HIGHLIGHTS

Claudins in kidney health and disease

Risk factors for overcorrection of severe hyponatremia: a post hoc analysis of the SALSA trial

Humoral response to viral vector COVID-19 vaccine in hemodialysis patients

System of integrating biosignals during hemodialysis: the CONTINUAL (Continuous mOnitoriNg viTal sIgN dUring hemodiALysis) registry

Elderly kidney transplant recipients have favorable outcomes but increased infection–related mortality
Aims and Scope

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

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

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

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The image on the front cover: Jo et al demonstrated the pathophysiology of claudins in the kidney disease. They showed the renal phenotypes according to the dysregulation of claudins along the nephron. Please see the text for more details (pp. 275-287).
Overcorrection versus osmotic demyelination syndrome: what should we watch out for during management of symptomatic chronic hyponatremia?

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Overcorrection of serum sodium level in symptomatic chronic hyponatremia is the major risk factor of ODS development [2,4]. Since the incidence of ODS is very low, overcorrection has been used as a study outcome (instead of ODS development) in studies for management of symptomatic hyponatremia [1,4,6]. To date, however, there are no universal definitions for hyponatremia overcorrection [4,6,7]. In the SALSA (Efficacy and Safety of Rapid Intermittent Correction Compared With Slow Continuous Correction With Hypertonic Saline In Patients With Moderately Severe or Severe Symptomatic Hyponatremia) trial, a randomized clinical trial for management of symptomatic hyponatremia [1], overcorrection was defined as an increase in serum sodium level by >12 mEq/L or >18 mEq/L within

Hyponatremia is common in hospitalized patients [1]. Since serum sodium level is a major determinant of serum osmolality, decreased serum sodium level means that there is a relative excess of free water in the extracellular space (Fig. 1A). This excessive water moves into the cells, causing cellular edema (Fig. 1B) [2]. Hyponatremia is intimately associated with mortality, even in milder forms [3,4]. To date, however, the mechanism behind the increased risk of mortality with hyponatremia is not known [3]. Although acute hyponatremia, hyponatremia which develops within 48 hours, can result in death due to catastrophic brain herniation [5], the hazard of mild chronic hyponatremia cannot be explained by this same mechanism. Therefore, further investigation is needed to determine whether the hazard of mild to moderate chronic hyponatremia is real [3,5].

Over a period of about 48 hours after hyponatremia onset, the brain’s cell volume returns to normal by expelling intracellular solutes and water (Fig. 1C) [2]. Despite this adaptation, chronic hyponatremia can result in several symptoms that reflect increased intracranial pressure (e.g., vomiting, stupor, coma, and seizure), which requires urgent management by increasing the serum sodium level [4]. Because intracellular solutes are expelled, overcorrection of the serum sodium level excessively increases serum osmolality to ultimately induce a further out-shift of intracellular water. This can result in cellular shrinkage during management of symptomatic chronic hyponatremia (Fig. 1D). This process is the main pathogenesis of osmotic demyelination syndrome (ODS) [2].

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the first 24 or 48 hours, respectively. As a post hoc analysis of the SALSA trial, in this issue, Yang et al. [8] attempted to identify risk factors of overcorrection and to develop a new score called the NASK (HypoNatreemia, Alcoholism, Severe symptoms, and hypoKalemia) score to calculate the probability of overcorrection during management of symptomatic hyponatremia. In this study, the variables that were included in the new score were initial serum sodium level and presence of hypokalemia, severe symptoms, and chronic alcoholism (Table 1).

There has only been one prior study to be compared with the study by Yang et al. [8]; Woodfine et al. [6] proposed the SHOR (Severe Hyponatremia Overcorrection Risk) score using data from a single-center retrospective cohort to predict overcorrection during management of hyponatremia. Unlike the study by Yang et al. [8], Woodfine et al. [6] did not have a predefined threshold for overcorrection. Therefore, they had to use a latent class analysis to define overcorrection based on 14 criteria, which resulted in four classes of overcorrection status: none, unlikely, possible, and definite. Using a multinomial instead of a binomial logistic regression, they suggested five risky components (lower initial serum sodium level, hypokalemia, vomiting, somnolence, and low urine osmolality) and three protective components (older age, volume overload, and chest tumor) of overcorrection (Table 1).

As shown in Table 1, some factors were similar, but others were different between the NASK and SHOR scores. Lower initial serum sodium, lower serum potassium, and symptoms suggesting severe signs were robust components to increase the risk of overcorrection. Although chronic alcoholism scored high points on the NASK, it had no role in the SHOR score (Table 1). Although age had no role in the NASK score, older age received negative points in the SHOR score (Table 1). Different clinical and statistical designs, ethnicities, and management strategies likely led to these discordant results. Therefore, future large prospective studies need to be undertaken to validate the usefulness of the NASK and SHOR scores in management of symptomatic chronic hyponatremia.

Although ODS is strongly associated with overcorrection of chronic hyponatremia, other conditions (including alcoholism, malnutrition, and liver transplantation) are also associated with ODS development, even in the absence of hyponatremia or overcorrection [2]. This trend might be due to the control of movement of free water between

Figure 1. Movement of free water and intracellular solutes according to extracellular sodium level. (A) Excessive extracellular free water, (B) cellular edema, (C) adaptive expelling, and (D) cellular shrinkage.
intracellular and extracellular spaces by serum osmolality and not by serum sodium level. Therefore, any solutes that abruptly increase the extracellular osmolality in the brain can cause ODS. In addition, some conditions (including chronic alcoholism and hypokalemia) can have a synergistic effect on development of ODS, even if the serum sodium level is within the recommended range [4].

What should we watch out for during management of symptomatic chronic hyponatremia? Overcorrection of serum sodium level itself has no clinical role unless it is tightly associated with ODS development. Development of further tools to predict overcorrection during management of hyponatremia will be helpful. However, prediction and prevention of overcorrection are not sufficient. Tailored approaches to treat symptomatic chronic hyponatremia need to be exercised in full consideration of various risk factors of ODS beyond overcorrection.

**Conflicts of interest**

The author has no conflicts of interest to declare.

**References**


Are older adults safe and suitable candidate donors or recipients for kidney transplantation?

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Because the prevalence of end-stage renal disease (ESRD) is increasing worldwide, the number of elderly dialysis patients has been consistently increasing. According to the Korean Renal Data System (KORDS), the mean age of patients on prevalent dialysis was 65.0 years, and patients aged ≥65 years represented more than half of dialysis patients in 2019 in Korea [1]. This trend is paralleled by an increasing rate of kidney transplant (KT) in the elderly population. Due to the shortage of kidney donors and both the increase in age and life expectancy of dialysis patients, many elderly ESRD patients are dying while waiting for KT. Based on the Korean Network for Organ Sharing (KONOS) 2019 annual report, the mean wait time for KT in elderly dialysis patients aged ≥75 years (2,706 days) and aged 65 to 74 years (1,944 days) was longer than for all dialysis patients (1,737 days). Although eligible older recipients reported longer survival than dialysis patients who remained on the waiting list [2], older dialysis patients often experience difficulties in undergoing KT due to high comorbidity and increased risk of early postoperative complications and immunosuppression. To date, there is no definitive upper age limit for KT, but it is still debated whether elderly patients should be placed on the waiting list for KT or remain on dialysis.

In addition, elderly donors have been gradually increasing over the past decades to expand the donor pool and reduce waiting times for KT. Historically, the discard rates of kidneys from elderly deceased donors (DDs) were high; however, longevity matching to provide kidneys of elderly DDs to elderly recipients or patients with a shorter life expectancy recently allowed better allocation of kidneys in current clinical practice. In living donor (LD) KT, selection and allocation of suitable elderly individuals as living KT donors are more difficult and complex. Lower baseline kidney function and high comorbidities of elderly donors can shift the balance of benefit and risk, with poorer graft function for the recipient and increased perioperative complications and longer-term risks for the donor. Therefore, acceptance of older adults as kidney donors remains controversial.

In the present issue of *Kidney Research and Clinical Practice*, Lim et al. [3] investigated the clinical effects of donor or recipient age on patient survival and graft outcomes of KT recipients over 20 years. A total of 1,023 KT recipients was divided into four groups of donors and recipients...
based on 60 years of age: old-to-old, young-to-old, old-to-young, and young-to-young groups. Among participants, 129 recipients (12.6%) were >60 years of age at the time of KT, and 154 patients (15.1%) received a kidney from older donors aged ≥60 years. During the follow-up period of 69.2 months, elderly recipients experienced significantly higher mortality, especially infection-related mortality, than younger recipients; however, the incidence of cardiovascular and cancer-related mortality did not differ between elderly and younger recipients. Elderly individuals receiving a kidney from elderly and younger donors were associated with 3.06- and 2.89-fold higher risk of all-cause mortality, respectively, compared with younger individuals receiving a kidney from younger donors. However, significant differences were not found regarding the incidence of delayed graft function, graft failure, acute and late rejection, and infection-related hospitalization between elderly and younger recipients.

