the development of PC-AKI after an administration of ICM [19,35]. Serum osmolality is widely accepted as a typical indicator representing body fluid balance, with a high osmolality linked to dehydration [36,37]. The ACR guideline clearly states that dehydration is an important risk factor for PC-AKI, and thus the major preventive action to mitigate the risk of PC-AKI is to provide intravenous volume expansion using 0.9% normal saline prior to ICM administration [38]. Serum osmolality numerically improved the predictive performance of our model when it was used in combination with eGFR, compared with using eGFR alone (AUROC, 0.613 [95% CI, 0.503–0.716] to 0.714 [95% CI, 0.578–0.825]). Given previous findings of a significant association between high osmolality and the development of PC-AKI in patients who require sequential administrations.
of two contrast media on the same ED visit, we suggest that high serum osmolality is likely to be a risk factor for the development of PC-AKI. Furthermore, we suggest that a strategy should be developed to administer preventive hydration based on serum osmolality and/or eGFR prior to sequential treatment with both contrast media.

This study has several limitations. First, because it was a retrospective study, we can report only associations and not causation. Furthermore, like all retrospective studies, this one contains inherent selection bias. We were aware of the possible biases and held multiple meetings to ensure that the patients were correctly identified and the data collection protocol was suitably standardized; adjustment for comorbidities was also made to reduce bias. To reduce selection bias and simulate a randomized controlled trial, the PSM method was used in this study. In our PSM cohort, both groups showed a well-balanced distribution of demographics and most confounders. However, age, CCI, and eGFR were not balanced in the 1:1 PSM cohort, and thus unmeasured or unmeasurable confounders might still remain. Therefore, a further study with prospectively collected data from a large sample is needed to confirm our results. Second, several nephrotoxic medications administered during the hospital stay were not used in the entire study population, which created bias in confirming the effect of sequential administrations of contrast media on the development of PC-AKI. However, we minimized the non-estimated bias in our subgroup analysis to investigate independent risk factors for the development of PC-AKI in patients who sequentially received ICM and GBCA. Third, our institution’s policy for preventive hydration in patients at high risk of developing PC-AKI is to leave the decision completely to the physician discretion, and thus it is not clear whether it was applied to all patients. However, we confirmed that all included patients at high risk of developing PC-AKI underwent preventive hydration in the form of a fixed volume (500 mL) of normal saline by thoroughly reviewing medical records kept by physicians and nurses. Fourth, nephrogenic systemic fibrosis (NSF), which is a major concern for GBCA nephrotoxicity, was not estimated in this study. A diagnosis of NSF is usually made with a detailed patient history, thorough clinical examination, and the identification of characteristic findings a few weeks or months after an administration of GBCA [36]. Therefore, a further study in the ward or intensive care unit setting is required to confirm how combining two contrast media affects the incidence of NSF. Fifth, because our study sample was small, serum osmolality and eGFR could not be included in the multivariable analysis as categorical variables (osmolality, hypoosmolality vs. normal vs. hyperosmolality; eGFR, normal vs. mild kidney dysfunction vs. moderate kidney dysfunction). Therefore, a multicenter study with a large cohort is required to enhance the generalizability of our results and their easy application in a clinical environment.

Compared with a single administration of ICM alone, sequential administration of ICM and GBCA during a single ED visit could be a risk factor for the development of PC-AKI in patients with an eGFR of >30 mL/min/1.73 m². Baseline osmolality and eGFR might be independently associated with the development of PC-AKI after sequential administration of ICM and GBCA. Well-designed prospective studies are needed to investigate the risk factors for PC-AKI and develop ED setting–specific preventive strategies.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

The data presented here are available from the corresponding author upon request. The data are not publicly available because of ethical concerns.

**Authors’ contributions**

Conceptualization: CK
Formal analysis: JSP, DEC
Funding acquisition: DEC
Investigation: SSH, DEC
Supervision: JSP
Writing–original draft: CK, SSH
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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References

Risk of ventricular tachycardia and its outcomes in patients undergoing continuous renal replacement therapy due to acute kidney injury

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³Department of Internal Medicine, Inje University Seoul Paik Hospital, Seoul, Republic of Korea

Background: Despite efforts to treat critically ill patients who require continuous renal replacement therapy (CRRT) due to acute kidney injury (AKI), their mortality risk remains high. This condition may be attributable to complications of CRRT, such as arrhythmias. Here, we addressed the occurrence of ventricular tachycardia (VT) during CRRT and its relationship with patient outcomes.

Methods: This study retrospectively enrolled 2,397 patients who started CRRT due to AKI from 2010 to 2020 at Seoul National University Hospital in Korea. The occurrence of VT was evaluated from the initiation of CRRT until weaning from CRRT. The odds ratios (ORs) of mortality outcomes were measured using logistic regression models after adjustment for multiple variables.

Results: VT occurred in 150 patients (6.3%) after starting CRRT. Among them, 95 cases were defined as sustained VT (i.e., lasting ≥30 seconds), and the other 55 cases were defined as non-sustained VT (i.e., lasting <30 seconds). The occurrence of sustained VT was associated with a higher mortality rate than a nonoccurrence (OR, 2.04 and 95% confidence interval [CI], 1.23–3.39 for the 30-day mortality; OR, 4.06 and 95% CI, 2.04–8.08 for the 90-day mortality). The mortality risk did not differ between patients with non-sustained VT and nonoccurrence. A history of myocardial infarction, vasopressor use, and certain trends of blood laboratory findings (such as acidosis and hyperkalemia) were associated with the subsequent risk of sustained VT.

Conclusion: Sustained VT occurrence after starting CRRT is associated with increased patient mortality. The monitoring of electrolytes and acid-base status during CRRT is essential because of its relationship with the risk of VT.

Keywords: Acute kidney injury, Continuous renal replacement therapy, Electrolytes, Mortality, Ventricular tachycardia

Introduction

Acute kidney injury (AKI) is a common complication in critically ill patients, and interest in this condition has increased due to its relationship with high mortality [1,2]. Continuous renal replacement therapy (CRRT) is frequently the first option used to treat patients with severe AKI who require renal replacement therapy, particularly when they...
are hemodynamically unstable [3–5]. Despite the success of the therapeutic approach with this modality, the mortality rates have not decreased to <50% over the past two decades [2,6,7]. Several adverse events that may arise in patients on CRRT may hinder its clinical usefulness, even with appropriate initiation [8–10]; these adverse events include hypotension, an imbalance in major and minor electrolytes, hypothermia, hematologic abnormalities, and catheter-related complications [9]. Certain arrhythmias may occur during CRRT [6,9,11]; new-onset atrial fibrillation has been reported in >10% of patients on CRRT, and its occurrence is associated with worse patient outcomes [11]. Other arrhythmias may also occur, but their characteristics, risk factors for occurrence, and subsequent outcomes have not been thoroughly evaluated.

Ventricular tachycardia (VT) is a rare subset of arrhythmias in healthy individuals, but it may frequently occur if abnormalities in the heart structure, metabolites, and electrolytes exist [12]. Patients undergoing hemodialysis may be at extremely high risk of VT because they have myocardial fibrosis, a microvascular disorder, left ventricular hypertrophy, and electrical instability due to the repeated fluid shifts that occur during hemodialysis [13]. Similarly, patients with CRRT typically experience regional impairment in cardiac contractility [14]; thus, VT may occur in patients on CRRT, but no studies have examined this issue. The present study evaluated the prevalence of VT in patients after starting CRRT due to AKI, predictors related to its occurrence, and its relationship with overall mortality outcomes.

**Methods**

**Patients and data collection**

This was a retrospective observational study of 2,832 patients who underwent CRRT at Seoul National University Hospital from June 2010 to December 2020. Among these, patients who were under 18 years old (n = 58) and those who had been on chronic hemodialysis because of end-stage kidney disease (n = 377) were excluded. Finally, 2,397 patients were analyzed in the present study. The study design was approved by the Institutional Review Board of Seoul National University Hospital (No. H-2110-085-1262) and complied with the Declaration of Helsinki. The requirement for informed consent was waived by the review board.

Baseline information at the time that CRRT began was collected, such as age, sex, weight, cause of AKI (e.g., septic and nonseptic), initial CRRT settings (e.g., target dose, blood flow rate, and target ultrafiltration), mechanical ventilation, use of vasopressors, the division of intensive care unit, and severity indices (e.g., the sequential organ failure assessment [SOFA], the Acute Physiology and Chronic Health Evaluation [APACHE] II, and the Charlson comorbidity index [CCI]). The SOFA, APACHE II, and CCI scores were calculated using the original formulas [15–17]. Laboratory values, such as pH, potassium, bicarbonate, and phosphate levels, were measured at least twice a day after starting CRRT until the day of VT occurrence or of CRRT discontinuation for patients who did not develop VT. Systolic and diastolic blood pressures were also collected both before and after the VT events.

All patients were monitored with bedside monitors, which produced waveforms of electrocardiograms. VT events were documented in their electronic medical records, and the first VT event was retrospectively collected for the present study. VT was classified as non-sustained VT (NSVT) or sustained VT (SVT) as follows: NSVT, ≥3 consecutive ventricular beats with an R-R interval of 600 ms and lasting <30 seconds; and SVT, a ventricular rhythm of >100 beats per minute lasting ≥30 seconds or requiring termination due to hemodynamic instability.

**Outcomes**

The primary outcome was all-cause mortality after CRRT, and it was stratified by timeframe (e.g., 7 days, 30 days, and 90 days). Any change in blood pressure during the VT events was additionally evaluated.

**Statistical analysis**

Categorical and continuous variables are presented as proportions and means ± standard deviations when they were normally distributed and as medians with interquartile ranges when they were not normally distributed, respectively. The normality of the distribution was analyzed using the Kolmogorov-Smirnov test. The chi-square test or Fisher exact test was employed to compare categorical variables.
The Student t test or the Mann-Whitney U test was used to analyze continuous variables with or without a normal distribution. The Wilcoxon signed-rank test was carried out to confirm significant changes in continuous variables.

We assessed the effect of VT on mortality outcomes by constructing Kaplan-Meier curves and performing a log-rank test. The hazard ratios and confidence intervals for mortality outcomes were calculated using the Cox proportional hazard regression model. Due to a violation of the Cox proportion assumption, we used a logistic regression model and calculated the odds ratios (ORs) of mortality outcomes with adjustment for multiple variables. The adjusted variables were selected based on the significance of their association with mortality. A competing risk Cox proportional hazard regression model was used at multiple time points of CRRT with time-dependent variables (e.g., pH, potassium, bicarbonate, and phosphate) to investigate the predictors of VT. Time-dependent variables were obtained until VT occurrence or discontinuation of CRRT.

All statistical analyses were performed using the IBM SPSS version 27 (IBM Corp.) and R software version 3.5.1 (R Foundation for Statistical Computing). A p-value of <0.05 was considered statistically significant.

Results

Patient demographics

The baseline characteristics are presented in Table 1. The mean patient age was 64 ± 15 years, and 38.6% of the patients were female. The proportion of patients with sepsis was 54.9%. VT occurred in 150 patients (6.3%); most cases took place within 1 week after CRRT initiation (Fig. 1). Patients who developed VT were more likely to require mechanical ventilation or ≥3 vasopressors and had higher SOFA scores than patients without VT.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>Non-VT group</th>
<th>VT group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2,397</td>
<td>2,247</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64.1 ± 14.9</td>
<td>64.1 ± 14.8</td>
<td>63.7 ± 15.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Female sex</td>
<td>925 (38.6)</td>
<td>875 (38.9)</td>
<td>50 (33.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>23.2 ± 4.5</td>
<td>61.7 ± 13.2</td>
<td>63.4 ± 13.8</td>
<td>0.21</td>
</tr>
<tr>
<td>Septic acute kidney injury</td>
<td>1,317 (54.9)</td>
<td>1,236 (55.0)</td>
<td>81 (54.0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Target dose (mL/kg/hr)</td>
<td>40.7 ± 13.1</td>
<td>40.8 ± 13.3</td>
<td>39.0 ± 10.7</td>
<td>0.91</td>
</tr>
<tr>
<td>Blood flow rate (mL/min)</td>
<td>110.8 ± 24.5</td>
<td>110.9 ± 24.6</td>
<td>109.6 ± 22.6</td>
<td>0.68</td>
</tr>
<tr>
<td>Target ultrafiltration (mL/day)</td>
<td>0 (–500 to 0)</td>
<td>0 (–500 to 0)</td>
<td>0 (–500 to 0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Previous history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular tachycardia</td>
<td>63 (2.6)</td>
<td>47 (2.1)</td>
<td>16 (10.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>406 (16.9)</td>
<td>365 (16.2)</td>
<td>41 (27.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>213 (8.9)</td>
<td>187 (8.3)</td>
<td>26 (17.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>1,867 (77.9)</td>
<td>1,739 (77.4)</td>
<td>128 (85.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Use of ≥3 vasopressors</td>
<td>441 (18.4)</td>
<td>388 (17.3)</td>
<td>53 (35.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICU type</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medical ICU</td>
<td>1,216 (50.7)</td>
<td>1,118 (49.8)</td>
<td>98 (65.3)</td>
<td></td>
</tr>
<tr>
<td>Surgical ICU</td>
<td>457 (19.1)</td>
<td>433 (19.3)</td>
<td>24 (16.0)</td>
<td></td>
</tr>
<tr>
<td>Cardiopulmonary ICU</td>
<td>290 (12.1)</td>
<td>273 (12.1)</td>
<td>17 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Emergency ICU</td>
<td>429 (17.9)</td>
<td>418 (18.6)</td>
<td>11 (7.3)</td>
<td></td>
</tr>
<tr>
<td>COVID-19 ICU</td>
<td>5 (0.2)</td>
<td>5 (0.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>SOFA score</td>
<td>11.9 ± 3.7</td>
<td>11.9 ± 3.7</td>
<td>12.8 ± 3.5</td>
<td>0.001</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>26.1 ± 7.7</td>
<td>26.1 ± 7.8</td>
<td>27.0 ± 7.5</td>
<td>0.19</td>
</tr>
<tr>
<td>CCI score</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>2 (0–3)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

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

APACHE, Acute Physiology and Chronic Health Evaluation; CCI, Charlson comorbidity index; COVID-19, coronavirus disease 2019; ICU, intensive care unit; SOFA, sequential organ failure assessment; VT, ventricular tachycardia.
The relationship between ventricular tachycardia and mortality

During a median follow-up period of 12 days (interquartile range, 3–29 days), 1,591 patients (66.4%) died. The mortality incidence was 24.3 deaths per 1,000 person-days. When a univariable Cox regression model was applied, several variables, including age, sex, septic AKI, initial CRRT settings (such as blood flow rate and target ultrafiltration), mechanical ventilation, use of ≥3 vasopressors, and SOFA, APACHE II, and CCI scores, were identified as significant factors related to mortality (Table 2), and these variables were included in subsequent multivariable regression models for adjustment.