Several researchers reported that increased recipient age has a critical effect on the clinical outcomes of KT. Elderly recipients experience more infection-related complications and are at increased risk of death due to infections [4]. In contrast, the incidence of acute rejection is generally thought to decline with increased recipient age [4]. The higher infectious complications and lower acute rejection in elderly recipients might be due to the combined effects of immunosenescence and immunosuppressive medications. Immunosenescence, defined as dysregulation of the immune system caused by aging, is associated with weaker immune responses and increased disease susceptibility such as tumors and infections in elderly transplant recipients [5]. Immunosenescence affects both the innate and adaptive immune systems, with the most notable changes observed in the adaptive T cell immune system in transplant recipients [6]. Aging-related thymic involution reduces the T cell thymic output and results in reduced numbers of naive T cells and T regulatory cells. Aging also leads to accumulation of memory T cells associated with increased cytokine production and defective CD4+ and CD8+ memory T cell function. Furthermore, commonly used immunosuppressive agents for KT have age-specific effects. Aging decreases total body clearance of calcineurin inhibitors and increases intracellular lymphocyte calcineurin inhibitor concentrations in transplant recipients [7]. Therefore, higher drug levels after similar dosing of immunosuppressive agents can induce greater immune compromise in elderly recipients. Because the classical immunosuppressive protocols have been established in clinical trials, from which elderly patients are often excluded, the optimal immunosuppressive regimens and use of microbial prophylaxis have not been established in elderly recipients [4]. The effects of aging on the immune system should be further investigated to assist with tailored immunosuppressive regimens and appropriate infection prophylaxis for elderly recipients.

Donor age has also been a crucial factor for reduced graft function and lower graft and patient survival [8]. Lim et al. [3] found that the proportion of transplant recipients with serum creatinine levels of ≥1.5 mg/dL at 1 year after KT was higher in recipients from elderly donors than in recipients from younger donors. Elderly individuals who received a kidney from elderly donors had lower kidney function than recipients who received a kidney from younger donors during the five years after KT. In addition, young individuals receiving a kidney from elderly donors were associated with 2.41-fold higher risk of death-censored graft failure compared with young individuals receiving a kidney from young donors. However, significant differences were not found in the incidence of delayed graft function and graft failure between recipients from elderly and younger donors.

The pathophysiologic mechanism for negative outcomes of increased donor age in KT can be explained by immunosenescence. When transplanting the kidney, activation of innate immune responses occurs in the donor organ, particularly in response to organs donated after brain death and graft ischemia. Aging affects the co-stimulation of dendritic cell precursors and dendritic cells and hinders the function of neutrophils and natural killer cells. These age-associated effects result in impaired function of the innate immune system [9]. Substantial number of passenger dendritic cells deriving from the transplanted kidney have been shown to disseminate into the recipient. The immunogenicity in an older transplanted kidney affects transplant recipients through passenger dendritic cells. In addition, aging can influence the detrimental consequences of renal ischemia/reperfusion injury (IR) during KT by increasing immunogenicity in the allograft [6]. Aging also enhances the development of atherosclerosis, which in an allograft, contributes to the risk of allograft vasculopathy by activating the production of monocyte- and T cell-attracting chemo-
Last, the major characteristics of aging kidney are a decrease in glomerular filtration rate, loss of functioning nephrons, and increase of glomerulosclerosis and tubular atrophy. These features can contribute to decreasing the functional reserve of kidneys received from elderly donors, which might increase their susceptibility to IR injury and decrease transplant kidney function. **Table 1** summarizes the effects of aging on donor kidney and transplant recipients after KT.

Although acceptance of older adults as living kidney donors appears appropriate, the decline in kidney function after donation in elderly donors requires precise pretransplant donor workup. Because most LD have reduced kidney function after donation, all older LD candidates should continue to be assessed and carefully selected to minimize the risk of post-donation adverse outcomes [10]. Thus, a living kidney donation from elderly donors who have been thoroughly screened and continuously followed up can be safe. In cases of DDs, the Kidney Donor Risk Index and Kidney Donor Profile Index scoring systems are widely used to predict posttransplant graft function. The use of these indicators can aid in selecting suitable elderly DDs. Taken together, these findings suggest that elderly kidney donors can be acceptable for KT with active preoperative surveillance and careful perioperative management.

Currently, elderly recipients and donors are no longer a contraindication of KT. Transplant programs should consider older patients with ESRD as acceptable KT candidates if any contraindications do not exist during the evaluation process. The decision regarding eligibility for KT in elderly recipients must be made in the best interest of recipients based on objective medical and surgical criteria. The use of selected kidneys from elderly donors can result in favorable patient and graft outcomes and expand the donor pool. Further research and public debate on patient selection and appropriate management of older donors and recipients are needed to improve patient and graft survival after receiving or donating a kidney.

### Conflicts of interest

The author has no conflicts of interest to declare.

### Funding

This work was supported by a National Research Foundation of Korea (NRF) grant, funded by the Korean government (MSIT) (No. 2021R1C1C1012208), and by a Cooperative Research Grant 2019 from the Korean Society of Nephrology.

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Claudins in kidney health and disease

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Claudins are strategically located to exert their physiologic actions along with the nephron segments from the glomerulus. Claudin-1 is normally located in the Bowman’s capsule, but its overexpression can reach the podocytes and lead to albuminuria. In the proximal tubule (PT), claudin-2 forms paracellular channels selective for water, Na⁺, K⁺, and Ca²⁺. Claudin-2 gene mutations are associated with hypercalciuria and kidney stones. Claudin-10 has two splice variants, -10a and -10b; Claudin-10a acts as an anion-selective channel in the PT, and claudin-10b functions as a cation-selective pore in the thick ascending limb (TAL). Claudin-16 and claudin-19 mediate paracellular transport of Na⁺, Ca²⁺, and Mg²⁺ in the TAL, where the expression of claudin-3/16/19 and claudin-10b are mutually exclusive. The claudin-16 or -19 mutation causes familial hypomagnesemia with hypercalciuria and nephrocalcinosis. Claudin-14 polymorphisms have been linked to increased risk of hypercalciuria. Claudin-10b mutations produce HELIX syndrome, which encompasses hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia. Hypercalciuria and magnesium in metabolic acidosis are related to downregulation of PT and TAL claudins. In the TAL, stimulation of calcium-sensing receptors upregulates claudin-14 and negatively acts on the claudin-16/19 complex. Claudin-3 acts as a general barrier to ions in the collecting duct. If this barrier is disturbed, urine acidification might be impaired. Claudin-7 forms a nonselective paracellular channel facilitating Cl⁻ and Na⁺ reabsorption in the collecting ducts. Claudin-4 and -8 serve as anion channels and mediate paracellular Cl⁻ transport; their upregulation may contribute to pseudohypoaldosteronism II and salt-sensitive hypertension.

Keywords: Claudinopathy, Collecting duct, Electrolytes, Glomerulus, Proximal tubule, Thick ascending limb

Introduction

Urinary excretion of solutes, ions, and water is determined by the tubular transport removed from the glomerular filtrates. Renal tubular transport (either via reabsorption or secretion) occurs through both transcellular and paracellular pathways. Traditionally, renal regulatory function for fluid and electrolyte balance is exerted by changes in transcellular transport across the renal tubular epithelial cells. However, the regulatory roles of paracellular transport in the kidney remain incompletely known.

The junctional complexes located in the paracellular route comprise tight junctions (TJs), adherence junctions, and desmosomes [1]. The TJ is composed of three compo-
nents of transmembrane bridging proteins: claudin, occludin, and junctional adhesion molecules. The C-terminus of each has a PDZ binding domain linked to scaffold zonula occludens (ZO) proteins. The ZO proteins can bind directly to cytoskeleton actin filaments [2]. In glomerular epithelial cells, the glomerular slit diaphragm has specialized transmembrane bridging proteins (nephrin and podocin) between podocyte foot processes [3].

Furuse et al. [4] identified occludin as the first component of TJs, and its abundance is related to the degree of sealing of the epithelia [5]. However, occludin knockout mice displayed well-developed TJs [6]. They also found another integral component of TJs that might have a critical role in paracellular transport. Using the same liver fraction as employed to identify occludin, a single 22-kD band was discovered by stepwise sucrose density gradient centrifugation. Peptide sequencing revealed two proteins in this band that were subsequently named claudin 1 and 2. The name “claudin” is derived from the Latin word “claudere,” which means to close [7].

Claudins can characterize TJs because they polymerize in a linear fashion and form TJ strands with paracellular barriers or pore functions [8]. The claudin family has 27 members [9], many of which are located in the mammalian nephron [10]. Claudins contain from 21- to 28-kDa proteins and consist of four transmembrane domains, two extracellular loops (ECLs), amino- and carboxy-terminal cytoplasmic domains, and a short cytoplasmic turn (Fig. 1). The paracellular ion selectivity is determined by the charged amino acid residues located in ECL1. The ECL2 has binding sites for claudin interactions [11]. Table 1 summarizes different claudins according to ion permeability and selectivity based on in vitro studies using cultured cell lines and ex vivo studies using knockout mice [12–42]. The results can be discrepant depending on the properties of the tested cells and animals.

**Glomerular claudins**

Claudins are located along with nephron segments from the glomerulus, where they exert their physiologic actions. Claudin-1 is mainly located in Bowman’s capsule or parietal epithelial cells [43]. Gong et al. [43] showed that claudin-1 overexpression was associated with overt albuminuria. In transgenic mice with claudin-1 overexpression, claudin-1 protein labeling extended to the glomerular tuft, localizing in the podocytes. Claudin-1 messenger RNA (mRNA) and protein levels also increased in the glomeruli of the representative animal model of nephrotic syndrome, puromycin aminonucleoside nephrosis (PAN). As nephrin expression declined, claudin-1 expression reached the glomerular tuft, colocalizing with nephrin in PAN glomeruli. Claudin-1 might interact with nephrin and podocin, disrupting the endogenous nephrin and podocin interactions that hold the slit diaphragm in place [43]. Thus, proteinuria can result from claudin-1 overexpression.

However, it remains unclear whether upregulation of claudin-1 is the cause of proteinuria or just a mediator of podocyte injury. In normal conditions, a high concentration of nicotinamide mononucleotide (NMN) leads to epigenetic silencing of the promoter of claudin-1 by Sirt1 in podocytes [44]. Hasegawa et al. [45] reported that, in diabetic mice, proximal tubule Sirt1 expression decreased and was followed by a decrease in NMN concentration. In the absence of NMN, the claudin-1 promoter was no longer silenced, leading to increased claudin-1 expression in podocytes and causing foot process effacement and albuminuria. Thus, proximal tubule Sirt 1 exerts regulatory action on claudin-1, but this scenario was not valid in nondiabetic
animals, such as 5/6 nephrectomized mice.

Claudin-5 and claudin-6 are expressed in glomerular podocytes. When claudin mRNA levels were quantified in isolated rat glomeruli, claudin-5 expression was most abundant [46]. Its podocyte localization was demonstrated by immunoelectron microscopy and might be altered in PAN rats [46].

According to Zhao et al. [47], claudin-6 is localized in the TJs of rat podocytes. Claudin-6 was expressed in most of the tubules and glomeruli in neonates, but the expression in tubules dwindled in adults and was well-preserved in the glomeruli during development. Immunoelectron microscopy revealed that claudin-6 was distributed along the glomerular capillary wall and colocalized with ZO-1, and that its level of expression was not significantly altered in PAN rats [47].

### Proximal tubule claudins

The major proximal tubule claudins are claudin-2 and claudin-10a. Claudin-2 forms paracellular channels selective for small cations such as Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\) and is also permeable to H\(_2\)O so that 20% to 25% of proximal water absorption can occur paracellularly. It appears that cations and water travel through the same pore, where the amino acid residues in the ECL1 of claudin-2 line the narrowest part [48].

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**Table 1. Ion permeability and selectivity of claudins**

<table>
<thead>
<tr>
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<th>Claudins (selectivity)</th>
<th>Tested cells or mice</th>
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<td></td>
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<td>[15,16]</td>
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<td></td>
<td>Claudin-12 (Ca(^{2+}))</td>
<td>Claudin-12 KO</td>
<td>[17]</td>
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<tr>
<td></td>
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<td></td>
<td></td>
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<td>FVB/N mice</td>
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<td>Claudin-19 (Ca(^{2+}), Mg(^{2+}))</td>
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<td>Claudin-7 (Cl(^{-}))</td>
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FVB, Friend leukemia virus B; HEK-293, human embryonic kidney 293; KD, knockdown; KO, knockout; LLC-PK1, Lilly Laboratories Culture-Porcine Kidney 1; MDCK, Madin-Darby canine kidney; MDCK-C7, MDCK-clone 7; mIMCD3, mouse inner medullary collecting duct cell line 3; M-1, mouse kidney cortical collecting duct cell line 1; OK, opossum kidney.
The function of claudin-2 can be inferred from knock-out animals. Net transepithelial reabsorption of Na\(^+\), Cl\(^-\), and H\(_2\)O was reduced from isolated perfused S2 segments of the proximal tubules in claudin-2 knockout mice [49]. These changes were associated with an increase in paracellular electrical resistance but no changes in the apical and basolateral membrane resistance that represents transcellular electrical resistance.

The transepithelial resistance (TER) is an indicator of permeability and varies inversely with paracellular permeability; it progressively increases from the proximal tubule or leaky epithelia to the collecting duct or tight epithelia. This finding is relevant because approximately two-thirds of the glomerular filtered fluid is reabsorbed in the proximal tubule, and fine tuning of tubular transport occurs in the distal nephron [50]. Previous claudin-2 knockdown or overexpression studies were mostly from Madin-Darby canine kidney (MDCK) or tight epithelial cells. We tested the effects of TJ protein depletion in truly leaky human kidney-2 (HK-2) cells. Fig. 2 shows TER and immunoblot results from HK-2 cells transfected with small-interfering RNAs against claudin-2, occludin, and ZO-1. With claudin-2 knockdown, an increase in occludin was associated with and might have led to a decrease in TER. When claudin was knocked down, claudin-2 was suppressed, leading to increased TER. Similarly, TER was increased by ZO-1 knockdown in association with a decrease in claudin-2 [51].

We concluded that integration of claudin-2, occludin and ZO-1 is necessary for maintaining the function of the proximal tubular epithelium.

Claudin-10 has two splice variants -10a and -10b, respectively located in the proximal tubule and the thick ascending limb (TAL). In the proximal tubule, claudin-10a acts as an anion-selective channel (e.g., chloride absorption), whereas claudin-2 functions as a cation-selective pore [52,53]. Further independent roles of claudin-10a in the kidney remain to be determined.

The paracellular sodium transport mediated by claudin-2 contributes to energy efficiency in the kidney. Pei et al. [54] showed that claudin-2 knockout mice had larger renal oxygen consumption amounts for tubular sodium transport and a consequently lower energy efficiency. In addition, medullary hypoxia was suggested in claudin-2 knockout mice as they demonstrated remarkable furosemide-induced improvement of oxygen tension in the outer medulla. In brief, proximal tubule and TAL sodium transport are interconnected and share their load of transport. If the paracellular sodium transport is blocked in the

![Figure 2](image-url)

**Figure 2.** The effects of claudin-2, occludin, and ZO-1 gene knockdown on TER and expression of other tight junction proteins in HK-2 cells. HK-2 cells were transfected with small-interfering RNAs (siRNA) against claudin-2, occludin, and ZO-1. (A) TER was significantly decreased by claudin-2 siRNA transfection but significantly increased by siRNA transfection against occludin or ZO-1. Data are mean ± standard deviation of three independent experiments. *p < 0.05 vs. vehicle by Student t test for unpaired data. (B) Claudin-2 deficiency elevated occludin expression, occludin deficiency reduced claudin-2 expression, and ZO-1 deficiency also reduced claudin-2 expression. Adapted from the article of Kim and Kim [51], according to the Creative Commons License.

HK-2, human kidney 2; TER, transepithelial electrical resistance; ZO-1, zonula occludens-1.
proximal tubule, the transport load is shifted to the TAL, where Na-K-Cl cotransporter 2 (NKCC2) hyperactivity can enhance energy consumption [55].

Claudin-2 also has pathophysiological significance in calcium metabolism. Claudin-2 knockout mice demonstrate hypercalciuria due to decreased proximal tubular calcium reabsorption, which leads to papillary nephrocalcinosis and kidney stones. These results can be accentuated by decreased colonic calcium secretion or increased intestinal calcium absorption. Two large population-based studies have shown that common polymorphisms in the claudin-2 gene were associated with increased risk of kidney stones. Finally, a family case study was described in which males with a rare missense mutation in claudin-2 had marked hypercalciuria and kidney stone disease [56].

Metabolic acidosis can be associated with increased urinary calcium excretion. The protein level of claudin-2 decreased in rats with chronic metabolic acidosis and in MDCK II and HK-2 cells in response to an acidic pH [57]. The authors interpreted these results as an attempt to compensate for the chronic state of metabolic acidosis because the downregulation of claudin-2 might be associated with an increase in Na+/H+ exchanger 3 (NHE3) activity in the proximal tubule. However, Pei et al. [54] reported that the total and phosphorylated NHE3 abundance decreased by 23% and 27%, respectively, in claudin-2 knockout kidneys.

**Thick ascending limb claudins**

The major TAL claudins are claudin-3, -10b, -14, -16, and -19. They mediate paracellular transport of cations such as Na+, Ca2+, and Mg2+. The transcellular transport system is composed of apical NKCC2 and renal outer medullary potassium channels (ROMK) and of basolateral Na+/K+-ATPase and ClC-Kb chloride channels. In the cortex and outer stripe of the outer medulla (OSOM), the lumen-positive voltage produced by apical K+ recycling drives paracellular reabsorption of divalent cations via the claudin-16/19 complex. Here, paracellular Na+ transport can act in reverse and add to the lumen-positive transepithelial voltage. In the inner stripe of the outer medulla, however, Na+ is paracellularly reabsorbed through claudin-10b to contribute to medullary hypertonicity [58,59].

In Fig. 3, immunofluorescence microscopy shows that localization of claudin-10 does not overlap with that of claudin-16. However, claudin-16 and -19 are colocalized [60]. Similarly, claudin-3 and claudin-19 can be colocalized with each other but not with claudin-10. This characteristic claudin expression in the TAL was reported as a mosaic pattern. According to Milatz et al. [13], claudin-3 and claudin-19 were expressed in the intracellular compartment of all cortex/OSOM TAL cells. However, claudin-16 was strictly localized to the TJs. In brief, the expression of claudin-3/16/19 and claudin-10b are mutually exclusive in the TAL, and the two arrangements respectively mediate divalent and monovalent cation transport [61].

Next to the proximal tubule, the TAL is the major site of paracellular calcium transport in the kidney [61]. Divalent cations Ca2+ and Mg2+ are reabsorbed through the claudin-16/19 complex, and claudin-14 negatively regulates claudin-16 and -19 via direct interaction. During upstream signaling, microRNAs (miR-9 and miR-374) regulate claudin-14 mRNA stability and suppress translational efficacy. Gene transcription of microRNAs is regulated by the transcriptional factor nuclear factor of activated T cells (NFAT) and also via deacetylation of nearby histone molecules [62]. Consequently, claudin-14 is upregulated by stimulation of the calcium-sensing receptor (CaSR) [63].

The downregulation of claudin-2 in metabolic acidosis was described above, and we further investigated the role of TAL claudins in metabolic acidosis-induced hypercalciuria and hypermagnesiuria [64]. Fig. 4 shows that, in acid-loaded rats, both claudin-16 and claudin-19 expression decreased compared with those in controls. However, claudin-14 and CaSR expression increased in acid-loaded rats. All these changes were reversed by coadministration of the CaSR antagonist NPS-2143 and were confirmed using immunofluorescence microscopy. Hypercalciuria and hypermagnesiuria in acid-loaded rats also were significantly ameliorated by NPS-2143 coadministration. Thus, claudin-16 and claudin-19 are downregulated by metabolic acidosis via the CaSR.