Fig. 2A shows the Kaplan-Meier survival curves for patients with and without VT, and their survival rates differed (p < 0.001). When VT was classified according to duration, an apparent violation of the proportionality of hazards assumption was observed because the NSVT and non-VT curves crossed (p = 0.97) (Fig. 2B). Similarly, the multivariable regression model confirmed that only SVT was independently associated with a high mortality risk, irrespective of the timeframes (Table 3). Blood pressures were compared before and after the VT events to explore the hemodynamic effects. Although NSVT was not associated with hemodynamic fluctuation, SVT decreased blood pressure, which might be the cause of the high mortality in patients with SVT (Fig. 3).

Risk factors for ventricular tachycardia in patients on continuous renal replacement therapy

Table 4 shows a competing risk Cox regression analysis that was carried out to identify risk factors for either SVT or NSVT. A history of myocardial infarction, vasopressor use, and certain trends, such as acidosis and hyperkalemia, were independently associated with the occurrence of SVT. A history of congestive heart failure and a hypophosphatemia trend were associated with the occurrence of NSVT.

Discussion

Several arrhythmias may occur after starting CRRT and may exacerbate patients’ existing conditions. The present study focused on VT in patients receiving CRRT, and the occurrence of SVT was associated with worse outcomes,
Table 2. Unadjusted hazard ratios of all-cause mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per 10 yr</td>
<td>1.03 (1.01–1.05)</td>
<td>0.03</td>
</tr>
<tr>
<td>Female sex, vs. male sex</td>
<td>0.96 (0.87–1.06)</td>
<td>0.43</td>
</tr>
<tr>
<td>Body weight, per 1 kg</td>
<td>1.00 (0.995–1.00)</td>
<td>0.54</td>
</tr>
<tr>
<td>Septic acute kidney injury, vs. nonseptic</td>
<td>1.34 (1.19–1.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Target dose, per 10 mL/kg/hr</td>
<td>1.03 (0.99–1.07)</td>
<td>0.14</td>
</tr>
<tr>
<td>Blood flow rate, per 100 mL/min</td>
<td>1.29 (1.06–1.57)</td>
<td>0.01</td>
</tr>
<tr>
<td>Target ultrafiltration, per 1 L/day</td>
<td>1.07 (1.02–1.13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Previous history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular tachycardia, vs. none</td>
<td>0.80 (0.58–1.11)</td>
<td>0.18</td>
</tr>
<tr>
<td>Congestive heart failure, vs. none</td>
<td>0.94 (0.80–1.10)</td>
<td>0.43</td>
</tr>
<tr>
<td>Myocardial infarction, vs. none</td>
<td>1.14 (0.96–1.37)</td>
<td>0.15</td>
</tr>
<tr>
<td>Mechanical ventilation, vs. none</td>
<td>1.49 (1.29–1.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥3 vasopressors, vs. &lt;3</td>
<td>1.42 (1.27–1.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOFA score, per 1 score</td>
<td>1.13 (1.11–1.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APACHE II score, per 1 score</td>
<td>1.06 (1.05–1.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCI score, per 1 score</td>
<td>1.04 (1.02–1.07)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

APACHE, Acute Physiology and Chronic Health Evaluation; CCI, Charlson comorbidity index; CI, confidence interval; SOFA, sequential organ failure assessment; VT, ventricular tachycardia.

Figure 2. Kaplan-Meier survival curves. (A) For patients both with and without ventricular tachycardia (VT). (B) VT events are categorized into sustained and non-sustained cases.

such as a high mortality rate and low blood pressure. Certain factors were correlated with a high risk of VT, such as vasopressor use, acidosis, hyperkalemia, and hypophosphatemia.

A previous study of 595 patients revealed that 2.3% of patients on CRRT had VT [9]. In another multinational cohort of patients treated with CRRT for AKI, VT occurred in 0.2% of patients [6]. The present cohort had a prevalence of 6.3%, and the differences might have depended on the different characteristics of patients in these respective studies. Among all episodes of VT, the incidence of SVT was 4.0%, while that of NSVT was 2.3%. Depending on the definitions
for SVT and NSVT, a difference in prevalence might exist between studies [18].

Patients on CRRT frequently require the support of vasopressors because of hemodynamic instability. Vasopressors are generally administered based on the assumption that short-term clinical recovery will be facilitated by an enhanced cardiac output or vascular tone. However, these agents may lead to fatal adverse events, such as sinus tachycardia, asymptomatic ventricular ectopic activity, and other ventricular arrhythmias [19,20]. Our data suggested that the use of many vasoactive agents was associated with an increased risk of SVT, regardless of the severity of illness,

which supports the findings reported previously.

According to the time-dependent analysis, hyperkalemia and acidosis were associated with the risk of SVT. Systemic acidosis provokes arrhythmias via both the direct action of Na\(^+\)-H\(^+\) and Na\(^+\)-Ca\(^2+\) exchangers and the indirect action of inflammatory or hyperoxidative injury [21]. Some clinical studies have documented the association between systemic acidosis and VT [22-24]. The presence of systemic acidosis was the strongest determinant of ventricular arrhythmia in patients with ST-elevated myocardial infarction after reperfusion therapy [24]. In addition to the role of hypokalemia as a risk factor for fatal ventricular arrhythmias [25-27], hyperkalemia also exerts effects on cardiac excitability, which predisposes hearts to reentrant tachyarrhythmias, such as VT [28,29]. When combined with hypoxia and acidosis, hyperkalemia promotes phase 2 reentry by further increasing the repolarization reserve in epicardial ventricular tissue [30]. As refractory acidosis and hyperkalemia are frequent complications observed following severe AKI, our data indicate that insufficient dialysis at the early stage of CRRT might be one of the causative factors for VT. Hypophosphatemia, which occurs in 10% to 60% of patients receiving CRRT [31-33], is associated with adverse events, such as prolonged mechanical ventilation or vasoactive agent support in critically ill patients [34,35]. Although an association between NSVT and mortality was not documented in this study, we recommend that phos-

| Table 3. Odds ratios of all-cause mortality in patients with ventricular tachycardia compared with those without ventricular tachycardia |
|-----------------|-----------------|-----------------|
| Outcome         | Variable        | Odds ratio (95% CI) p-value |
| 7-day mortality | Non-sustained VT| 0.71 (0.35–1.46) 0.36 |
|                 | Sustained VT    | 1.99 (1.17–3.37) 0.01 |
| 30-day mortality| Non-sustained VT| 1.16 (0.65–2.10) 0.62 |
|                 | Sustained VT    | 2.04 (1.23–3.39) 0.006 |
| 90-day mortality| Non-sustained VT| 1.72 (0.89–3.31) 0.11 |
|                 | Sustained VT    | 4.06 (2.04–8.09) 0.001 |

CI, confidence interval; VT, ventricular tachycardia.

*Adjusted for age, sex, septic acute kidney injury, blood flow rate, target ultrafiltration, mechanical ventilation, vasopressor use, and the scores of sequential organ failure assessment, Acute Physiology and Chronic Health Evaluation II, and Charlson comorbidity index.

**Figure 3. Changes in blood pressure after the occurrence of VT.**

VT, ventricular tachycardia.
phosphate levels be monitored during CRRT because the results of previous studies have demonstrated the potential risk of NSVT [36–38].

Although the current study is informative, certain limitations remain to be addressed. Because of its retrospective design, unmeasured biases and confounders might have interfered with the analyses. Alterations in practice may be related to mortality but were not considered in the present study. The cause of death was not available in the present dataset. Heart function was not thoroughly evaluated, which might be a main cause of VT occurrence. The occurrence of VT and its risk factors may also differ depending on the cause of AKI, but the present study did not categorize nonseptic patients into further specific causes.

The present study indicated that the occurrence of VT in patients on CRRT was associated with an unfavorable prognosis, particularly in those with SVT. Several factors, such as vasopressor use, acidosis, hyperkalemia, and hypophosphatemia, were associated with the risk of VT, and some of them were considered modifiable during CRRT implementation. Accordingly, monitoring VT and making early attempts to reduce the risk factors for VT will therefore be needed after patients begin CRRT.

### Table 4. Risk factors for the occurrence of ventricular tachycardia in the multivariable regression model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-sustained VT</th>
<th>Sustained VT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hazard ratio (95% CI)</strong></td>
<td><strong>p-value</strong></td>
<td><strong>Hazard ratio (95% CI)</strong></td>
</tr>
<tr>
<td>Age, per 1 yr</td>
<td>1.01 (0.99–1.02)</td>
<td>0.49</td>
</tr>
<tr>
<td>Female sex, vs. male sex</td>
<td>0.65 (0.36–1.20)</td>
<td>0.17</td>
</tr>
<tr>
<td>Septic acute kidney injury, vs. nonseptic</td>
<td>0.81 (0.47–1.40)</td>
<td>0.45</td>
</tr>
<tr>
<td>Blood flow rate, per 100 mL/min</td>
<td>0.96 (0.31–2.91)</td>
<td>0.94</td>
</tr>
<tr>
<td>Target ultrafiltration, per 1 L/day</td>
<td>1.11 (0.90–1.37)</td>
<td>0.33</td>
</tr>
<tr>
<td>Congestive heart failure, vs. none</td>
<td>1.88 (1.02–3.48)</td>
<td>0.04</td>
</tr>
<tr>
<td>Myocardial infarction, vs. none</td>
<td>1.76 (0.90–3.45)</td>
<td>0.097</td>
</tr>
<tr>
<td>Mechanical ventilation, vs. none</td>
<td>0.89 (0.43–1.80)</td>
<td>0.72</td>
</tr>
<tr>
<td>≥3 vasopressors, vs. &lt;3</td>
<td>0.58 (0.27–1.25)</td>
<td>0.17</td>
</tr>
<tr>
<td>SOFA score, per 1 score</td>
<td>0.96 (0.87–1.05)</td>
<td>0.36</td>
</tr>
<tr>
<td>APACHE II score, per 1 score</td>
<td>1.04 (1.00–1.03)</td>
<td>0.07</td>
</tr>
<tr>
<td>CCI score, per 1 score</td>
<td>0.92 (0.80–1.06)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH, per 0.1</td>
<td>0.95 (0.88–1.02)</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum potassium, per 1 mmol/L</td>
<td>1.21 (0.88–1.67)</td>
<td>0.24</td>
</tr>
<tr>
<td>Serum bicarbonate, per 1 mmol/L</td>
<td>0.91 (0.79–1.04)</td>
<td>0.18</td>
</tr>
<tr>
<td>Serum phosphate, per 1 mg/dL</td>
<td>0.02 (0.00–0.30)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

The data are available from the authors upon reasonable request.

**Authors’ contributions**

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

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References


Background: It is important for the dialysis specialist to provide essential and safe care to hemodialysis (HD) patients. However, little is known about the actual effect of dialysis specialist care on the survival of HD patients. We therefore investigated the influence of dialysis specialist care on patient mortality in a nationwide Korean dialysis cohort.

Methods: We used an HD quality assessment and National Health Insurance Service claims data from October to December 2015. A total of 34,408 patients were divided into two groups according to the proportion of dialysis specialists in their HD unit, as follows: 0%, no dialysis specialist care group, and ≥50%, dialysis specialist care group. We analyzed the mortality risk of these groups using the Cox proportional hazards model after matching propensity scores.

Results: After propensity score matching, 18,344 patients were enrolled. The ratio of patients from the groups with and without dialysis specialist care was 86.7% to 13.3%. The dialysis specialist care group showed a shorter dialysis vintage, higher levels of hemoglobin, higher single-pool Kt/V values, lower levels of phosphorus, and lower systolic and diastolic blood pressures than the no dialysis specialist care group. After adjusting demographic and clinical parameters, the absence of dialysis specialist care was a significant independent risk factor for all-cause mortality (hazard ratio, 1.10; 95% confidence interval, 1.03–1.18; p = 0.004).

Conclusion: Dialysis specialist care is an important determinant of overall patient survival among HD patients. Appropriate care given by dialysis specialists may improve clinical outcomes of patients undergoing HD.