Genetic defects in TAL claudins are directly linked to human diseases. The calcium- and magnesium-wasting disorder caused by either a claudin-16 or -19 mutation is called familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). The claudin-19 disorder is accompanied by severe ocular defects and is classified as type 2 FHHNC. The phenotype of the claudin-14 mutation is characterized by deafness without renal manifestations.
Claudin-10b mutations produce HELIX syndrome, which encompasses hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia and is suggestive of abnormalities in renal ion transport, ectodermal gland homeostasis, and epidermal integrity [58].

It is interesting that claudin-14 channelopathy has no renal manifestations. However, claudin-14 knockout mice have demonstrated reduced fractional excretion of calcium and magnesium in response to high dietary calcium intake [65]. Consistent with this, claudin-14 gene polymorphisms have been associated with differences in urinary calcium excretion, whereas no associations were found with claudin-16 and -19 polymorphisms [66].

The claudin-10 mutation HELIX syndrome is characterized by lack of sweat, saliva, and tears, and it has an autosomal recessive inheritance pattern. Renal manifestations include hypokalemia, hypocalciuria, and hypermagnesemia, as shown in a case series [67]. The data from claudin-10 knockout mice can explain this renal phenotype. Conditioned knockout mice deficient in claudin-10b were generated, and the absence of claudin-10b decreased Na⁺ permeability and increased Mg²⁺ and Ca²⁺ permeability in isolated perfused TALs [14]. Sodium wasting might be linked to an increase in fractional excretion of potassium, and increased magnesium and calcium reabsorption could lead to hypermagnesemia and hypocaliuria in claudin-10b knockout mice. A different feature of claudin-10b knockout mice from HELIX syndrome in humans was the presence of nephrocalcinosis. Interestingly, upregulation of both claudin-16 and claudin-19 was induced in claudin-10b knockout mice and can explain these results [14].

Figure 3. The distinct expression of claudin-10, claudin-16, and claudin-19 in the mouse cortical thick ascending limb. (A) Immunofluorescence microscopy reveals that localization of claudin-10 (Cldn 10, green) does not overlap with that of claudin-16 (Cldn 16, red). (B) Claudin-16 (Cldn 16, red) and claudin-19 (Cldn 19, green) are colocalized in the mouse cortical thick ascending limb. Bar = 20 μm. Adapted from the article of Prot-Bertoye and Houillier [60], according to the Creative Commons License.
Figure 4. Alteration of the thick ascending limb claudins in metabolic acidosis. (A) Immunoblots were performed from rat kidneys after a 7-day experiment. Each lane was loaded with a protein sample from a different rat and reacted with a specific antibody. (B) Densitometric analysis of the immunoblot bands reveals decreased claudin-16 and claudin-19 and increased claudin-14 and calcium-sensing receptor (CaSR) protein in NH₄Cl-loaded rats. These changes were reversed by coadministration of the CaSR antagonist NPS-2143. (C) Immunofluorescence microscopy shows the altered expression of claudin-16, claudin-19, claudin-14, and CaSR in the thick ascending limb from each group of animals (magnification, ×400). *p < 0.05 vs. control; #p < 0.05 vs. cinacalcet; §p < 0.05 vs. NH₄Cl by Mann-Whitney U test. Adapted from the article of Oh et al. [64] with original copyright holder’s permission.
Collecting duct claudins

Claudin-3, -4, -7, and -8 are mainly located in the collecting duct. Claudin-3 acts as a general barrier for ions, and it can promote urinary acidification due to blockage of H^+ back-leak [68]. The sealing effect of claudin-3 against ions of either charge and uncharged solutes was demonstrated by its overexpression in MDCK II cells, which induced a marked increase in paracellular resistance and decreases in permeability of sodium, chloride, and larger molecules, such as 4-kDa dextran [41].

In the collecting duct, a transepithelial voltage of −25 mV with respect to the basolateral side drives Cl^- transport through the paracellular channel, which is made up of claudin-4, -7, or -8 [10]. Thus, claudin-4 and -8 serve as selective anion channels, mediating a “chloride shunt,” which is coupled with transcellular Na^+ reabsorption via the epithelial Na^+ channel (ENaC). They may also act as Na^+ barriers [68]. Collecting duct-specific knockout of either claudin-4 or claudin-8 causes hypotension, hypochloremia, metabolic alkalosis, and renal salt wasting [69,70].

Claudin-7 can form a nonselective paracellular channel that facilitates Cl^- and Na^+ reabsorption in the collecting duct [71]. Claudin-7 knockout mice die shortly after birth due to severe renal salt wasting and dehydration, which is suggestive of the essential roles of claudin-7 and the collecting duct paracellular NaCl transport in maintaining fluid balance [72].

We postulated that claudin-4 or -8 upregulation contributes to salt-sensitive hypertension, and this hypothesis was tested in Dahl salt rats (Fig. 5). Compared with Dahl salt-resistant rats, Dahl salt-sensitive rats had higher blood pressure and lower urinary NaCl excretion. Claudin-4 and -8 also induce a decrease in occludin and an increase in zonula occludens-1 (ZO-1) and claudin-4 mRNA in SS rats compared with SRs. Data are mean ± standard error. Immunoblot results also reveal that occludin decreased and claudin-4 protein increased in SS compared with SRs. *p < 0.05 by the Mann-Whitney U test. Adapted from the article of Jo et al. [73], according to the Creative Commons License. BW, body weight; SBP, systolic blood pressure.
pressure and reduced sodium excretion. In the kidney, claudin-4 protein and mRNA levels increased, and occludin protein and mRNA decreased [73]. These results might be responsible for salt retention or impaired pressure natriuresis because claudin-4 is a chloride pore, and occludin is a nonspecific or sodium barrier located along the tubule.

Hou et al. [23] reported that claudin-4 requires claudin-8 for TJ localization. Claudin-4 protein expression was suppressed by claudin-8 gene knockdown in polarized M-1 cells, whereas claudin-3 and -7 expression were not affected. In the absence of the claudin-8 gene, claudin-4 expression was confined to the endoplasmic reticulum and the Golgi apparatus and was not observed in the apical cell membrane where TJs are located.

Another regulatory factor of claudin-4 is channel-activating protease-1 (CAP1). When the cells were treated with CAP1, the expression of claudin-4 at the TJs was reduced, whereas ZO-1 expression was not affected. CAP1 decreased the cell membrane expression levels of claudin-4 and reduced paracellular Cl⁻ permeability by disrupting claudin-4 trans-interaction [69].

In the collecting duct principal cells, aldosterone stimulates transcellular Na⁺ reabsorption and K⁺ secretion via ENaC and ROMK, respectively. In addition, aldosterone can affect paracellular Cl⁻ absorption by regulating claudins [68]. Aldosterone activates CAP1, which inhibits claudin-4, as previously mentioned. Aldosterone also induces phosphorylation of with-no-lysine kinase-4 (WNK4), and activated WNK4 phosphorylates claudin-4 on threonine residues to promote the chloride shunt [68]. Deletion of the claudin-7 gene in collecting duct cells induced upregulation of WNK4 and ENaC [71].

We previously tested this theme in cyclosporine-treated rats because hyperchloremic metabolic acidosis is often

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**Figure 6. The pathophysiology of claudins in the mammalian kidney.** Different phenotypes or claudinopathies can be produced by dysregulation of claudins along the nephron. FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HELIX presents as hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia. ▲, overexpression; ▼, knockdown; ◆, polymorphism.
encountered in patients using cyclosporine [74]. In the kidney, the protein expression of the Na-Cl cotransporter (NCC) was decreased by cyclosporine treatment, which suggested a decrease in transepithelial chloride transport. Instead, WNK4 increased in cyclosporine-treated rat kidneys. WNK4 upregulation was confirmed by in vitro cell culture studies and in vivo immunohistochemistry [75]. However, claudin-4 phosphorylation was not demonstrated in this study.

Transcellular and paracellular transport are interlinked in the collecting duct as well. Normally, transcellular sodium absorption occurs via ENaC, and paracellular Na⁺ back-leak is prevented by claudin-8 barrier. When the ENaC is hyperactive, the claudin-8 barrier is strengthened to block Na⁺ back-leak. In contrast, when the ENaC is inactivated, the claudin-8 barrier is weakened to promote Na⁺ back-leak [76]. Thus, claudin-8 combines with ENaC to enable unidirectional sodium transport across the collecting duct.

**Claudinopathy**

As the regulatory function and pathophysiology of claudins continue to be explored in the kidney, diseases associated with defective claudins have been termed “claudinopathies” [77]. Fig. 6 illustrates different claudinopathies along the nephron that have been described in previous experimental and clinical studies. Claudin-1 overexpression in the glomerular podocytes might have a role in albuminuria [43]. Claudin-2 and claudin-14 polymorphisms are associated with altered urine calcium excretion [56,66], which is suggestive of a role in idiopathic hypercalciuria. Hypercalciuria in metabolic acidosis is related to downregulation of both proximal tubule and TAL claudins [57,64]. Claudin-16 and claudin-19 mutations lead to FHHNC type 1 and type 2, respectively. Claudin-10b mutations can cause HELIX syndrome, which presents with hypokalemia, hypermagnesemia, and hypocalciuria [58,67]. Upregulation of claudin-4 and/or -8 may play a role in the chloride shunt, producing pseudohypoaldosteronism II and salt-sensitive hypertension [69,70].

**Conclusion**

Recent data from claudin studies have indicated that the paracellular pathways along the nephron are actively involved in renal physiology and pathophysiology. As the ion permeability and selectivity of different claudins continue to be defined, further studies will be required to show the regulatory and pathogenic roles of claudins in various electrolyte disorders. Understanding the interactions between paracellular and transcellular transport pathways will provide deeper insight into integrative renal physiology.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

The work was supported by grants from the National Research Foundation of Korea (NRF-2018R1D1A1A02047590 and 2020R1I1A1A01074620).

**Authors’ contributions**

Conceptualization: GHK
Formal analysis, Investigation: CJ, GHK
Experiments, Visualization: CJ, SK
Writing–Original Draft: GHK
Writing–Review & Editing: CJ, GHK
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Diagnosing metabolic acidosis in chronic kidney disease: importance of blood pH and serum anion gap

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Metabolic acidosis is one of the most common complications of chronic kidney disease (CKD). It is associated with the progression of CKD, and many other functional impairments. Until recently, only serum bicarbonate levels have been used to evaluate acid-base changes in patients with reduced kidney function. However, recent emerging evidence suggests that nephrologists should reevaluate the clinical approach for diagnosing metabolic acidosis in patients with CKD based on two perspectives; pH and anion gap. Biochemistry and physiology textbooks clearly indicate that blood pH is the most important acid-base parameter for cellular function. Therefore, it is important to determine if the prognostic impact of hypobicarbonatemia varies according to pH level. A recent cohort study of CKD patients showed that venous pH modified the association between a low bicarbonate level and the progression of CKD. Furthermore, acidosis with a high anion gap has recently been recognized as an important prognostic factor, because veverimer, a nonabsorbable hydrochloride-binding polymer, has been shown to improve kidney function and decrease the anion gap. Acidosis with high anion gap frequently develops in later stages of CKD. Therefore, the anion gap is a time-varying factor and renal function (estimated glomerular filtration rate) is a time-dependent confounder for the anion gap and renal outcomes. Recent analyses using marginal structural models showed that acidosis with a high anion gap was associated with a high risk of CKD. Based on these observations, reconsideration of the clinical approach to diagnosing and treating metabolic acidosis in CKD may be warranted.

Keywords: Anion gap, Hydrogen-ion concentration, Metabolic acidosis

Introduction

Metabolic acidosis is one of the most common complications in patients with chronic kidney disease (CKD) \cite{1}. This condition should not be overlooked by nephrologists in clinical settings because it has been associated with a wide range of poor outcomes including bone demineralization \cite{2}, insulin resistance \cite{3}, muscle protein proteolysis \cite{4}, functional limitations in older individuals \cite{5}, and cognitive impairment \cite{6}. Importantly, metabolic acidosis is also associated with cardiovascular outcomes and mortality in CKD patients \cite{7-9}. Basic studies showed that acid retention induced by nephron loss or dietary acid load causes kidney tissue injury through endothelin-1 activation, the...
renin-angiotensin-aldosterone system, and the alternative complement pathway [10–13]. In contrast, in several clinical cohort studies, low serum bicarbonate levels were shown to be associated with a faster progression of CKD [14–18]. Indeed, randomized controlled trials and corresponding meta-analyses revealed that alkali therapy conferred beneficial effects against the progression of CKD to kidney failure with replacement therapy (KFRT) [19–24].

According to current guidelines, alkali therapy initiation is recommended when serum bicarbonate levels are <22 mEq/L [25,26]. However, this recommendation is based exclusively on serum bicarbonate levels (Fig. 1). Additionally, clinical trials of veverimer, a recent novel approach for treating metabolic acidosis, have highlighted the possibility that anion gap acidosis is an important cause of CKD progression. In this review, our objective was to reconsider the effects of metabolic acidosis on the progression of CKD from two different perspectives: blood pH and the anion gap.

**Blood pH modulates the association between low bicarbonate level and progression of chronic kidney disease to kidney failure with replacement therapy**

Normal H\(^+\) concentration in extracellular fluid is almost one-millionth of the concentrations of N\(^+\), K\(^+\), Cl\(^-\), and HCO\(_3^-\). However, compared with larger cations, such as Na\(^+\) or K\(^+\), small H\(^+\) ions have stronger affinities for small and negatively charged parts of molecules. Therefore, smaller fluctuations in H\(^+\) concentrations are required for normal cellular functions [27].

A textbook on acid-base physiology outlines that an initial diagnosis of acid-base disorders should begin with measuring the blood pH [28]. However, blood pH is not frequently measured in certain clinical settings, such as in the United States. Rather, the serum total CO\(_2\) (TCO\(_2\)) is measured to screen for acid-base disturbances, because TCO\(_2\) is affected by both metabolic and respiratory disorders. The screening test ranges from US$26–$33 per test. In contrast, in Japan, a venous blood gas test, that includes measuring Na\(^+\), K\(^+\), Cl\(^-\), pH, pO\(_2\), pCO\(_2\), and HCO\(_3^-\), is routinely performed to diagnose acid-base disorders in the clinical outpatient setting [29]. This venous blood gas test costs US$126 based on current exchange rates (US$1 is almost 110 Japanese yen). Importantly, this cost was established by the Japanese government and all Japanese patients are required to have medical insurance; therefore, Japanese clinicians may not be aware of the direct cost as this is covered by patient insurance.

In the United States, serum TCO\(_2\) is used as a surrogate marker of HCO\(_3^-\). In Japan, pH and pCO\(_2\) in venous blood are measured in clinical laboratories and blood HCO\(_3^-\) is calculated using the Henderson-Hasselbalch equation [30]. In the United States, blood gas measurements are rarely performed in the main clinical laboratory and typically assessed in a blood gas laboratory [30], which may not be located in a convenient location for patient referrals from clinics. Further, to minimize errors, the measurements should be taken soon after the blood gas samples are obtained. These barriers to measuring blood gases may account for why blood pH is not frequently measured in the outpatient settings.

As described above, acid-base disorder diagnoses and

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**Figure 1. Recommendations for metabolic acidosis management in CKD patients.**

CKD, chronic kidney disease; KDIGO, Kidney Disease: Improving Global Outcomes; K/DOQI, Kidney Disease Outcomes Quality Initiative.
prescriptions for alkali therapy were based exclusively on blood HCO$_3^-$ test values. However, the degree to which alterations in blood pH affect CKD progression remains unclear. Recently, Kajimoto et al. [31] addressed this important issue by conducting a retrospective cohort study of Japanese CKD patients. In this approach, they used pH data that were measured along with other parameters in blood gas analyses and analyzed hazard ratios for incident KFRT using Cox proportional hazard models with/without acidemia (pH $\leq 7.32$). Kajimoto et al. [31] evaluated pulmonary diseases, such as chronic obstructive pulmonary disease or interstitial pneumonia in CKD patients and estimated respiratory compensation capacity using venous blood gas data (Fig. 2). They calculated respiratory compensation capacity using large amounts of blood gas data. We plotted the pressure of carbon dioxide against bicarbonate levels and calculated the slope of the regression line using a mixed-effect model. In this context, the slope of the regression line represents the respiratory compensation capacity and represents how much the carbon dioxide pressure can be reduced for each 1-mmol/L decrease in bicarbonate (Fig. 3).

The cohort included 1,058 CKD patients, among which a total of 374 developed KFRT during the median follow-up of 3.0 years. This study determined that 38% of CKD patients with hypobicarbonatemia (HCO$_3^-$ $\leq 21.5$) had normal pH ($7.32 \leq \text{pH} \leq 7.42$), whereas 59% with the same HCO$_3^-$ values had acidemia (pH $< 7.32$). These data indicated that approximately 40% of CKD patients with hypobicarbonatemia (HCO$_3^-$ $\leq 21.5$) did not have acidemia, which resulted from an adequate respiratory compensation capacity. This implies that a substantial proportion of patients within the

Figure 2. A schematic summary of the study conducted by Kajimoto et al. [31].

Figure 3. Quantification of respiratory compensation capacity in each CKD patient in the study by Kajimoto et al [31]. The authors assessed the respiratory compensation capacity by using a large number of blood gas data samples. They plotted the pressure of carbon dioxide and bicarbonate levels and calculated the slope of regression line by using a mixed effect model. Here, respiratory compensation capacity is the slope of the regression line, which also reflects the amount of pressure of carbon dioxide that can be reduced if 1 mmol/L of bicarbonate is reduced.

CKD, chronic kidney disease.
indicated target range for alkali therapy do not exhibit acidemia. The same observation was made in healthy individuals described in the Health ABC study. Approximately 60% of individuals with low bicarbonate levels were determined not to have acidemia [32]. Among CKD patients with acidemia (pH < 7.32), the lowest bicarbonate quartile exhibited a 2.29-fold higher risk of KFRT compared with the highest bicarbonate quartile. In contrast, among patients without acidemia (pH ≥ 7.32), the risk of KFRT in the lowest bicarbonate quartile was not significantly different from that in the highest bicarbonate quartile. In summary, a substantial proportion of CKD patients with hypobicarbonatemia may not be at risk for KFRT, but these patients should be considered as targets for alkali therapy.