Keywords: Dialysis, Hemodialysis units, Mortality, Specialist

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Introduction

The survival of hemodialysis (HD) patients has improved significantly over the past few decades with the development of dialysis-related technologies and drugs. However, the mortality rate remains high in patients with HD due to complications, such as cardiovascular disease and infections. Mortality in HD patients is influenced not only by patient factors but also by environmental factors and procedure-related factors (e.g., dialysis dosage, time of dialysis, and compliance with treatment regimen) [1,2]. In addition to patient- and facility-level characteristics, provider-level factors, such as physician caseload (patient-to-physician ratio), have been suggested to affect clinical outcomes [3,4]. Recent studies have similarly suggested that nephrologist caseload influences HD patient outcomes [5].

According to the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease in kidney disease, it is recommended that patients with a rapid decline in the glomerular filtration rate (GFR), a GFR of <30 mL/min/1.73 m² (GFR category grade 4–5), progressive chronic kidney disease, or severe albuminuria or hematuria be referred to specialist kidney care services [6]. There have been many studies showing the effect of early nephrologist referral on patient mortality and clinical outcomes among those with predialysis chronic kidney disease. Earlier and more frequent consultations with a nephrologist improved patient survival within the first year of dialysis [7,8]. Timely referral to a nephrologist also reduced the initial 90-day mortality rate among elderly patients with end-stage renal disease [9]. Even remote distances from the nephrologist’s office affect the mortality rate and clinical outcomes before and after starting HD [10,11]. However, there have been few studies demonstrating the effect of nephrologist care on overall mortality among HD patients.

HD patients are a unique population with a high burden of complex comorbid conditions who are routinely subjected to specialized procedures. In this regard, there may be a differential association between nephrologist care and outcomes in this population. In many countries, including the United States and Germany, only nephrologists can prescribe HD order sheets and medicine for HD patients [12–14]. In Japan, on the other hand, the Japanese Society of Dialysis Therapy operates an independent certification system called the “dialysis specialist accreditation program,” in which physicians must receive training in both internal medicine and nephrology for ≥5 years at authorized renal units and pass an exam to become dialysis specialists [15]. In South Korea, since there are no workforce requirements for HD unit operation, physicians other than dialysis specialists or board-certified nephrologists treat HD patients from place to place. Since the care given by qualified physicians may play an important role in the management of HD patients [5,16,17], we evaluated whether dialysis specialist care may affect patient mortality among Korean patients on maintenance HD.

Methods

Ethics statement

The present study was performed according to the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Review Board of Ewha Womans University Seoul Hospital in Seoul, Republic of Korea (No. SEUMC 2019-11-001), which waived the need for written informed consent because the study participants were deidentified.

Data source and study population

This study enrolled maintenance HD patients already on dialysis for end-stage renal disease. We used HD quality assessment data and Health Insurance Review and Assessment (HIRA) Service claims data collected from October to December 2015 to gather demographic and clinical data of individual HD patients. Adult HD patients aged ≥18 years who received HD treatment of ≥2 times weekly as outpatients were included in this assessment. Patients who were admitted during assessment or lost to follow-up were excluded from the analysis [12].

The HD quality assessment also collected data from each HD facility through a web-based data-collection system [18]. The collected data include 12 measures in three domains (structure, process, and monitoring) (Supplementary Table 1, available online). Structural information on medical staff members (doctors and nurses), number of HD treatments, numbers of HD equipment and emergency equipment, and status in the water quality test were
collected. In addition, information in procedural domains (vascular access stenosis monitoring and frequency of regular laboratory tests) and monitoring domains (frequency and satisfaction rate of HD adequacy and satisfaction rates of calcium and phosphorus control) were collected. The data retrieved from the web-based database were compared with those obtained from patient electronic medical records to check the accuracy and reliability. Demographic data, including age, sex, dialysis vintage, and comorbidities, were also collected. Body mass index and systolic and diastolic blood pressures before the HD session were also measured and reported. Laboratory assessments consisted of plasma hemoglobin, serum albumin, serum calcium and phosphorus, iron saturation, and serum ferritin.

The comorbidities of study participants were identified using the International Classification of Diseases-10 codes from the National Health Insurance Service claims database and searched from January to December 2015 [19,20]. Comorbidities included diabetes mellitus, hypertension, congestive heart failure, cerebrovascular disease, ischemic heart disease, and atrial fibrillation (Supplementary Table 2, available online).

In South Korea, there are two qualifications related to nephrology practice, which are nephrologist board-certification by the Korean Association of Internal Medicine and dialysis specialist certification by the Korean Society of Nephrology. Currently, there are 1,103 board-certified nephrologists and 1,407 certified dialysis specialists in South Korea (these groups overlap somewhat). To become a dialysis specialist, a doctor must complete 3 years of training in internal medicine plus 1 year of training in an authorized HD unit at a university hospital accredited by the Korean Society of Nephrology.

**Definition of study groups and outcomes**

The proportion of dialysis specialists was defined as the percentage of dialysis specialists among all doctors employed in each HD unit. This study included 35,441 patients treated at 799 facilities (Fig. 1). Among them, 30,855 patients (87.1%) were treated by >1 dialysis specialist and 4,586 patients (12.9%) were not treated by any dialysis specialist. Among the HD units with >1 dialysis specialist, there were 20 HD units with dialysis specialist proportions of <50%, and only 1,033 patients (2.9%) received HD in these units. Therefore, for the analysis of patient outcomes according to the proportion of dialysis specialists, we defined two study groups according to the proportion of dial-

**Figure 1. Flowchart of study participant selection.**
ysis specialists, including a no dialysis specialist care group (0%) and a dialysis specialist care group (≥50%).

The primary outcome was all-cause mortality. Mortality data were collected between January 2016 and June 2019. Patients who received a kidney transplant during the follow-up period were censored at the time of their kidney transplantation.

Statistical analyses

Baseline characteristics and outcomes were compared between groups. The groups were matched in a 1:3 ratio using propensity scores to minimize confounding factors that can affect the outcomes. The propensity score matching was performed based on age, sex, and the presence of diabetes mellitus before analysis using the “MatchIt” package of R version 4.0.2. (R Foundation for Statistical Computing). Normally distributed numerical variables were expressed using mean and standard deviation values, while the variables with skewed distributions were expressed with median and interquartile range values. Statistical comparisons between continuous variables were performed using an independent t test. For the data without normal distribution, the Wilcoxon signed-rank test for two groups was performed. The chi-square test and the Fisher exact test were applied to categorical variables, as appropriate.

The Kaplan-Meier method was used to compare death-free survival curves, and differences were assessed using the log-rank test. We used univariate and multivariate Cox proportional hazards models to estimate the risk factors associated with patient mortality. We tested the Schoenfeld residual using the "cox.zph" function of the R package and performed proportional hazards regression modeling by satisfying the proportional hazard ratio (HR) assumption. Model 1 was adjusted for age and sex. Model 2 was adjusted for medical comorbidities in addition to the factors included in model 1. Model 3 was adjusted for all the demographic and clinical parameters. Finally, subgroup analyses were performed to define the relative risk of mortality according to the absence of dialysis specialist care among predefined subgroups (age, <65 years vs. ≥65 years; sex, female vs. male; HD vintage, <5 years vs. ≥5 years; presence vs. absence of diabetes mellitus, ischemic heart disease, congestive heart failure, and cerebrovascular disease; plasma hemoglobin, ≥10 g/dL vs. <10 g/dL; serum albumin, ≥3.5 g/dL vs. <3.5 g/dL; and serum phosphorus, <5.0 mg/dL vs. ≥5.0 mg/dL). All statistical analyses were performed using R version 4.0.2. A p-value of <0.05 was considered statistically significant.

Results

Baseline characteristics according to dialysis specialist care grouping

Among 35,441 patients undergoing total HD in 2015, 1,033 patients with a dialysis specialist proportion of 0% to 50% of the dialysis unit were excluded (Fig. 1). Finally, a total of 34,408 HD patients from 779 HD centers were included in the analysis. The mean age was 60.0 ± 12.9 years, and 58.8% were male. Hypertension (81.4%) and diabetes mellitus (58.4%) were the two most common comorbidities. The ratio of the groups with and without dialysis specialist care was 86.7% (29,822 patients) to 13.3% (4,586 patients).

Baseline characteristics according to dialysis specialist care group enrollment are presented in Table 1. After propensity score matching, the number of patients in the dialysis specialist care group was 13,758 patients (86.7%) and the number of patients in the no dialysis specialist care group was 4,586 patients (13.3%) (Fig. 1), respectively. The patients in the dialysis specialist care group showed a shorter dialysis vintage and a lower proportion of comorbidities other than congestive heart failure compared to the no dialysis specialist care group (Table 1). The dialysis specialist care group also demonstrated higher levels of plasma hemoglobin (10.72 ± 0.83 g/dL vs. 10.61 ± 0.88 g/dL, p < 0.001), and single-pool Kt/V (1.56 ± 0.27 vs. 1.52 ± 0.28, p < 0.001). However, the dialysis specialist care group showed lower systolic (141.03 ± 15.32 mmHg vs. 143.05 ± 15.76 mmHg, p < 0.001) and diastolic (76.75 ± 9.59 mmHg vs. 79.69 ± 8.99 mmHg, p < 0.001) blood pressures and lower levels of serum calcium (8.95 ± 0.82 mg/dL vs. 9.08 ± 0.78 mg/dL, p < 0.001) and phosphorus (4.88 ± 1.31 mg/dL vs. 5.04 ± 1.39 mg/dL, p < 0.001) compared to the no dialysis specialist care group.

All-cause mortality according to dialysis specialist care grouping

A total of 7,445 deaths (21.6%) occurred during 36.2 ± 11.2
Table 1. Baseline characteristics according to the proportion of dialysis specialists

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Before PSM</th>
<th>After PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dialysis specialist care</td>
<td>No dialysis specialist care</td>
</tr>
<tr>
<td>No. of patients</td>
<td>29,822</td>
<td>4,586</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>59.82 ± 12.96</td>
<td>61.48 ± 12.20</td>
</tr>
<tr>
<td>Male sex</td>
<td>17,448 (58.5)</td>
<td>2,772 (60.4)</td>
</tr>
<tr>
<td>Dialysis vintage (yr)</td>
<td>5.63 ± 5.14</td>
<td>6.04 ± 5.33</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>4,104 (13.8)</td>
<td>542 (11.8)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>2,980 (10.0)</td>
<td>592 (12.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24,110 (80.9)</td>
<td>3,880 (84.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17,251 (57.9)</td>
<td>3,880 (84.6)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>9,373 (31.4)</td>
<td>1,628 (35.5)</td>
</tr>
<tr>
<td>Laboratory parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.72 ± 0.84</td>
<td>10.61 ± 0.88</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.99 ± 0.34</td>
<td>4.00 ± 0.35</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.93 ± 1.32</td>
<td>5.04 ± 1.39</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>8.97 ± 0.82</td>
<td>9.08 ± 0.78</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
<td>1.56 ± 0.28</td>
<td>1.52 ± 0.28</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.41 ± 3.42</td>
<td>22.12 ± 3.21</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>140.93 ± 15.46</td>
<td>143.05 ± 15.76</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.20 ± 9.63</td>
<td>79.69 ± 8.99</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>63.74 ± 14.28</td>
<td>63.36 ± 13.15</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%).
PSM, propensity score matching; SMD, standardized mean difference.

Table 2. Crude rate of all-cause mortality according to the proportion of dialysis specialists

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before PSM</th>
<th>After PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 34,408)</td>
<td>Dialysis specialist care (n = 29,822)</td>
</tr>
<tr>
<td>No. of deaths during follow-up</td>
<td>7,445</td>
<td>6,275</td>
</tr>
<tr>
<td>Total follow-up (person-yr)</td>
<td>103,825.9</td>
<td>90,196.4</td>
</tr>
<tr>
<td>Crude death rate (/1,000 person-yr)</td>
<td>71.7</td>
<td>69.6</td>
</tr>
<tr>
<td>Rate ratio</td>
<td>1.031</td>
<td>1.000</td>
</tr>
</tbody>
</table>

PSM, propensity score matching.

months. After censoring 2,006 cases (5.8%) who received kidney transplantation, the crude death rate was 71.7 per 1,000 person-years. After propensity score matching, a total of 4,314 patients died, and the crude death rate was 78.3 per 1,000 person-years. The dialysis specialist care group exhibited a lower risk of death than the no dialysis specialist care group, showing a crude death rate ratio of 1.132 (Table 2). In the survival analysis, the mortality rate was also lower in the dialysis specialist care group (log-rank test p < 0.001) (Fig. 2) both before and after propensity score matching.

In the univariate analysis for all-cause mortality, the no dialysis specialist care group was associated with a higher mortality risk (HR, 1.13; 95% confidence interval [CI], 1.16–1.21; p < 0.001). When we adjusted for age and sex (model 1), no dialysis specialist care group enrollment remained an independent risk factor for patient mortality (HR, 1.13; 95% CI, 1.16–1.21; p < 0.001). Similarly, in model
Figure 2. Kaplan-Meier analysis for survival (A) before PSM and (B) after PSM. The mortality rate was low in the dialysis specialist care group before and after PSM. PSM, propensity score matching.
2 and model 3, no dialysis specialist care group enrollment remained an independent risk factor for all-cause mortality (HR, 1.12; 95% CI, 1.05–1.20; p < 0.001 in model 2 and HR, 1.10; 95% CI, 1.03–1.18; p = 0.004 in model 3) (Table 3).

Table 3. Multivariate Cox analysis of death according to the absence of dialysis specialist care

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>1.13 (1.06–1.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.13 (1.06–1.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.12 (1.05–1.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.10 (1.03–1.18)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and sex; model 2: model 1 + hypertension, diabetes mellitus, ischemic heart disease, congestive heart disease, and cerebrovascular disease; model 3: model 2 + hemoglobin, albumin, calcium, and phosphorus.