With respect to the possibility of increased blood pressure and sodium retention induced by alkali therapy, previous physiologic studies have suggested that NaHCO$_3$ is easier to excrete than NaCl because HCO$_3^-$ is excreted predominantly as NaHCO$_3$ and not as KHCO$_3$ [33]. Therefore, when dietary sodium intake was restricted to approximately 200–700 mg/day, alkali therapy (200 mEq/day, 16.8 g/day NaHCO$_3$) did not induce increases in blood pressure or body weight in a small number of CKD patients [33]. However, a comparable intake of NaHCO$_3$ (100 mEq/day, 8.4 g/day) and NaCl (100 mEq/day, 5.85 g/day) still induced increases in blood pressure and weight gain [34]. In the general clinical setting, CKD patients do not typically adhere to recommendations to follow very strict restrictions for dietary sodium intake. A recent analysis of CKD patients found that the median salt intake was 8 g/day [35]. Indeed, recent alkali therapy trials excluded patients with uncontrolled hypertension and/or obvious congestive heart failure [36] and patients with decompensated heart failure [22]. Accordingly, CKD patients that meet criteria for alkali therapy should be selected carefully. The report by Kajimoto et al. [31] may provide important guidance for selecting the most appropriate CKD patients for alkali therapy. According to the study, CKD patients with low bicarbonate levels without acidemia may not require sodium bicarbonate. However, subclinical metabolic acidosis with normal serum bicarbonate has recently emerged and is suggested to have clinical significance [37]. In addition, a previous study revealed that alkali therapy caused greater renal function preservation in patients with normal venous total CO$_2$ [20]. Therefore, additional clinical evidence is needed to address the question of which patients will benefit the most from alkali therapy.

**Anion gap levels impact the progression of chronic kidney disease to kidney failure with replacement therapy**

Two categories of metabolic acidosis have been defined based on anion gap levels; normal anion gap (hyperchloremic) acidosis and high anion gap acidosis. Normal anion gap acidosis is usually identified during the early course of CKD, whereas high anion gap acidosis occurs in later stages of CKD owing to the accumulation of nonchlo-ride anions, including phosphate, sulfate, and a wide range of organic acids [38]. As previously described, low bicarbonate levels have been associated with rapid progression of CKD [14–18]. However, the current clinical understanding of how high anion gap acidosis affects renal outcomes, notably in the later stages of CKD is limited. Some initial studies have reported that uremic acids such as indoxyl sulfate, p-cresyl sulfate, and trimethylamine N-oxide cause renal fibrosis that is induced by kidney injury [39–42].

A recent series of clinical trials of veverimer, a nonabsorbable binding polymer for hydrochloric acid, showed intriguing results that are relevant for therapeutic strategies for metabolic acidosis in CKD [43–45]. Veverimer (TRC101) was developed as a treatment for metabolic acidosis in CKD patients. Veverimer is an orally administered, sodium- and counterion-free hydrochloric acid binder and hydrochloric acid binding is a novel therapeutic concept for treating metabolic acidosis that does not add problematic counterions, such as sodium or potassium. Veverimer selectively captures and removes hydrochloric acid from the gastrointestinal tract and increases serum bicarbonate [44]. In a multicenter randomized controlled trial, treatment with veverimer improved renal outcomes, identified as occurrence of renal replacement therapy, or a decline in estimated glomerular filtration rate (eGFR) of at least 50%, over 52 weeks [43]. Initial research in the veverimer trials hypothesized that chloride ions would increase with veverimer treatment. However, chloride ion levels did not increase in response to veverimer. Interestingly, administration of veverimer reduced the anion gap in CKD patients at 5, 12, and 52 weeks after trial initiation [43,44,46] (Table 1). These data may suggest that veverimer improves kidney function through anion gap reduction.
Asahina et al. [47] examined the association between anion gap and renal outcomes using cohort data from 1,168 Japanese CKD patients. It is well established that high anion gap acidosis develops during the later stages of CKD and the anion gap changes with progression of CKD. The anion gap has been repeatedly measured in conjunction with eGFR in Japanese outpatients and kidney function (eGFR) directly affects the anion gap. Moreover, an elevated anion gap may subsequently affect kidney function (eGFR). In this manner, exposure-confounder feedback is generated between the anion gap and eGFR (Fig. 4). Therefore, eGFR is believed to be a time-dependent confounder of the anion gap and renal outcomes. When the association between the anion gap and renal outcomes was analyzed in the presence of a time-dependent confounder, application of the conventional time-dependent Cox proportional hazard model alone was noted as an insufficient method for analysis as it can provide biased estimates [48,49]. Therefore, G-methods should be used for analysis in these situations.

G-methods include marginal structural models (MSMs) and the G-formula. MSM is explained herein for simplicity (Fig. 5); MSM is a counterfactual outcome model that is applied to account for time-dependent confounding. When applying MSMs, analysts establish imaginary pseudo-populations with or without exposure. The pseudo-populations that are established are balanced by inverse probability weighting in terms of baseline covariates and other time-dependent confounders. Asahina et al. [47] obtained time-varying inverse probability weights based on the inverse probability of treatment weights and the inverse probability of censoring weights. By analyzing differences between these pseudo-populations, they were able to determine an assumption for the exposure effect on outcomes.

As previously indicated [50], significantly elevated high anion gap acidosis occurred after stage 4 CKD [47]. In the MSM analysis, metabolic acidosis with a high anion gap was associated with a 3.04-fold rate of KFRT and a 5.56-fold rate of all-cause death, compared with the normal anion gap.

### Table 1. Differences in electrolyte levels compared with baseline data in the veverimer trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>No. of patients</th>
<th>Δ[Na⁺] (mEq/L)</th>
<th>Δ[Cl⁻] (mEq/L)</th>
<th>Δ[HCO₃⁻] (mEq/L)</th>
<th>Δ[AG⁻] (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bushinsky et al.</td>
<td>2018</td>
<td>104</td>
<td>0</td>
<td>0</td>
<td>3.3</td>
<td>-3.3</td>
</tr>
<tr>
<td>Wesson et al.</td>
<td>2019</td>
<td>112</td>
<td>-0.4</td>
<td>-0.4</td>
<td>4.7</td>
<td>-4.7</td>
</tr>
<tr>
<td>Wesson et al.</td>
<td>2019</td>
<td>124</td>
<td>0.3</td>
<td>-0.2</td>
<td>4.5</td>
<td>-4.0</td>
</tr>
</tbody>
</table>

Figure 4. Exposure-confounder feedback. Exposure-confounder feedback is generated between the anion gap (AG) and estimated glomerular filtration rate (eGFR). The eGFR can be a time-dependent confounder. Consequently, if a time-dependent confounder is identified in a statistical model, further assessment cannot be made by using conventional regression models and G-methods, like a marginal structural model or G-formula, must be adapted.

ESKD, end-stage kidney disease.

G-methods (counterfactual model)
- Marginal structural model
- G-formula
However, in the conventional multivariate Cox proportional hazard models, high anion gap acidosis was not associated with a significantly higher rate of KFRT or all-cause death compared with normal anion gap acidosis, suggesting that analyses using Cox proportional hazard models may underestimate the association between the anion gap and renal outcome/mortality.

In a previous study of 1,145 patients with moderate CKD \[51\], patients in the highest traditional anion gap tertile (11.8–maximum mEq/L) had a higher risk of end-stage renal disease compared with adults in the middle tertile (8.1–11.8 mEq/L) using a frailty model (relative hazard, 1.76; 95% confidence interval [CI], 1.16–2.32). The highest tertile of the full anion gap (19.54–maximum mEq/L) was also associated with a higher risk of all-cause mortality compared with adults in the middle tertile (15.93–19.54 mEq/L) based on a frailty model (relative hazard, 1.20; 95% CI, 1.01–1.39). A possible explanation for the smaller effect size in the analyses by Banerjee et al. \[51\], compared with those of Asahina et al. \[47\], may be the difference in renal function of participants (eGFR: 30–60 vs. 10–60 mL/min/1.73 m\(^2\), respectively), rather than the difference in statistical methods. The re-analyses that were stratified by eGFR in the study by Asahina et al. \[47\] revealed that high anion gap patients with an eGFR of ≥30 mL/min/1.73 m\(^2\) did not have a significantly higher risk of KFRT compared with normal anion gap patients within the same renal function range.

Anion gap constituents in chronic kidney disease

In the study by Asahina et al. \[47\], the association between a high anion gap and an increased occurrence of KFRT remained significant when the analysis was adjusted for albumin and phosphate, both of which are primary constituents of the anion gap in CKD patients, suggesting that substances other than albumin and phosphate may be involved in the progression of CKD in response to a high anion gap. In recent metabolomic analyses of human samples identified 492 uremic solutes from patients that continued on hemodialysis compared with age-matched control patients \[52\]. This included 214 with unknown chemical structure and 278 with known chemical structure, including well-known uremic solutes, 3-indoxyl sulfate, p-cresol sulfate, and trimethylamine N-oxide, all of which were reported...
to induce renal injury [39–42]. However, 3-indoxyl sulfate, p-cresol sulfate, and trimethylamine N-oxide were only observed at low levels among the 492 uremic solutes, suggesting that other uremic solutes that comprise the anion gap in CKD patients may accelerate the progression of CKD.

Uremic solutes are effectively excreted through both glomerular filtration and tubular secretion [53,54], suggesting that tubular malfunction may lead to accumulation of uremic solutes, and accumulation of solutes may induce kidney injury. Indeed, in the Chronic Renal Insufficiency Cohort study of 3,416 CKD patients, those with reduced tubular secretion of organic acids including kynurenic acid, pyridoxic acid, indoxyl sulfate, xanthosine, isovalerylglucine, and cinnamoylglycine, were found to have a significantly higher risk of progression to CKD [55].

Recently, the human intestinal flora was reported to play a pivotal role in the production of uremic solutes [56]. Renal insufficiency itself strongly affects the colonic microenvironment and alters the composition of intestinal flora to the extent that the environment easily produces toxic uremic retention solutes [57,58]. Several small interventional studies targeting the intestinal flora in patients with renal insufficiency have been performed in response to these data on the association between renal function loss and changes in intestinal flora (known as dysbiosis). Such interventions, including probiotics, prebiotics, and synbiotics, were reported to decrease indoxyl sulfate or p-cresol sulfate in predialysis and dialysis patients [59–64]. However, it is largely unknown if such interventions can in fact reduce anion gap levels in patients. Therefore, further studies to elucidate the mechanism and clinical implications will be needed.