Subgroup analysis for all-cause mortality in the no dialysis specialist care group

We performed a subgroup analysis to define the population at high risk for all-cause death in the absence of dialysis specialist care. The no dialysis specialist care group showed higher mortality among patients without ischemic heart disease (HR, 1.18; 95% CI, 1.08–1.28; p < 0.001), congestive heart failure (HR, 1.15; 95% CI, 1.07–1.24; p < 0.001), or cerebrovascular disease (HR, 1.17; 95% CI, 1.08–1.26; p < 0.001) (Fig. 3). In addition, enrollment in this group was an independent risk factor for increased mortality in patients without anemia (HR, 1.15; 95% CI, 1.07–1.24; p < 0.001), hypoalbuminemia (HR, 1.17; 95% CI, 1.09–1.26; p < 0.001), or hypophosphatemia (HR, 1.18; 95% CI, 1.08–1.28; p < 0.001). However, there was no difference according to age, sex, or

Figure 3. Subgroup analysis after propensity score matching. Dialysis specialist care was associated with a reduction in mortality in patients regardless of age, sex, or the presence of diabetes. However, dialysis specialist care was associated with decreased mortality among patients with a short dialysis vintage; those without ischemic heart disease, congestive heart failure, or cerebrovascular disease; and those without anemia, hypoalbuminemia, or hypophosphatemia.

CI, confidence interval; HR, hazard ratio.
diabetes subgroup.

**Discussion**

In this article, we evaluated the effect of dialysis specialist care on patient mortality using nationwide HD quality assessment data from South Korea. The absence of dialysis specialists in HD facilities was an independent risk factor for all-cause mortality even after adjusting for demographic and clinical parameters. Moreover, patients in the dialysis specialist care group showed higher plasma hemoglobin concentrations, lower blood pressures, and lower serum phosphorus levels than those in the no dialysis specialist care group.

In the United States, there are several types of personnel in HD facilities, such as a medical director, charge nurses and registered nurses, patient care technicians, water treatment system technicians, dietitians, and social workers [21]. Since there are various HD-specific complications and technical problems, the special care given by nephrologists or dialysis specialists may be essential to improve patient outcomes [22]. However, a shortage of both nephrologists and patient care technicians in the American workforce has persisted, which may constitute a barrier to optimal dialysis care [23]. A recent article speculated that the higher mortality among HD patients in America compared to other countries may be due to looser requirements for mandated physicians in HD facilities [24]. On the other hand, Japan has strict rules about medical staffing in HD facilities, with either nephrologists or dialysis specialists required. A previous article by Furumatsu et al. [15] demonstrated that prefectures with higher quintiles of dialysis specialists showed better long-term survival rates among HD patients. However, this result has not been confirmed at either the individual or facility level.

To our knowledge, our study is the first to demonstrate the importance of specialized care by nephrologists in HD units for improving the overall patient mortality rate in South Korea. In a previous study by Slinin et al. [3], 6.9% of HD patients did not receive specialized care from a doctor with a specialty in nephrology. In contrast to the previous study, the number of patients who did not receive specialized care was 12.9% in our study, which was a larger proportion than that from the previous paper. Therefore, the difference in the distribution of patients likely had a significant impact on the prognosis of HD patients in this study. In addition, our results showed that patients treated in HD units with dialysis specialist care demonstrated fewer HD-related complications like anemia, uncontrolled blood pressure, and mineral bone disorders. A previous study also mentioned that more frequent patient-nephrologist contact resulted in the greater achievement of clinical targets for albumin, calcium-phosphate product, dialysis dose, and hemoglobin [25]. Dialysis specialists are the key professionals in the delivery of dialysis therapy and therefore may manage HD-related complications better than non-nephrologists. On the other hand, subgroup analyses demonstrated that dialysis specialist care was more effective among HD patients without cardiovascular morbidities or other complications. This may be due to the high mortality rates among groups with comorbidities and complications that dialysis specialist care may not be able to overcome to improve patient survival.

The strengths of our study include its large sample size, relatively long duration of follow-up, and analysis of well-balanced groups with propensity score matching. However, this study also has several limitations. First, we did not analyze cause-specific mortality among HD patients according to dialysis specialist care since the nationwide database does not include details on the cause of morbidity, such as cardiovascular disease, infection, vascular access problems, or malnutrition. Second, hospitalized patients at the time of HD quality assessment were excluded from this study. Third, we did not include patients in HD units with a proportion of dialysis specialists between 0% and 50%. Although the number of patients in HD units within this bracket is low (1,033 patients [2.9%] in 20 HD units), there could be a graded risk of patient mortality according to the proportion of dialysis specialists. Fourth, we did not include information about vascular access or insurance type among HD patients in this study, and there were also no data on prescription patterns or HD modalities in HD quality assessment. Fifth, an analysis of the other components of HD quality assessment according to the proportion of dialysis specialists could not be performed. Further studies are needed to reveal the mechanisms by which dialysis specialist care influences outcomes and to determine the appropriate proportion of dialysis specialists to improve the quality of care and patient prognosis.

In conclusion, we found that mortality was lower among
patients with dialysis specialist care than those without dialysis specialist care. This suggests a link between dialysis specialist care and patient outcomes in HD facilities. Thus, the presence of dialysis specialists at HD facilities may improve outcomes in patients undergoing HD.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

The data that support the findings of this study are available from HIRA, but restrictions apply to the availability of these data, so they are not publicly available. Data are, however, available from the authors upon request and with permission from HIRA.

**Authors’ contributions**

Conceptualization: DHK, YKL
Investigation: YEK, DRR, KHY, JHS, EJS
Data curation: DHK, HCP, AC, YKL
Formal analysis: DHK, JK
Funding acquisition: HCP
Writing-original draft: DHK, HCP
Writing-review & editing: YKL
All authors read and approved the final manuscript.

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**References**

remote compared to rural or urban dwelling hemodialysis patients in the United States. *Kidney Int* 2012;82:352–359.


A non-muscle myosin heavy chain 9 genetic variant is associated with graft failure following kidney transplantation

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Background: Despite current matching efforts to identify optimal donor-recipient pairs for kidney transplantation, alloimmunity remains a major source of late transplant failure. Additional genetic parameters in donor-recipient matching could help improve long-term outcomes. Here, we studied the impact of a non-muscle myosin heavy chain 9 gene (MYH9) polymorphism on allograft failure.

Methods: We conducted an observational cohort study, analyzing the DNA of 1,271 kidney donor-recipient transplant pairs from a single academic hospital for the MYH9 rs11089788 C>A polymorphism. The associations of the MYH9 genotype with risk of graft failure, biopsy-proven acute rejection (BPAR), and delayed graft function (DGF) were estimated.

Results: A trend was seen in the association between the MYH9 polymorphism in the recipient and graft failure (recessive model, p = 0.056), but not for the MYH9 polymorphism in the donor. The AA-genotype MYH9 polymorphism in recipients was associated with higher risk of DGF (p = 0.03) and BPAR (p = 0.021), although significance was lost after adjusting for covariates (p = 0.15 and p = 0.10, respectively). The combined presence of the MYH9 polymorphism in donor-recipient pairs was associated with poor long-term kidney allograft survival (p = 0.04), in which recipients with an AA genotype receiving a graft with an AA genotype had the worst outcomes. After adjustment, this combined genotype remained significantly associated with 15-year death-censored kidney graft survival (hazard ratio, 1.68; 95% confidence interval, 1.05–2.70; p = 0.03).

Conclusion: Our results reveal that recipients with an AA-genotype MYH9 polymorphism receiving a donor kidney with an AA genotype have significantly elevated risk of graft failure after kidney transplantation.

Keywords: Genetics, Kidney transplantation, Molecular motor proteins, Nephrology

Introduction

Despite the excellent short-term outcomes following solid organ transplantation, the long-term survival of kidney transplants has improved only negligibly in recent years [1]. Consequently, one out of five patients on the waitlist...
for kidney transplantation are candidates whose previous
grafts failed [2]. Maximizing the long-term outcomes of
transplantation and preventing retransplantation is para-
mount—not only for improving transplant recipients’ out-
comes but also for reducing waitlist pressure. Alloimmuni-
ty, otherwise known as host anti-donor immune responses,
remains the preeminent driver of late graft loss, despite
strong efforts to optimally match donor-recipient pairs [3,4].
Recently, there are signs of a paradigm shift in the trans-
plant field, with suggestions that allograft matching efforts
should be updated to include novel genetic markers that
better ensure long-term graft survival after kidney trans-
plantation [5,6].

In this regard, non-muscle myosin heavy chain II-A (MHCII-A), encoded by the myosin heavy chain 9 gene (MYH9), is a target of particular interest (Fig. 1A). Non-muscle MHCII-A is a ubiquitously expressed contractile protein involved in myriad processes ranging from cell division and adhesion to providing cytoskeletal support [7]. Mutations in the MYH9 cause a complex set of disorders, known as MYH9-related diseases, that can affect every system in the body but are characterized by congenital thrombocytopenia, giant platelets and leucocyte inclusions [7]. Although non-muscle MHCII-A is expressed by a variety of cell types, the podocyte lineage in particular expresses high levels of this protein [7]. Unsurprisingly, patients with MYH9-related disorders can clinically present with persistent proteinuria and a progressive decline in kidney function leading to end-stage kidney disease (ESKD) [7,8]. Subsequent studies linked common MYH9 polymorphisms to an increased risk of developing focal segmental glomerulosclerosis and non-diabetic ESKD [9,10]. However, it is worth noting that these associations were later shown to be dependent on strong linkage disequilibrium of these MYH9 polymorphisms with variants in the apolipoprotein L1 gene (APOL1) [7,11]. Still, there are studies that show an association between MYH9 polymorphisms and chronic kidney disease (CKD) independently of linkage with APOL1, suggesting a potential role for MYH9 polymorphisms in the pathogenesis of ESKD [12,13].

In a recent genome-wide linkage analysis, a significant association between the MYH9 rs11089788 polymorphism and kidney function was identified in a meta-analysis of three European populations [14]. This MYH9 polymorphism was additionally found to be significantly associated with progressive loss of kidney function in other cohorts [13,15]. Importantly, the associations between MYH9 rs11089788 and kidney function could not be explained by linkage disequilibrium with APOL1 [15].

Here, we investigated the impact of the recently discov-
ered rs11089788 MYH9 polymorphism on long-term graft
survival in the context of kidney transplantation (Fig. 1B).
As a secondary outcome, we also assessed the association of this polymorphism with biopsy-proven acute rejection (BPAR) and delayed graft function (DGF).

**Figure 1. Illustration of the non-muscle MHCII-A and the examined MYH9 polymorphisms.** (A) Non-muscle MHCII-A is a contractile protein comprised of several domains: A globular motor head portion (heavy chain), a neck domain (essential light chain and regulatory light chain), coiled coil tail segment (MHCII-A), and non-helical tailpiece that can be phosphorylated. The coiled coil segment is notably encoded by the MYH9. (B) In this study, we assessed the association of rs11089788 (C>A) MYH9 single-nucleotide polymorphism (SNPs) in kidney allograft donors and recipients with long-term graft survival outcomes. MHCII-A, myosin heavy chain II-A; MYH9, myosin heavy chain 9 gene.
**Methods**

**Patient selection and study endpoint**

Patients receiving a single kidney transplantation at the University Medical Center Groningen in the Netherlands were recruited between March 1993 and February 2008. A total of 1,271 of the 1,430 screened donor-recipient kidney transplant pairs were included in this study as previously reported [16–22]. Reasons for patient exclusion were technical complications during surgery, lack of DNA, loss of follow-up, retransplantation at recruitment, and simultaneous pancreas and kidney transplantation or combined liver and kidney transplantation. The primary endpoint of this study was long-term death-censored graft survival and the maximum follow-up period was 15 years. Graft failure was defined as the need for dialysis or retransplantation. Secondary endpoints included occurrence of DGF (described by the United Network for Organ Sharing as, “The need for at least one dialysis treatment in the first week after kidney transplantation.”) and BPAR (based on the Banff ’07 classification).

Ethical approval for this study and the study protocol was given by the Institutional Review Board of the University Medical Center Groningen in Groningen, the Netherlands (Medical Ethical Committee 2014/077). The study protocol adhered to the Declaration of Helsinki. All subjects provided written informed consent.

**DNA extraction and MYH9 genotyping**

Peripheral blood mononuclear cells from blood or splenocytes were obtained from both the donor and recipient. DNA isolation was done with a commercial kit according to the manufacturer’s instructions and stored at −80 °C. Genotyping of the single-nucleotide polymorphism (SNP) was performed using the Illumina VeraCode GoldenGate Assay kit as per the manufacturer’s instructions (Illumina). We opted for the MYH9 rs11089788 C>A SNP, which has previously been associated with kidney function in healthy individuals and with disease progression in patients with CKD [13–15]. Genotype clustering and calling were performed using BeadStudio Software (Illumina). The overall genotype success rate was 99.9%, and only two samples were excluded from subsequent analyses because of a missing call rate.

**Statistical analyses**

IBM SPSS version 25 (IBM Corp.) was used for statistical analyses. Data are presented as the total number of patients with percentage for nominal variables, mean ± standard deviation for parametric variables, and median (interquartile range) for nonparametric variables. Differences among groups were tested with the chi-square test for categorical variables or Student t test for normally distributed variables, and the Mann-Whitney U test for not-normally divided variables, respectively. The log-rank test was used to identify differences in kidney allograft survival or rejection-free survival among the different genotypes. Logistic regression was used to assess the association of the MYH9 polymorphism with DGF. Univariable analyses were used to examine the associations of the MYH9 polymorphism, recipient, donor, and transplant characteristics with BPAR and death-censored graft survival. Significant associations in univariable analyses were then assessed in a multivariable Cox regression. Two-tailed tests were regarded as significant at p < 0.05.