**Conclusions**

Assessment of venous pH may lead to a reduction in alkali therapy targets for CKD patients with metabolic acidosis. Therefore, it may be possible to reduce the number of cases with adverse effects from sodium bicarbonate supplementation.

Compared with sodium bicarbonate, anion gap-reducing reagents such as veverimer are a novel therapeutic approach for metabolic acidosis and may improve renal outcomes in CKD patients.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Acknowledgments**

We thank Richard Robins, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

**Authors’ contributions**

Conceptualization: JYK, YS, YI
Data curation: SK, YA, TO, KH, YD
Formal analysis: YS, SK, YA
Project administration: YS
Writing–original draft: JYK
Writing–review & editing: JYK

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Background: Hyponatremia overcorrection can result in irreversible neurologic impairment such as osmotic demyelination syndrome. Few prospective studies have identified patients undergoing hypertonic saline treatment with a high risk of hyponatremia overcorrection.

Methods: We conducted a post hoc analysis of a multicenter, prospective randomized controlled study, the SALSA trial, in 178 patients aged above 18 years with symptomatic hyponatremia (mean age, 73.1 years; mean serum sodium level, 118.2 mEq/L). Overcorrection was defined as an increase in serum sodium levels by >12 or 18 mEq/L within 24 or 48 hours, respectively.

Results: Among the 178 patients, 37 experienced hyponatremia overcorrection (20.8%), which was independently associated with initial serum sodium level (≤110, 110–115, 115–120, and 120–125 mEq/L with 7, 4, 2, and 0 points, respectively), chronic alcoholism (7 points), severe symptoms of hyponatremia (3 points), and initial potassium level (<3.0 mEq/L, 3 points). The NASK (hyponatremia, Alcoholism, Severe symptoms, and hypoKalemia) score was derived from four risk factors for hyponatremia overcorrection and was significantly associated with overcorrection (odds ratio, 1.41; 95% confidence interval, 1.24–1.61; p < 0.01) with good discrimination (area under the receiver-operating characteristic [AUROC] curve, 0.76; 95% CI, 0.66–0.85; p < 0.01). The AUROC curve of the NASK score was statistically better compared with those of each risk factor.

Conclusion: In treating patients with symptomatic hyponatremia, individuals with high hyponatremia overcorrection risks were predictable using a novel risk score summarizing baseline information.

Keywords: Hyponatremia, Novel risk score, Overcorrection, Prediction, Risk factors
Introduction

Hyponatremia is the most common electrolyte imbalance encountered in clinical practice, with a prevalence of 14% to 42% in hospitalized patients. Moreover, hyponatremia has a high mortality rate and long hospitalization period [1,2]. Hyponatremia can induce various clinical manifestations that range from mild (fatigue, nausea, vomiting, headache, gait disorder, and confusion) to severe symptoms (seizures, coma, and brain hypoxia) [3–6]. Hypertonic saline has been used to treat symptomatic hyponatremia [7,8]. An increase in serum sodium levels by 4 to 6 mEq/L is generally sufficient to improve the symptoms caused by cerebral edema. However, overcorrection of hyponatremia may result in irreversible neurologic disability such as osmotic demyelination syndrome (ODS) [9–12].

According to an American expert panel recommendation, overcorrection is defined as an increase in serum sodium levels of >10–12 mEq/L in any 24 hours period or >18 mEq/L in any 48 hours period, with a more stringent limit of >8 mEq/L in 24 hours for patients at a high risk of developing ODS [13]. A European clinical practice guideline defined overcorrection as an increase in serum sodium levels of >10 mEq/L during the first 24 hours or >8 mEq/L in any 24 hours period thereafter [1]. The distinction between both guidelines indicates that there is still no consensus regarding the definition of hyponatremic overcorrection [1,10,13–17]. Nevertheless, the incidence of overcorrection has been reported to be as high as 20% to 41% [10,14,18–21].

Several studies and guidelines have recommended a specific amount and rate of hypertonic saline for effective hyponatremia treatment and overcorrection prevention [1,10,13,16,18,20,22–24]. However, it remains unclear why overcorrection occurs in some patients that received the required amount of hypertonic saline at the recommended rate. A few studies have examined the risk factors for overcorrection following symptomatic hyponatremia treatment, and these studies were limited by their retrospective design [14,17,18,21]. Therefore, we conducted a post hoc analysis of a prospective randomized controlled study, in which we investigated risk factors for overcorrection in patients receiving treatment for severe hyponatremia. Our goal was to enable physicians to recognize individuals at a high risk of overcorrection and to prevent ODS by careful correction with hypertonic saline.

Methods

Study population

We performed a post hoc analysis of a multicenter, prospective randomized controlled study: the SALSA (Efficacy and Safety of Rapid Intermittent Correction Compared With Slow Continuous Correction With Hypertonic Saline In Patients With Moderately Severe or Severe Symptomatic Hyponatremia) trial. The study included 178 participants who were admitted at Seoul National University Bundang Hospital, SMG-SNU Boramae Medical Center, and Hallym University Dongtan Sacred Heart Hospital between August 2016 and August 2019. The detailed study protocol has been described elsewhere (ClinicalTrials.gov; NCT02887469) [19].

Patients aged above 18 years, with moderate to severe symptoms, and with glucose-corrected serum sodium levels of ≤125 mEq/L were included in this study [25]. Patients with the following conditions were excluded: pseudohyponatremia (serum osmolality, ≥275 mOsm/kg), primary polydipsia (urine osmolality, ≤100 mOsm/kg), current pregnancy, breastfeeding, anuria, arterial hypotension (systolic blood pressure, <90 mmHg and mean arterial pressure, <70 mmHg), liver disease (transaminase levels of > three times the upper limit of normal, known decompensated liver cirrhosis with ascites or diuretic use, hepatic encephalopathy, and esophageal varices), uncontrolled diabetes mellitus (glycated hemoglobin, >9), a history of cardiac surgery, acute myocardial infarction, sustained ventricular tachycardia, ventricular fibrillation, acute coronary syndrome, cerebral trauma, and increased intracranial pressure. This post hoc analysis was approved by the Institutional Review Boards of three centers: Seoul National University Bundang Hospital (No. B-2101-660-101), SMG-SNU Boramae Medical Center (No. 10-2021-6), and Hallym University Dongtan Sacred Heart Hospital (No. 2020-10-012). Written consent was obtained from all participants or a legal guardian, when applicable. The study complied with principles of the Declaration of Helsinki.

Data collection and definitions

Baseline demographics, alcohol consumption information, and anthropometric measurements were recorded. Chron-
ic alcoholism was defined as the consumption of at least 4 and 3 glasses of alcohol per day in males and females, respectively, regardless of the types of alcohol. We assessed the presence of comorbidities such as hypertension, diabetes mellitus, congestive heart failure, and cancer by screening for the I10–115; E10–14; I11.0, I13.0, I13.2, I50; and C codes based on the International Classification of Disease, the 10th revision as well as by a self-reported or confirmed history of antihypertensive and antidiabetic drug use. The definition of hyponatremia was based on biochemical severity: ‘severe (profound)’ was defined as serum sodium levels of ≤125 mEq/L [1,13]. Clinical manifestations of hyponatremia were divided into moderate and severe based on the clinical presentation of the patient at initial hyponatremia. Moderate symptoms included nausea, drowsiness, headache, general weakness, and malaise. Severe symptoms included vomiting, stupor, coma (Glasgow Coma Scale score, ≤8), and seizures. We determined the underlying cause of hyponatremia using a structured diagnostic approach based on history, physical examination, and laboratory test findings. Patients were divided into five categories: 1) decreased extracellular fluid (ECF) volume due to renal sodium loss (e.g., diuretics, especially thiazides), 2) decreased ECF volume due to nonrenal sodium loss (e.g., gastrointestinal sodium loss or third spacing), 3) increased ECF volume (e.g., heart failure, liver cirrhosis, and nephrotic syndrome), 4) normal ECF volume with adrenal insufficiency, and 5) normal ECF volume fulfilling essential diagnostic criteria for the syndrome of inappropriate antidiuresis (SIAD) [20,26].

Two infusion methods for hypertonic saline have been described in published protocols; rapid intermittent bolus (RIB) and slow continuous infusion (SCI) [19,20,27]. The initial infusion rate was determined based on hyponatremia symptom severity. The treatment guidelines for the two groups are detailed in Supplementary Methods (available online) and Supplementary Fig. 1 (available online). The treatment goals were to increase serum sodium level by 5–9 mEq/L and achieve symptom relief within the first 24 hours, as well as increase serum sodium level by 10–17 mEq/L or to ≥130 mEq/L and to achieve symptom relief within the first 48 hours. Serum sodium levels were measured every 6 hours for 2 days using indirect ion-selective electrodes at the following three centers with the indicated equipment: Seoul National University Bundang Hospital, AU5800 (Beckman Coulter, Indianapolis, IN, USA) and Dimension Vista 1500 (Siemens Healthineers, Erlangen, Germany); SMG-SNU Borame Medical Center, Modular DP (Roche Diagnostics, Indianapolis, IN, USA) and Unicel DxC 800 (Beckman Coulter); and Hallym University Dongtan Sacred Heart Hospital, AU5800 (Beckman Coulter).

Study outcomes

The primary outcome was the incidence of hyponatremia overcorrection at any given period, which was defined as an increase in serum sodium levels by >12 mEq/L or >18 mEq/L within the first 24 or 48 hours, respectively. The secondary outcomes represented the time-specific increase in cumulative hyponatremia overcorrection rates specified by time. These were defined as an increase in serum sodium levels by >12 mEq/L within 6, 12, and 24 hours.