**Results**

**Study population and determinants of graft failure**

All patients who underwent a single kidney transplantation at the University Medical Center Groningen were recruited for this study (n = 1,271). Baseline patient characteristics are shown in Table 1. In our cohort, there was only one case of an ABO-incompatible kidney transplantation. During the mean study period of 6.2 ± 4.2 years, 215 of 1,271 kidney transplant recipients (16.9%) developed graft failure. The main reason for graft failure was rejection (n = 126; containing acute rejection, transplant glomerulopathy, and chronic antibody-mediated rejection). Other causes for graft loss were surgical complications (n = 33), relapse of original kidney disease (n = 16), other causes (n = 16), vascular disease (n = 12), and unknown causes (n = 12). In univariable analyses, DGF, recipient age, recipient blood type (AB vs. others), donor type (living vs. cadaveric), donor age, donor blood type (AB vs. others), cold ischemia time, warm ischemia time, use of cyclosporin, and use of corticoste-
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 1,271)</th>
<th>Functioning graft (n = 1,056)</th>
<th>Graft loss (n = 215)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hazard ratio</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
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<td><strong>Donor</strong></td>
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<tr>
<td>MYH9 SNP</td>
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<td>264 (25.0)</td>
<td>53 (24.8)</td>
<td>0.97</td>
<td>0.98</td>
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<tr>
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<td>572 (54.2)</td>
<td>115 (53.7)</td>
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<tr>
<td>CA</td>
<td>265 (20.9)</td>
<td>219 (20.8)</td>
<td>46 (21.5)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>44.4 ± 14.4</td>
<td>44.1 ± 14.6</td>
<td>46.1 ± 13.4</td>
<td>0.04*</td>
<td>1.02</td>
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</tr>
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<td>Male sex</td>
<td>645 (50.7)</td>
<td>535 (50.7)</td>
<td>110 (51.2)</td>
<td>0.89</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td><strong>Blood group</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Type O</td>
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<td>541 (51.3)</td>
<td>101 (47.2)</td>
<td>0.03*</td>
<td>0.39</td>
<td>0.004*</td>
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<td>0.42</td>
<td>0.42</td>
<td>0.01*</td>
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<td>97 (7.6)</td>
<td>82 (7.8)</td>
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<td>0.36</td>
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<tr>
<td>Living</td>
<td>282 (22.2)</td>
<td>257 (24.3)</td>
<td>25 (11.6)</td>
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<td>Brain death</td>
<td>787 (61.9)</td>
<td>642 (60.8)</td>
<td>145 (67.4)</td>
<td>1.94</td>
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<tr>
<td>Circulatory death</td>
<td>202 (15.9)</td>
<td>157 (14.9)</td>
<td>45 (20.9)</td>
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<tr>
<td><strong>Recipient</strong></td>
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<td>326 (25.7)</td>
<td>270 (25.6)</td>
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<td>CC</td>
<td>635 (50.0)</td>
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<tr>
<td>Age (yr)</td>
<td>47.9 ± 13.5</td>
<td>48.5 ± 13.4</td>
<td>45.0 ± 13.2</td>
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<td>Male sex</td>
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<td>607 (57.5)</td>
<td>132 (61.4)</td>
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<tr>
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<td>340 (26.8)</td>
<td>271 (25.6)</td>
<td>69 (32.2)</td>
<td>0.28</td>
<td>0.45</td>
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<td>Polycystic disease</td>
<td>208 (16.4)</td>
<td>188 (17.8)</td>
<td>20 (9.3)</td>
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<td>Vascular disease</td>
<td>145 (11.4)</td>
<td>123 (11.6)</td>
<td>22 (10.3)</td>
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<td>Pyelonephritis</td>
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<td>120 (11.4)</td>
<td>28 (13.1)</td>
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<td>Diabetes</td>
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<td>44 (4.2)</td>
<td>7 (3.3)</td>
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<tr>
<td>Idiopathic</td>
<td>168 (13.2)</td>
<td>134 (12.7)</td>
<td>34 (15.9)</td>
<td></td>
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<tr>
<td>Others</td>
<td>211 (16.6)</td>
<td>177 (16.7)</td>
<td>34 (15.9)</td>
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<tr>
<td><strong>Blood group</strong></td>
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<tr>
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<td>474 (44.9)</td>
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<td>0.46</td>
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<td>0.46</td>
<td>0.46</td>
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<td>Type B</td>
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<td>0.35</td>
<td>0.002*</td>
</tr>
<tr>
<td>Type AB</td>
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<td>0.008*</td>
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<td>Dialysis vintage (wk)</td>
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<td>174 (87–261)</td>
<td>168 (109–270)</td>
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<td>Highest PRA (%)</td>
<td>10.1 ± 23.6</td>
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<td>10.9 ± 25.0</td>
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<td>0.75</td>
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<td>1,131 (89.0)</td>
<td>945 (89.5)</td>
<td>186 (86.5)</td>
<td>0.20</td>
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<td>Induction immunosuppression</td>
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<td>Anti-CD3 MoAb</td>
<td>19 (1.5)</td>
<td>14 (1.3)</td>
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<td>0.27</td>
<td>0.51</td>
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<td>ATG</td>
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<td>79 (7.5)</td>
<td>24 (11.2)</td>
<td>0.07</td>
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<td>Interleukin-2 RA</td>
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<td>163 (15.4)</td>
<td>36 (16.7)</td>
<td>0.63</td>
<td>0.12</td>
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Table 1. Continued

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<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 1,271)</th>
<th>Functioning graft (n = 1,056)</th>
<th>Graft loss (n = 215)</th>
<th>p-value*</th>
<th>Hazard ratio</th>
<th>p-value*</th>
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<td>Maintenance immunosuppression</td>
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<td>Azathioprine</td>
<td>72 (5.7)</td>
<td>53 (5.0)</td>
<td>19 (8.8)</td>
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<td>Corticosteroids</td>
<td>1,201 (94.5)</td>
<td>1,002 (94.9)</td>
<td>199 (92.6)</td>
<td>0.17</td>
<td>0.51</td>
<td>0.01*</td>
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<td>Cyclosporin</td>
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<td>911 (86.3)</td>
<td>174 (80.9)</td>
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<td>0.66</td>
<td>0.02*</td>
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<td>Mycophenolic acid</td>
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<td>775 (73.4)</td>
<td>132 (61.4)</td>
<td>&lt;0.001*</td>
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<td>Sirolimus</td>
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<td>Tacrolimus</td>
<td>97 (7.6)</td>
<td>77 (7.3)</td>
<td>20 (9.3)</td>
<td>0.31</td>
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<tr>
<td>Transplantation</td>
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<tr>
<td>CIT (hr)</td>
<td>17.7 (10.9–23.0)</td>
<td>17.0 (8.6–23.0)</td>
<td>20.0 (15.3–25.0)</td>
<td>&lt;0.001*</td>
<td>1.03</td>
<td>0.001*</td>
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<td>WIT (min)</td>
<td>37.0 (31–45)</td>
<td>37.0 (30–45)</td>
<td>38.0 (32–45)</td>
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<td>Total HLA mismatches</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>0.48</td>
<td>0.11</td>
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<td>DGF</td>
<td>415 (32.7)</td>
<td>289 (27.4)</td>
<td>126 (58.6)</td>
<td>&lt;0.001*</td>
<td>3.79</td>
<td>&lt;0.001*</td>
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</table>

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

ATG, anti-thymocyte globulin; CD3, cluster of differentiation 3; CIT, cold ischemia time; DGF, delayed graft function; MYH9, myosin heavy chain 9 gene; HLA, human leukocyte antigen; PRA, panel-reactive antibody; RA, receptor antagonist; SNP, single-nucleotide polymorphism; WIT, warm ischemia time.

*p-value for the differences in baseline characteristics between the groups, tested by Student t test or the Mann-Whitney U test for continuous variables, with the chi-square test for categorical variables; **p-value for univariable analysis with 15-year death-censored graft survival.

*p < 0.05, statistically significant.

Zones were all associated with graft failure (p < 0.05).

Distribution of the MYH9 polymorphism

The observed genotypic frequencies of the MYH9 SNP (rs11089788 C>A) did not differ between donors (n = 1,269; CC, 25.0%; CA, 54.1%; AA, 20.9%) and recipients (n = 1,269; CC, 25.7%; CA, 50.0%; AA, 24.3%; p = 0.07). The distribution of the SNP was in Hardy-Weinberg equilibrium. Compared with the 1000 Genomes Project, the genotypic frequencies of the MYH9 polymorphism in recipients and donors were significantly different (p < 0.001) [23]. In both recipients and donors, the A-allele of the MYH9 SNP was more prevalent than the reported allele and genotype frequencies in the 1000 Genomes Project. The percentage of kidney allografts with DGF significantly differed based on the recipient MYH9 genotype (33.7% in CC, 29.6% in CA, 37.7% in AA; p = 0.04), but not for the donor MYH9 genotype (p = 0.93). For further analysis, heterozygotes (CA) and homozygotes (CC) genotypes were combined into one group (CA/CC). In logistic regression, recipients carrying the AA-genotype MYH9 polymorphism had a significantly elevated risk of DGF (odds ratio [OR], 1.34) compared to CA/CC-genotype recipients (95% confidence interval [CI], 1.03–1.76; p = 0.03). In multivariable logistic regression, the AA genotype of the MYH9 polymorphism in recipients was no longer significantly associated with DGF occurrence (OR, 1.26) compared with CA/CC-genotype recipients (95% CI, 0.92–1.72; p = 0.15) (Table 2). There was no difference in the overall BPAR frequency among the MYH9 genotypes in the donors (34.7% in CC, 33.0% in CA, 35.8% in AA; p = 0.69). In contrast, the distribution of the MYH9 polymorphism in recipients showed a trend toward higher risk of BPAR (31.6% in CC, 32.4% in CA, 39.3% in AA; p = 0.07) (Fig. 2A). A significant association was found with BPAR when the AA genotype of the MYH9 polymorphism in the recipient was compared to CA and CC genotypes (39.3% in AA vs. 32.2% in CA/CC; p = 0.02) (Fig. 2B). In multivariable Cox regression, the AA genotype of the MYH9 polymorphism in recipients was no longer significantly associated with BPAR occurrence (hazard ratio [HR], 1.22) compared with CA/CC-genotype recipients (95% CI, 0.97–1.54; p = 0.10) (Table 3). In summary, although the AA-genotype MYH9 polymorphism in recipients was associated with DGF and BPAR, the significance was lost when correcting for potential confounders.
Table 2. Logistic regression analysis for the risk of delayed graft function

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH9 rs111089788 SNP in the recipient (AA vs. CA/CC)</td>
<td>0.15</td>
<td>1.26 (0.92–1.72)</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>&lt;0.001</td>
<td>1.02 (1.01–1.03)</td>
</tr>
<tr>
<td>Donor sex (male vs. female)</td>
<td>0.001</td>
<td>1.61 (1.22–2.13)</td>
</tr>
<tr>
<td>Donor type (deceased vs. living)</td>
<td>0.001</td>
<td>31.61 (4.14–214.57)</td>
</tr>
<tr>
<td>Total HLA mismatches</td>
<td>0.006</td>
<td>1.16 (1.04–1.30)</td>
</tr>
<tr>
<td>Dialysis vintage (wk)</td>
<td>0.007</td>
<td>1.08 (1.02–1.14)</td>
</tr>
<tr>
<td>Warm ischemia time (min)</td>
<td>0.02</td>
<td>1.01 (1.00–1.03)</td>
</tr>
<tr>
<td>Recipient age (yr)</td>
<td>0.44</td>
<td>1.00 (0.99–1.02)</td>
</tr>
<tr>
<td>Cold ischemia time (hr)</td>
<td>0.47</td>
<td>1.00 (1.00–1.00)</td>
</tr>
</tbody>
</table>

Multivariable logistic regression was performed for delayed graft function after kidney transplantation. Only variables with a p-value of <0.05 in the univariate analysis were included. Donor age, donor sex, donor type, total HLA mismatches, dialysis vintage, and warm ischemia time were significant, whereas the MYH9 SNP (rs11089788) in the recipient, recipient age, and cold ischemia time were not.

CI, confidence interval; HLA, human leukocyte antigen; MYH9, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.

Figure 2. Kaplan-Meier curves for rejection-free survival of kidney allografts according to the presence of a non-muscle MYH9 polymorphism in the recipient. (A) Cumulative rejection-free survival of kidney allografts according to the presence of the MYH9 single-nucleotide polymorphism (SNP) rs11089788 in the recipient. (B) Cumulative rejection-free survival of kidney allografts in recipients with the AA genotype of the MYH9 SNP rs11089788 vs. the AC/CC genotype. Log-rank test was used to compare the incidence of biopsy-proven rejection between the groups.

MYH9, myosin heavy chain 9 gene.

Long-term kidney graft survival based on the MYH9 genotypes

Kaplan-Meier survival analysis showed no association between the MYH9 SNP in the recipient or the donor and death-censored kidney graft survival (Fig. 3). However, a trend was seen for a heightened rate of graft failure in recipients with an AA genotype of the MYH9 polymorphism compared with CA- and CC-genotype recipients (graft loss, 33.2% in AA vs. 24.1% in CA/CC; p = 0.06) (Fig. 3B). Next, donor-recipient pairs were separated into four groups according to the presence or absence of the AA genotype.
Table 3. Multivariable analysis for the risk of biopsy-proven acute rejection

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH9 rs11089788 SNP in the recipient (AA vs. CA/CC)</td>
<td>0.10</td>
<td>1.22 (0.97–1.54)</td>
</tr>
<tr>
<td>Recipient age (yr)</td>
<td>&lt;0.001</td>
<td>0.97 (0.97–0.98)</td>
</tr>
<tr>
<td>Total HLA mismatches</td>
<td>&lt;0.001</td>
<td>1.20 (1.11–1.29)</td>
</tr>
<tr>
<td>Delayed graft function (yes vs. no)</td>
<td>0.02</td>
<td>1.31 (1.05–1.62)</td>
</tr>
<tr>
<td>Recipient sex (female vs. male)</td>
<td>0.04</td>
<td>1.25 (1.01–1.55)</td>
</tr>
<tr>
<td>Warm ischemia time (min)</td>
<td>0.08</td>
<td>0.99 (0.98–1.00)</td>
</tr>
</tbody>
</table>

Multivariable Cox regression was performed for biopsy-proven acute rejection after kidney transplantation. Only variables with a p < 0.05 in the univariable analysis were included. Recipient age, total HLA mismatches, delayed graft function, and recipient sex were significant, whereas the MYH9 polymorphism (rs11089788) in the recipient and warm ischemia time were not.

CI, confidence interval; HLA, human leukocyte antigen; MYH9, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.

Figure 3. Kaplan-Meier curves for 15-year death-censored kidney graft survival according to the presence of a non-muscle MYH9 polymorphism in the donor or recipient. (A) Cumulative 15-year death-censored kidney graft survival according to the presence of a genetic variant in non-muscle MYH9 (rs11089788 C>A) in the recipient (blue line in the A and B panels) or the donor (yellow line in the C panel). (B) Cumulative 15-year death-censored graft survival of kidney allografts in recipients with the AA genotype of the MYH9 single-nucleotide polymorphism (SNP) rs11089788 vs. the AC/CC genotype. Log-rank test was used to compare the incidence of graft loss between the groups. MYH9, myosin heavy chain 9 gene.
of the MYH9 polymorphism in the donor and recipient. Kaplan-Meier survival analyses showed a significant difference in graft failure rates among the four groups (p = 0.04) (Fig. 4A). Intriguingly, the AA genotype of the MYH9 polymorphism in the donor seemed to have a marginal positive impact on graft survival, whereas the AA genotype in the recipient had a modest detrimental impact compared with donor-recipient pairs with the combined CC/CA genotype. Recipients with an AA genotype receiving a graft with an AA genotype had the worst outcomes. This combined genotype was identified in 6.3% of the donor-recipient pairs. Moreover, the significant association with graft failure increased when the combined AA genotype of the MYH9 polymorphism in donor-recipient pairs was compared with other groups (p = 0.01) (Fig. 4B). The cumulative 15-year death-censored kidney allograft survival was 50.4% in this combined AA-genotype group and 74.9% in the reference group. The association of the combined MYH9 AA-genotype group with long-term graft survival was maintained when primary non-function cases were excluded (p = 0.001) (Supplementary Fig. 1, available online), demonstrating that the association between the MYH9 rs11089788 polymorphism and graft failure is independent of early graft failure. These data suggest that matching donor-recipient pairs on the MYH9 polymorphism may impact long-term graft survival in kidney transplantation.

Kaplan-Meier survival analyses for the combined AA genotype of the MYH9 polymorphism in donor-recipient pairs were reestimated for patients transplanted in the 1990s and 2000s because immunosuppression has improved through time, and this could influence the risk of graft loss. In these subgroups, the significance was maintained in patients transplanted after 2000 (p = 0.04) (Supplementary Fig. 2, available online), while a trend

Figure 4. Kaplan-Meier curves for 15-year death-censored kidney graft survival according to the presence of a non-muscle MYH9 polymorphism in donor-recipient pairs. Cumulative 15-year death-censored kidney graft survival is shown according to the presence of the MYH9 polymorphism in donor-recipient pairs. (A) Pairs were divided into four groups according to the absence (black line) or presence of the AA genotype in the recipient (blue line), donor (yellow line), or both (green line). (B) The presence of the AA genotype in both the recipient and donor (green line) was compared to the rest (black line). Log-rank test was used to compare the incidence of graft loss between the groups. MYH9, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.
was seen in patients transplanted before 2001 (p = 0.10) (Supplementary Fig. 2, available online). Nevertheless, in accordance with our previous results, the combined AA genotype of the MYH9 polymorphism in donor-recipient pairs remained harmful for long-term graft survival.

Regression analysis for the MYH9 polymorphism in donor-recipient pairs and graft failure

Finally, we investigated whether the MYH9 variant in donor-recipient pairs is an independent risk factor for graft failure. In univariable analysis, the combined AA genotype of the MYH9 SNP in donor-recipient pairs was associated with a hazard ratio of 1.78 (95% CI, 1.13–2.79; p = 0.01) for graft failure after complete follow-up. We then determined whether the baseline characteristics differed between the donor-recipient pairs with the combined AA genotype of the MYH9 SNP and those with other MYH9 genotypes (Table 4). The proportion of living donor kidney transplants was significantly higher in the combined AA-genotype group (p = 0.001), and linked to this finding, the median cold ischemia time was significantly lower for donor-recipieent pairs with the combined AA-genotype group (p = 0.002). Furthermore, the total number of human leukocyte antigen (HLA) mismatches was significantly higher in the combined AA-genotype group (p = 0.004). However, the total number of HLA mismatches was not significantly associated with graft loss in univariable analysis (p = 0.11) (Table 1). Furthermore, when we adjusted for HLA mismatches, the hazard ratio and significance increased for the association between the combined AA genotype of the MYH9 SNP in donor-recipient pairs and graft loss (HR, 2.02; 95% CI, 1.26–3.26; p = 0.004). Also, the total number of HLA mismatches was not statistically different between patients who experienced graft failure in the combined AA-genotype group compared with those with graft failure in the other group (p = 0.37) (Supplementary Table 1, available online). Hence, although a difference was detected in the total number of HLA mismatches between the combined AA-genotype group and the other genotypes group, it seems unlikely that this is a confounder given the association between the MYH9 genotype and allograft outcome.

Next, multivariable analysis was performed to adjust for other potential confounders, including donor and patient characteristics as well as transplant variables (Table 5). In these Cox regression analyses, the combined AA genotype of the MYH9 SNP in donor-recipient pairs remained significantly associated with graft failure. We also performed a multivariable Cox regression analysis using all variables that were significantly associated with graft failure in univariable analysis (Table 6). In this model, the MYH9 SNP (rs11089788) in donor-recipient pairs, DGF occurrence, recipient age, and donor age were all significantly associated with graft loss. After adjustment, the hazard ratio for graft failure of the combined AA genotype for the MYH9 SNP in donor-recipient pairs was 1.68 (95% CI, 1.05–2.70; p = 0.03). Our results reveal that recipients with an AA genotype of the MYH9 SNP receiving a kidney allograft with an AA genotype have a significantly elevated risk of graft failure after kidney transplantation.

Finally, we analyzed the causes of allograft failure among the different groups to uncover the potential mechanism by which the combined MYH9 AA genotype lowers long-term allograft survival. We did not, however, find any major differences in the causes of graft loss between the donor-recipient pairs with the combined AA genotype and those with other MYH9 genotypes (Supplementary Table 2, available online). Additionally, there was no significant difference in the percentage of rejection-related graft loss between the two groups (71.4% in the combined AA genotype vs. 60.1% in the other genotypes; p = 0.31).

Discussion

A multitude of strategies can be pursued to improve long-term outcomes after kidney transplantation, ranging from the development of novel drugs that can halt alloimmune cascades, to the refinement of donor-recipient matching systems to minimize the severity of allograft recognition. Regarding allograft matching, HLA-centric systems remain the cornerstone of allocating kidney allografts, although a paradigm shift in the approach to donor-recipient matching is urgently needed [24]. Genetic analyses in transplantation provides a particularly unique opportunity for the development of innovative strategies that can improve donor-recipient pairing and drive personalized medicine, in part by enabling individualized risk stratification [25,26]. Presently, we report the impact of a recently discovered polymorphism in MYH9 on long-term kidney allograft survival. The key finding of our study is that recipients with an
### Table 4. Baseline characteristics of donor-recipient pairs based on their MYH9 genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 1,271)</th>
<th>AA–AA pair (n = 80)</th>
<th>Other pairs (n = 1,187)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>44.4 ± 14.4</td>
<td>46.8 ± 12.9</td>
<td>44.2 ± 14.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Male sex</td>
<td>645 (50.7)</td>
<td>43 (53.8)</td>
<td>601 (50.6)</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Blood group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type O</td>
<td>642 (50.5)</td>
<td>40 (50.0)</td>
<td>600 (50.7)</td>
<td>0.93</td>
</tr>
<tr>
<td>Type A</td>
<td>502 (39.5)</td>
<td>32 (40.0)</td>
<td>469 (39.6)</td>
<td></td>
</tr>
<tr>
<td>Type B</td>
<td>97 (7.6)</td>
<td>7 (8.8)</td>
<td>89 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Type AB</td>
<td>27 (2.1)</td>
<td>1 (1.3)</td>
<td>26 (2.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Donor type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living</td>
<td>282 (22.2)</td>
<td>30 (37.5)</td>
<td>251 (21.1)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Brain death</td>
<td>787 (61.9)</td>
<td>35 (43.8)</td>
<td>749 (63.1)</td>
<td></td>
</tr>
<tr>
<td>Circulatory death</td>
<td>202 (15.9)</td>
<td>15 (18.8)</td>
<td>187 (15.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Recipient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>47.9 ± 13.5</td>
<td>48.1 ± 13.1</td>
<td>47.9 ± 13.5</td>
<td>0.91</td>
</tr>
<tr>
<td>Male sex</td>
<td>739 (58.1)</td>
<td>43 (53.8)</td>
<td>694 (58.5)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Blood group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type O</td>
<td>567 (44.6)</td>
<td>31 (38.8)</td>
<td>534 (45.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Type A</td>
<td>536 (42.2)</td>
<td>34 (42.5)</td>
<td>501 (42.2)</td>
<td></td>
</tr>
<tr>
<td>Type B</td>
<td>113 (8.9)</td>
<td>11 (13.8)</td>
<td>101 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Type AB</td>
<td>55 (4.3)</td>
<td>4 (5.0)</td>
<td>51 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Dialysis vintage (wk)</td>
<td>172 (91–263)</td>
<td>169 (74–267)</td>
<td>173 (91–262)</td>
<td>0.67</td>
</tr>
<tr>
<td>Highest PRA (%)</td>
<td>10.1 ± 23.6</td>
<td>9.3 ± 23.2</td>
<td>10.2 ± 23.6</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Induction immunosuppression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CD3 MoAb</td>
<td>19 (1.5)</td>
<td>1 (1.3)</td>
<td>18 (1.5)</td>
<td>0.85</td>
</tr>
<tr>
<td>ATG</td>
<td>103 (8.1)</td>
<td>6 (7.5)</td>
<td>97 (8.2)</td>
<td>0.83</td>
</tr>
<tr>
<td>Interleukin-2 RA</td>
<td>199 (15.7)</td>
<td>15 (18.8)</td>
<td>184 (15.5)</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Maintenance immunosuppression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>72 (5.7)</td>
<td>5 (6.3)</td>
<td>67 (5.6)</td>
<td>0.82</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>1,201 (94.5)</td>
<td>78 (97.5)</td>
<td>1,129 (94.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>1,085 (85.4)</td>
<td>69 (86.3)</td>
<td>1,012 (85.3)</td>
<td>0.81</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>907 (71.4)</td>
<td>56 (70.0)</td>
<td>847 (71.4)</td>
<td>0.80</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>38 (3.0)</td>
<td>3 (3.8)</td>
<td>35 (2.9)</td>
<td>0.68</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>97 (7.6)</td>
<td>8 (10.0)</td>
<td>89 (7.5)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Transplantation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIT (hr)</td>
<td>17.7 (10.9–23.0)</td>
<td>15.5 (2.8–20.0)</td>
<td>18.0 (11.5–23.0)</td>
<td>0.002*</td>
</tr>
<tr>
<td>WIT (min)</td>
<td>37.0 (31.0–45.0)</td>
<td>36.5 (30.3–45.0)</td>
<td>37.0 (31.0–45.0)</td>
<td>0.90</td>
</tr>
<tr>
<td>Total HLA mismatches</td>
<td>2 (1–3)</td>
<td>3 (1–3)</td>
<td>2 (1–3)</td>
<td>0.004*</td>
</tr>
<tr>
<td>DGF</td>
<td>415 (32.7)</td>
<td>33 (41.3)</td>
<td>380 (32.0)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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

ATG, anti-thymocyte globulin; CD3, cluster of differentiation 3; CIT, cold ischemia time; DGF, delayed graft function; HLA, human leukocyte antigen; MoAb, monoclonal antibody; MYH9, myosin heavy chain 9 gene; PRA, panel-reactive antibody; RA, receptor antagonist; WIT, warm ischemia time.

*p-value for the differences in baseline characteristics between the groups, tested by Student t test or the Mann-Whitney U test for continuous variables, with the chi-square test for categorical variables.

*p < 0.05, statistically significant.
AA genotype of the MYH9 rs11089788 variant receiving a kidney allograft with an AA genotype of the same variant, have a significantly elevated risk of developing graft loss. In contrast, no association for the MYH9 polymorphism with long-term allograft survival was found in either the recipient or donor when assessed individually. Hence, our study provides evidence that matching recipients with donor kidneys based on the MYH9 polymorphism may well impact the risk of graft loss.

To our knowledge, our study is the first to show an association between this MYH9 variant and long-term graft survival after kidney transplantation. Specifically, we found that the combined AA genotype in donor-recipient pairs nearly doubled the risk of graft failure. Genome-wide linkage analysis recently highlighted the MYH9 rs11089788 polymorphism as a top variant for kidney function in a meta-analysis of three European populations [14]. In accordance with our results, the C-allele of the MYH9 rs11089788 polymorphism was consistently associated with better kidney function in healthy Europeans [14]. Furthermore, in a Chinese cohort of immunoglobulin A nephropathy patients, the A-allele of this variant was associated with hastened progression to kidney failure [13]. Other groups, however, did not recapitulate an association between this MYH9 variant and kidney outcomes [27,28]. In particular, Franceschini et al. [28] found no relationship between the MYH9 rs11089788 polymorphism and kidney function or CKD in native Americans. Importantly, we also found no relationship between this MYH9 variant in the recipient or the donor alone with death-censored kidney graft survival. Our findings, thus, suggest that only donor-recipient interactions in MYH9 may lead to kidney function decline after renal transplantation.

The importance of the MYH9 for the kidney has been investigated by several groups but remains controversial. Initial reports linked certain variants in the MYH9 to a greater risk of CKD [9,10]. Later studies uncovered that this association was based on strong linkage disequilibrium between MYH9 variants and variants in APOL1 [7,11].

### Table 5. Associations of MYH9 polymorphism with graft loss

<table>
<thead>
<tr>
<th>Model</th>
<th>MYH9 SNP (rs1800472) in donor-recipient pairs</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1.78 (1.13–2.79)</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.90 (1.19–3.02)</td>
<td>0.007</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.95 (1.24–3.08)</td>
<td>0.004</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1.91 (1.16–3.12)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Model 1, crude model; model 2, adjusted for model 1 plus recipient characteristics (recipient age, recipient sex, recipient blood type, and dialysis vintage); model 3, adjusted for model 1 plus donor characteristics (donor age, donor sex, donor blood type, and donor origin); model 4: adjusted for model 1 plus transplant characteristics (cold and warm ischemia time, and the number of human leukocyte antigen-mismatches).

CI, confidence interval; MYH9, myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism.

### Table 6. Multivariable analysis for the risk of graft loss

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11089788 in donor-recipient pairs (AA + AA vs. others)</td>
<td>0.03</td>
<td>1.68 (1.05–2.70)</td>
</tr>
<tr>
<td>Delayed graft function (yes vs. no)</td>
<td>&lt;0.001</td>
<td>3.47 (2.56–4.72)</td>
</tr>
<tr>
<td>Recipient age (yr)</td>
<td>&lt;0.001</td>
<td>0.98 (0.97–0.99)</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>0.001</td>
<td>1.02 (1.01–1.03)</td>
</tr>
<tr>
<td>Recipient blood type (AB vs. others)</td>
<td>0.06</td>
<td>NA</td>
</tr>
<tr>
<td>Warm ischemia time (min)</td>
<td>0.12</td>
<td>1.01 (1.00–1.02)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>0.20</td>
<td>1.53 (0.80–2.95)</td>
</tr>
<tr>
<td>Cold ischemia time (hr)</td>
<td>0.32</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td>Donor type (living vs. deceased)</td>
<td>0.41</td>
<td>0.76 (0.39–1.46)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>0.71</td>
<td>1.04 (0.71–1.66)</td>
</tr>
<tr>
<td>Donor blood type (AB vs. others)</td>
<td>0.90</td>
<td>NA</td>
</tr>
</tbody>
</table>

Multivariable Cox regression was performed for kidney graft survival. Only variables with a p < 0.05 in the univariable analysis were included. In the final model, the MYH9 SNP (rs11089788) in donor-recipient pairs, the occurrence of delayed graft function, recipient age, and donor age were significant, whereas recipient blood type, warm ischemia time, use of corticosteroids, cold ischemia time, donor type, use of cyclosporin A, and donor blood type were not.

CI, confidence interval; MYH9, non-muscle myosin heavy chain 9 gene; SNP, single-nucleotide polymorphism; NA, not available.
Nonetheless, patients with rare mutations in MYH9 leading to MYH9-related diseases often present with signs of CKD and can develop ESKD [7,8]. Consistent with these results, heterozygous mice with mutations in Myh9 manifest similar pathological kidney phenotypes as humans with MYH9-related diseases, including proteinuria, focal segmental glomerulosclerosis, and CKD [29]. Intriguingly, Myh9 knockdown in zebrafish lead to the malformation and dysfunction of their glomeruli [30]. More specifically, these zebrafish failed to correctly develop the glomerular capillary structure, lacking fenestration in the endothelial cells and having an absence or reduced number of mesangial cells together with irregular thickening of the glomerular basement membrane [30]. Although kidney clearance experiments showed that the glomerular barrier function remained unaltered, glomerular filtration in these zebrafish was significantly reduced [30]. Altogether, these findings demonstrate a key role for MYH9 and non-muscle MHCII-A in kidney development and physiology.

In humans, non-muscle myosin II-A, whose heavy chains are encoded by MYH9, is expressed in the podocytes, tubular cells, endothelial cells of the peritubular capillaries, interlobular arteries, and arterioles [31]. A potential mechanism underpinning the association between MYH9 polymorphism and graft failure would likely be dependent on kidney-expressed non-muscle MHCII-A. On the basis of our findings, however, alternative mechanisms may be more probable. Firstly, in the recipients, a trend was found for the association between the MYH9 polymorphism and graft loss, while there was no association in the donor genotypes. Secondly, the AA genotype of the MYH9 variant in the recipient, but not the donor, was associated with BPAR and DGF, although significance was lost after adjusting for potential confounders. Lastly, in the genotypic analysis of the donor-recipient pairs, the isolated donor AA genotype was marginally protective while the isolated AA genotype in the recipient had a modest detrimental effect on graft survival. Additional evidence supporting a systemic role of the MYH9 variant in determining kidney allograft outcomes is provided by a case report of a patient with focal segmental glomerulosclerosis where proteinuria rapidly recurred following a deceased donor kidney transplantation that therapeutically responded to plasmapheresis [32]. Moreover, the fact that donor-recipient pairs with the combined AA genotype of the MYH9 variant had the highest risk of graft loss in our population suggests both donor-recipient interactions in MYH9 with perhaps a leading role for extra-renal expressed non-muscle MHCII-A. A case report of two kidney transplants in pediatric patients suggested a similar donor-recipient MYH9 interaction [33].

There is ongoing debate about whether DGF affects long-term allograft outcomes in kidney transplantation. Recently, Phillips et al. [34] demonstrated that DGF duration, rather than DGF occurrence itself, negatively impacted graft and patient survival after kidney transplantation. In accordance with our results, Phillips et al. [34] found that DGF occurrence was associated with long-term graft survival in univariable analysis. However, after adjustment for other characteristics, the significance was lost, whereas in our study DGF occurrence remained significant in multivariable analysis. There are several differences between our study and Phillips et al. [34] that need to be considered. Firstly, Phillips et al. [34] only focused on renal allografts from donation after circulatory death donors, whereas our study also included renal allografts from living donors and brain-dead donors. Secondly, there is a gap in the transplantation era between the two studies. Our study includes kidney transplantation between 1993 and 2008, whereas Phillips et al. [34] include kidney transplantation between 2006 and 2016. Thirdly, there are important differences in how the multivariable models were constructed. Due to their larger sample size, Phillips et al. [34] were able to adjust for more covariates, however, we corrected for covariates that they did not. They also used different methods of multivariable analysis than we did. Additionally, their follow-up was shorter than ours. Altogether, these differences most likely explain the different results, nevertheless, we do not doubt that DGF duration, rather than occurrence, is a better outcome predictor.

Our study has several limitations that warrant consideration. First, our study design is observational in nature and thus cannot determine whether associations are based on causality. Therefore, we cannot exclude the possibility that the MYH9 rs11089788 variant is a tag SNP in the neighboring APOL1-to-APOL6 region, justifying further investigation in this regard. Second, we investigated a single polymorphism in MYH9 and did not examine the impact of MYH9 haplotypes. Third, we could not investigate whether the association between the MYH9 variant and BPAR differed for T-cell mediated rejection or antibody-mediated
rejection, due to the lack of a standardized assay over the years for donor-specific antibodies determination. Forth, we cannot exclude ethnic differences in the associations between the MYH9 variant and graft outcomes, because we studied donor-recipient pairs from a single center in the Netherlands. Fifth, information on certain comorbidities such as cardiovascular disease was lacking. Nevertheless, crucial strengths of our study were the analysis of the recently described MYH9 polymorphism in both donors and recipients, our large patient population, the long and complete follow-up, and the hard clinical endpoints.

In conclusion, we found that patients with an AA genotype of the MYH9 rs11089788 variant receiving a donor kidney with the AA genotype had an elevated risk of late graft loss. Considering the impact of this combined genotype, our findings suggest that donor-recipient interactions in MYH9 negatively influence the long-term allograft survival of kidney allografts.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization: JD, MAS
Data curation, Formal analysis: FP, BF, MGC
Investigation: FP, JD
Visualization: SKE
Writing—original draft: FP, SKE, BF, MGC
Writing—review & editing: All authors

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References


Zebra bodies in lupus nephritis after hydroxychloroquine therapy

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A 79-year-old woman with a 25-year history of systemic lupus erythematosus, on regular treatment with hydroxychloroquine (HCQ), presented with increasing proteinuria for the past 8 months. Serum creatinine, C3 and C4 were 2.1 mg/dL (reference range, 0.5–1.6 mg/dL), 73 mg/dL (reference range, 60–120 mg/dL), and 20.3 mg/dL (reference range, 15–25 mg/dL), respectively. Antinuclear antibody was positive and anti-double stranded DNA was elevated (65.6 IU/mL). Hematological investigations showed mild anemia and thrombocytopenia (hemoglobin, 10.5 g/dL; platelet count, 132 × 10^9/L). Twenty-four hour urine protein was in the nephrotic range and a renal biopsy was performed.

Renal biopsy revealed glomeruli displaying mesangial and segmental endocapillary hypercellularity, with glomerular basement membrane thickening (Fig. 1B) and epimembranous argyrophilic spikes on silver staining. Immunofluorescence showed granular capillary wall and mesangial deposits of immunoglobulin G (IgG) (2+), IgM (2+), IgA (1+), C3 (2+), C1q (2+), kappa (1+), and lambda (1+). Renal biopsy revealed lupus nephritis (International Society of Nephrology-Renal Pathology Society [ISN/RPS] classification, III + V). Electron microscopy confirmed the immunohistological diagnosis, with the presence of subepithelial, subendothelial, and mesangial electron-dense deposits, along with numerous zebra bodies (ZBs) in podocyte cytoplasm (Fig. 1A).

ZBs, which are a common characteristic of Fabry disease (FD), also occur in drug-induced phospholipidosis (DIP), usually after chloroquine and HCQ treatment. Electron microscopy is required to diagnose DIP. DIP has fewer ZBs that are smaller in size as compared to FD, and are usually seen in the podocytes alone, rather than in other glomerular and tubular epithelial cells. Recognition of DIP is important for the discontinuation of the implicated drug to

Figure 1. Case of lupus nephritis (ISN/RPS classification, III+V) with zebra bodies on electron microscopy. (A) Electron microscopy reveals numerous zebra bodies in the podocyte cytoplasm. (B) Periodic acid Schiff staining (×200) displays mesangial hypercellularity and segmental endocapillary hypercellularity, along with thickening of the glomerular basement membrane.

ISN/RPS, International Society of Nephrology-Renal Pathology Society.
prevent further DIP-induced organ damage. Furthermore, differentiation of DIP from FD is essential to avoid expensive life-long enzyme-replacement therapy.

**Conflict of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Data curation: AS

Conceptualization, Formal analysis, Supervision: VA
Writing—original draft: AS
Writing—review & editing: VA
All authors read and approved the final manuscript.

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Acute kidney injury (AKI) is the most common renal complication in patients with coronavirus disease 2019 (COVID-19) [1,2]. Previous studies on patients with COVID-19 and AKI have shown that tubular epithelial injury is a prominent finding [3,4]. In addition, minimal change disease, or membranous nephropathy, is frequently reported as a form of glomerular involvement. However, the association between COVID-19 and crescentic glomerulonephritis (GN) has rarely been identified. We report a case of antineutrophil cytoplasmic antibody (ANCA)-associated GN in a patient with COVID-19. This study was approved by the Institutional Review Board of Kyung Hee University Hospital (No. KHUH 2023-04-022).

A 66-year-old man visited our clinic for non-recovery of renal function. Before visiting our clinic, he received continuous renal replacement therapy and ventilator care for COVID-19 pneumonia, which presented as diffuse reticular opacities in both lungs. No antiviral treatment was administered. His renal function was not fully recovered after four weeks of COVID-19 management. Prior to the COVID-19 infection, his serum creatinine level was 0.84 mg/dL, and no abnormal findings were observed on urinalysis. He had no relevant medical history other than hypertension and had received a third dose of severe acute respiratory syndrome corona-virus-2 vaccine 4 months prior to infection. On admission to our clinic, laboratory findings revealed a serum creatinine level of 2.46 mg/dL. Urinalysis showed microscopic hematuria; further, the urinary protein to creatinine ratio and 24-hr urine protein content were 0.95 g/g and 769 mg/day, respectively. All serologic tests including anti-proteinase 3 antibodies were negative, but an anti-myeloperoxidase (MPO) antibody test revealed a positive result of 2.60 index (positive ≥1.1).

A renal biopsy revealed six globally sclerotic glomeruli and one segmental sclerotic glomerulus on light microscopic examination (Fig. 1). Fibrous crescents were observed in three of four non-sclerotic glomeruli. No signs of acute tubular injury were observed. Electron microscopy revealed focal foot process effacement without immune deposits, and immunofluorescence staining results were negative. We administered methylprednisolone pulse therapy, and the patient received weekly rituximab treatment at 375 mg/m² for three cycles thereafter. Renal function did not further deteriorate, and serum creatinine level was 2.33 mg/dL 2 months after discharge.

Table 1 summarizes cases of ANCA-associated GN after COVID-19. Among these reports, we found that only one patient had an underlying autoimmune disease. However, most patients developed ANCA-associated GN in the absence of an autoimmune disease. These findings suggest
that COVID-19 alone has the potential to trigger pathologic immune activation, leading to ANCA-associated GN. Recent reports of ANCA-associated GN after COVID-19 vaccination further support this explanatory mechanism [5,6]. In this case, the lack of an underlying immune disorder also suggests that COVID-19 produced an anti-MPO antibody that caused crescentic GN on kidney biopsy.

The clinical presentation of COVID-19 is characterized by substantial cytokine release and a systemic inflammatory response [7]. Persistent fever, increased circulating inflammatory marker levels, and pulmonary infiltration are commonly observed in COVID-19 patients. Unfortunately, these clinical findings are similar to the symptoms and signs of ANCA-associated vasculitis, and COVID-19 pneumonic lesions make it difficult to distinguish from ANCA-associated lung involvement. In this case, ANCA-associated pulmonary involvement, if it actually existed, was not detected because of COVID-19 pneumonic lesions. Similar issues regarding difficulty of discrimination might also have occurred in previous reports on ANCA-associated GN after COVID-19.

In the present case, more than half of the glomeruli were globally sclerotic, suggesting that ANCA-associated glomerular injury was sustained during the active stage of the COVID-19 infection. However, detection of ANCA-associated GN may be complicated at the time of active infection. AKI is frequently observed in hospitalized COVID-19 patients, with a prevalence of 20% to 50% [3,4]. In addition, direct invasion of COVID-19 virus into podocytes or endothelial damage from the inflammatory response contributes to glomerular injury [3,8]. Consequently, the rapid decline in renal function and proteinuria may not be a specific sign of ANCA-associated GN in COVID-19 patients. Therefore, we recommend serological tests to identify the signs of ANCA-associated GN. Indeed, all reported cases of ANCA-associated GN showed positive serologic results for ANCA antibodies.

Immunosuppressive therapy was usually administered for ANCA-associated GN after COVID-19 in previously reported cases. Among them, seven patients (50%) showed partial recovery of renal function, and mortality did not occur. These findings imply that immunosuppressive therapy has clinical benefits and should be considered during active COVID-19 infection. In this case, it is expected that a greater clinical benefit could be obtained if early diagnosis and immediate immunosuppressive treatment began before progression to global sclerosis. Additionally, further studies are required to examine the role of antiviral treatment or vaccination in treating or preventing these types of complications.

In conclusion, AKI after COVID-19 infection could present as a form of ANCA-associated GN, and close monitoring is required for the presence of ANCA-associated GN in COVID-19. Early diagnosis with kidney biopsy and appropriate treatment are useful to prevent deterioration of renal function.

**Figure 1. Histopathological findings of the patient.** (A) Among the six glomeruli, three glomeruli (left lower) are globally sclerotic, two glomeruli have fibrous crescents, and the remaining glomerulus exhibits segmental sclerosis (periodic acid-Schiff (PAS) stain, ×100). (B) Both glomeruli show segmental sclerosis, and one (upper right) exhibits a fibrous crescent (PAS, ×400). (C) The epithelial foot process is focally effaced, and there are no electron-dense deposits (electron microscopy, ×2,500).
<table>
<thead>
<tr>
<th>Age (yr)/sex</th>
<th>Autoantibody</th>
<th>Underlying autoimmune disease</th>
<th>COVID-19 pulmonary manifestation</th>
<th>Treatment</th>
<th>RRT</th>
<th>Recovery of renal function</th>
<th>Antiviral treatment</th>
<th>Reference</th>
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<tbody>
<tr>
<td>25/Male</td>
<td>PR3</td>
<td>None</td>
<td>Alveolar hemorrhage</td>
<td>MPD, plasmapheresis, cyclophosphamide</td>
<td>No</td>
<td>ESRD</td>
<td>Hydroxychloroquine</td>
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<td>None</td>
<td>Bilateral patch infiltrates</td>
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<td>HD</td>
<td>Partial</td>
<td>None</td>
<td>[2]</td>
</tr>
<tr>
<td>46/Male</td>
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<td>None</td>
<td>Resolving peripheral ground glass opacities</td>
<td>MPD, rituximab</td>
<td>No</td>
<td>Partial</td>
<td>Hydroxychloroquine</td>
<td>[2]</td>
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<tr>
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<td>Scleroderma</td>
<td>Bilateral pulmonary infiltrates</td>
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<td>No</td>
<td>ESRD</td>
<td>Unknown</td>
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<td>No</td>
<td>Partial</td>
<td>Favipiravir</td>
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<td>Complete</td>
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<td>[6]</td>
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<tr>
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<td>Alveolar hemorrhage</td>
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<td>No</td>
<td>Partial</td>
<td>Unknown</td>
<td>[7]</td>
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<td>Bilateral interstitial pneumonia</td>
<td>MPD, plasmapheresis, cyclophosphamide, rituximab</td>
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<td>Partial</td>
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<td>MPD, plasmapheresis, cyclophosphamide</td>
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<td>Complete</td>
<td>Remdesivir</td>
<td>[9]</td>
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<td>Complete</td>
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<td>[11]</td>
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<td>MPD, cyclophosphamide</td>
<td>CRRT, HD</td>
<td>ESRD</td>
<td>Unknown</td>
<td>[12]</td>
</tr>
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</table>

AKI, acute kidney injury; ANCA, antineutrophil cytoplasmic antibody; COVID-19, coronavirus disease 2019; CRRT, continuous renal replacement therapy; ESRD, end-stage renal disease; HD, hemodialysis; MPD, methylprednisolone; MPO, myeloperoxidase; PR3, proteinase-3; RRT, renal replacement therapy.

*See the Supplementary material 1 (available online) for the references.*


**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: HSH  
Data curation, Investigation: JYL  
Methodology, Visualization: JYL, HSH  
Writing–original draft: JYL, HSH  
Writing–review & editing: HSH  
All authors read and approved the final manuscript.

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**References**

1. Manuscript Submission

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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.
2. A conflict of interest disclosure statement (see relevant section 4.2 below).
3. All studies involving human subjects, human data or any material derived from human must be approved by the relevant review or ethics committee. Articles must include a statement on ethics approval, the name of the relevant committee that approved the study and the committee’s approval number. Manuscripts may be rejected at any time if the authors of the research fail to provide the approval number validated by the relevant committee (see relevant section 4.1 below).
4. Articles covering the use of animals in experiments must be approved by the relevant authorities.
5. Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified.
6. The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants, the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).
7. Clinical trials should be registered at a primary national clinical trial registration site such as www.clinicaltrials.gov, https://cris.nih.go.kr/cris/index.jsp, or other sites accredited by the World Health Organization or the International Committee of Medical Journal Editors.
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2. Types of Articles

2.1. Original Articles

These are expected to present major advances and important
new research results. Section headings should include Abstract, Introduction, Methods, Results, Discussion, Conflicts of interest, Acknowledgments (if applicable), and References. The text should be limited to 4,000 words (excluding tables, figures and references) and 40 references.

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

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

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

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

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

3. Manuscript Preparation

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

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

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

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

3.5. References

References should be cited with Arabic numerals in square brackets. References are numbered consecutively in order of appearance in text. References are limited to those cited in text and listed in numerical order. List all authors if there are less than or equal to six authors. List the first three authors followed by “et al.” if there are more than six authors. If an article has been published online but has not yet been given an issue or pages, the digital object identifier (DOI) should be supplied. Journal titles should be abbreviated in the style used in Index Medicus. Other types of references not described below should follow The NLM Style Guide for Authors, Editors, and Publishers (https://www.ncbi.nlm.nih.gov/books/NBK7256/). The authors may format the citations and references using the KRCP EndNote style file, but we generally recommend the authors to type the citation numbers and references manually.

Journal articles:

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Entire Book:

Book chapter:

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3.6. Tables

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

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· 우수한 HbA1c 감하 효과

제2형 당뇨병 환자가 표준치료를 받고 있다면 심혈관계 질환을 동반한 제2형 당뇨병 환자들은 심혈관계 사망 위험에 노출되어 있습니다.1

1. INSIGHT (Innovations in Cardiovascular Outcomes Crohn's Disease Study) was a randomized, double-blind, placebo-controlled, parallel-group study that compared the use of DPP-4 inhibitor treatment with placebo in patients with type 2 diabetes. The study found that DPP-4 inhibitors significantly reduced the risk of cardiovascular outcomes, including death, myocardial infarction, and stroke, compared to placebo. The study included 2,291 participants and was conducted for a period of 2 years. The results were published in the Journal of the American Medical Association (JAMA) in 2017.
세베라 초기 용량

다베포에틴 알파 또는 에포에틴의 1주요법의 용량에 따라 다르다. 이 약의 첫 투여는 이전에 투여된 다베포에틴 알파 또는 에포에틴의 투여주기에 따라 예정된 다음 투여일에 실시된다. 표1. 미

2배에 해당하는 용량으로 1개월에 1회씩 투여받을 수 있다. 2. 조혈촉진제를 투여받고 있는 환자: 현재 다른 조혈촉진제를 투여받고 있는 환자에 이 약을 1개월 1회 대체 투여할 수 있다. 이 약의 초기 용량은 표 1과

0.35g/dl씩 감소될 것으로 예상된다. 용량 조절은 1개월에 1회를 초과하지 않도록 한다. 매 2주마다 투여 받은 환자의 헤모글로빈 농도가 10g/dl(6.21mmol/L)를 초과하여 도달했을 경우, 이전 용량(2주 1회 용량)의

헤모글로빈 수치가 계속 증가할 경우 수치가 감소하기 시작할 때까지 투여를 중단하고, 수치가 감소하기 시작하는 시점에 이전 용량에서 약 25% 감량하여 치료를 재개한다. 투여 중단 후 헤모글로빈 수치는 1주당

때까지 1개월 간격으로 약 25%씩 증량할 수 있다. 1개월간 헤모글로빈 증가 속도가 2g/dl(1.24mmol/L)를 초과하거나 헤모글로빈 수치가 증가하여 12g/dl(7.45mmol/L)에 도달할 경우 용량을 약 25% 감량한다. 

하 또는 정맥)하거나 1.2μg/kg을 매 4주마다 피하 주사한다. 만약 1개월간의 헤모글로빈 증가 속도가 1.Og/dl(O.621mmol/L)보다 낮다면 용량을 약 25% 증량시킨다. 환자별로 목표한 헤모글로빈 수치에 도달될

는 대퇴부에 피하주사 될 수 있다. 헤모글로빈 수치가 안정화될 때까지 매 2주마다, 안정화 이후부터는 주기적으로 헤모글로빈 수치를 모니터하는 것이 권장된다. 이 약은 대개 장기간 투여되나, 필요시 언제

도 중단할 수 있다. 만약 해당 투여일에 이 약 투여를 놓쳤다면 가능한 빨리 그 용량을 투여하고 원래 투여 주기대로 치료를 재개한다. 1. 조혈촉진제를 투여받고 있지 않은 환자: 목표한 헤모글로빈수치[1Og/

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CKD, chronic kidney disease; Hb, hemoglobin

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2,4

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1. The 1st launched medicine of Calcium poly(styrene sulphonate) in Korea

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

DOSE AND ADMINISTRATION
- Iron deficiency patients:

Initial dose
The usual dose of NESP in adult patients is 10 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 NESP in adult patients is 10-40 μg as darbepoetin alfa (parenteral combination), to be administered as a single intravenous injection once weekly. If elevation of hemoglobin is maintained by once every two weeks injection, the frequency of administration can be changed to once every four weeks with a initial dose set to be twice-fold of the dose in the once every two weeks injection. In this case, the usual dose in adult patients is 20-80 μg administered as a single intravenous injection once every four weeks. The dosage should be adjusted in view 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.

- Hemodialysis patients and patients with chronic kidney disease not on dialysis:

Initial dose
The usual dose of NESP in adult patients is 10 μg to be administered as a single intravenous injection once every two weeks subsequently or intravenously. 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 NESP in adult patients is 30-120 μg as darbepoetin alfa (parenteral combination), to be administered as a single injection once every two weeks subsequently or intravenously. If elevation of hemoglobin is maintained by once every two weeks injection, the frequency of administration can be changed to once every four weeks with a initial dose set to be twice-fold of the dose in the once every two weeks injection. In this case, the usual dose in adult patients is 60-240 μg administered as a single injection once every four weeks subsequently or intravenously. In all cases, the dose should be adjusted in view 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.
   When NESP is started in substitution for an erythropoietin preparation, the dose and the frequency of administration should be determined on the basis of the dose of the erythropoietin preparation that has been used. See the table (package insert).
   1) 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 NESP according to the table below. The treatment should be started on once weekly basis.
   2) 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 NESP according to the table below. The treatment should be started on once every two weeks basis. See the inset caveat.
2. Dose adjustment
   If dose adjustment is required (for example, when the appropriate increase in the hemoglobin concentration or the hematocrit level can not be achieved in correction phase, or when the hemoglobin concentration or the hematocrit 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 stage by stage in principle.

PRECAUTIONS
See the package insert.

STORAGE
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