Statistical analysis

Baseline characteristics and laboratory data are expressed as mean and standard deviation for continuous variables and frequency and percentage for discrete variables. Differences in continuous variables were analyzed using the Student t test and Mann-Whitney test, and the chi-square and Fisher exact tests were used for discrete variables. Univariable logistic regression was used to analyze each variable to identify significant risk factors for hyponatremia overcorrection. We retained variables with p < 0.05 in the multivariable model using backward selection. Odds ratios (ORs) and 95% confidence intervals (CIs) for hyponatremia overcorrection occurrence were calculated after stepwise adjustment for multiple confounders. We multiplied parameter estimates of discrete variables in the model by a constant to obtain scores. We multiplied coefficients of continuous variables by the constant, which then represented a risk score for each unit increase in an individual continuous variable. The risk score was summarized as the arithmetic sum of the points for each variable. We evaluated model discrimination using the area under the receiver-operating characteristic (AUROC) curve to compare our predictive model to an older overcorrection model (The Severe Hyponatremia Overcorrection Risk [SHOR] score) [17]. The SHOR score has eight risk factors for overcorrection: age, vomiting, somnolence, volume overload, initial
serum sodium level, initial serum potassium level, urine osmolality, and chest tumor. We adjusted our prospective data to compare the predictive abilities for overcorrection in the SHOR scoring system and our predictive model. Patients aged under 40 years were included in the 40 to 50 years group, and those with serum sodium levels of >116 mEq/L were included in the serum sodium of 114–116 mEq/L group. We defined somnolence as drowsiness or stupor. We obtained two scores (SHOR1 score: somnolence as stupor, SHOR2 score: somnolence as drowsiness), and each SHOR score was compared with that of our predictive model. Variables with p < 0.05 were considered statistically significant. All analyses and calculations were performed using IBM SPSS version 24.0 (IBM Corp., Armonk, NY, USA), and STATA version 14.0 (StataCorp LP, College Station, TX, USA).

Results

Study population

We enrolled 178 patients who were admitted for symptomatic hyponatremia between August 24, 2016 and August 21, 2019. The mean patients’ age was 73.1 ± 12.2 years and 44.9% of the patients were male. The most common causes of hyponatremia were thiazide diuretic use (n = 53 [29.8%]), and SIAD (n = 52 [29.2%]), with almost the same incidence. These were followed by adrenal insufficiency (n = 44 [24.7%]), decreased ECF volume due to nonrenal sodium loss (n = 39 [21.9%]), and increased ECF volume (n = 19 [10.7%]). Five individuals had a history of chronic alcoholism (n = 5 [2.8%]). Forty-four patients (24.7%) had severe symptoms of hyponatremia. Serum sodium and potassium levels at admission were 118.2 ± 5.0 and 4.0 ± 0.8 mEq/L, respectively.

Incidence and risk factors for hyponatremia overcorrection

Hyponatremia overcorrection occurred in 20.8% (37 of 178) of patients. Patients were classified into overcorrection and no-overcorrection groups, and their baseline characteristics are shown in Table 1. Patients in the overcorrection group were more likely to exhibit chronic alcoholism (8.1% vs. 1.4%), severe symptoms (45.9% vs. 19.1%), and thiazide use (43.2% vs. 26.2%) than those in the no-overcorrection group. The overcorrection group demonstrated lower levels of serum sodium, potassium phosphorus, and osmolality compared with the no-overcorrection group. The overcorrection group also showed higher serum albumin, aspartate aminotransferase, alanine aminotransferase, and total bilirubin levels than those of the no-overcorrection group. The cumulative amount of hypertonic saline administered during 48 hours was 554 mL. The cumulative amounts of hypertonic saline infused during the first 1/6 hours did not differ between the groups. Interestingly, the cumulative amounts of hypertonic saline administered for 24/48 hours were significantly smaller in the overcorrection group than that in the no-overcorrection group (24 hours, 350 mL vs. 416.3 mL, p = 0.045; 48 hours, 388.5 mL vs. 598.1 mL, p < 0.001). The 48 hours urine output was higher in the overcorrection than that in the no-overcorrection group (5,663 mL vs. 3,401 mL, p < 0.001).

We conducted univariable and multivariable logistic regression analyses separately to identify independent risk factors for hyponatremia overcorrection (Table 2). The univariate analysis showed that chronic alcoholism (OR, 6.13; 95% CI, 0.99–38.16; p = 0.05), severe symptoms of hyponatremia (OR, 3.59; 95% CI, 1.66–7.76; p < 0.01), low serum potassium level (OR, 0.37; 95% CI, 0.21–0.65; p < 0.01), low serum sodium level (OR, 0.84; 95% CI, 0.77–0.90; p < 0.01), high total bilirubin level (OR, 3.06; 95% CI, 1.42–6.58; p < 0.01), high albumin level (OR, 2.38; 95% CI, 1.19–4.76; p = 0.02), and thiazide use (OR, 2.14; 95% CI, 1.01–4.54; p = 0.05) were significant risk factors for overcorrection. Multivariate analysis showed that only chronic alcoholism (OR, 15.27; 95% CI, 1.46–159.28; p = 0.02), severe symptoms of hyponatremia (OR, 2.83; 95% CI, 1.14–7.02; p = 0.03), initial serum potassium level (OR, 0.86; 95% CI, 0.79–0.94; p < 0.01), and initial serum sodium level (OR, 0.34; 95% CI, 0.17–0.67; p < 0.01) were significant risk factors for overcorrection after adjusting for all variables.

Novel risk score for hyponatremia overcorrection (NASK score)

We created a scoring system to facilitate the manual calculation of hyponatremia overcorrection risk, and we used coefficients to change each impact to integer scores for four statistically significant variables. We calculated the scores...
<table>
<thead>
<tr>
<th>Characteristics and progression</th>
<th>Total</th>
<th>No overcorrection</th>
<th>Overcorrection</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>178</td>
<td>141</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>80 (44.9)</td>
<td>65 (46.1)</td>
<td>15 (40.5)</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>73.1 ± 12.2</td>
<td>73.7 ± 11.8</td>
<td>70.5 ± 13.4</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>57.1 ± 11.6</td>
<td>56.7 ± 11.4</td>
<td>58.6 ± 12.4</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>22.6 ± 4.3</td>
<td>22.4 ± 3.9</td>
<td>23.3 ± 5.3</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Causes of hyponatremia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased ECF d/t nonrenal Na loss</td>
<td>39 (21.9)</td>
<td>32 (22.7)</td>
<td>7 (18.9)</td>
<td>0.62</td>
</tr>
<tr>
<td>Increased ECF</td>
<td>19 (10.7)</td>
<td>17 (12.1)</td>
<td>2 (5.4)</td>
<td>0.37</td>
</tr>
<tr>
<td>SIAD</td>
<td>52 (29.2)</td>
<td>43 (30.5)</td>
<td>9 (24.3)</td>
<td>0.46</td>
</tr>
<tr>
<td>Thiazide use</td>
<td>53 (29.8)</td>
<td>37 (26.2)</td>
<td>16 (43.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>44 (24.7)</td>
<td>36 (25.5)</td>
<td>8 (21.6)</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Comorbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>59 (33.1)</td>
<td>46 (32.6)</td>
<td>13 (35.1)</td>
<td>0.77</td>
</tr>
<tr>
<td>Hypertension</td>
<td>123 (69.1)</td>
<td>93 (66.0)</td>
<td>30 (81.1)</td>
<td>0.08</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>31 (17.4)</td>
<td>25 (17.7)</td>
<td>6 (16.2)</td>
<td>0.83</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>11 (6.2)</td>
<td>8 (5.7)</td>
<td>3 (8.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>4 (2.2)</td>
<td>4 (2.8)</td>
<td>0 (0)</td>
<td>0.58</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>18 (10.1)</td>
<td>15 (10.6)</td>
<td>3 (8.1)</td>
<td>0.77</td>
</tr>
<tr>
<td>Malignancy</td>
<td>42 (23.6)</td>
<td>35 (24.8)</td>
<td>7 (18.9)</td>
<td>0.45</td>
</tr>
<tr>
<td>Chronic alcoholism</td>
<td>5 (2.8)</td>
<td>2 (1.4)</td>
<td>3 (8.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Infusion mode, bolus/continuous</td>
<td>87/91</td>
<td>72/69</td>
<td>15/22</td>
<td>0.25</td>
</tr>
<tr>
<td>Symptoms, moderate/severe</td>
<td>134/44</td>
<td>114/27</td>
<td>20/17</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium sparing diuretics</td>
<td>6 (3.4)</td>
<td>6 (4.3)</td>
<td>0 (0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Furosemide</td>
<td>13 (7.3)</td>
<td>11 (7.8)</td>
<td>2 (5.4)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>SSRI</td>
<td>13 (7.3)</td>
<td>8 (5.7)</td>
<td>5 (13.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>AED</td>
<td>22 (12.4)</td>
<td>14 (9.9)</td>
<td>8 (21.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>NSAID</td>
<td>42 (23.6)</td>
<td>33 (23.4)</td>
<td>9 (24.3)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>139.2 ± 25.3</td>
<td>138.3 ± 25.2</td>
<td>142.6 ± 25.7</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>75.7 ± 13.8</td>
<td>75.2 ± 13.0</td>
<td>77.4 ± 16.8</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Laboratory values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (mEq/L)</td>
<td>118.2 ± 5.0</td>
<td>119.2 ± 4.2</td>
<td>114.5 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum osmolality (mOsm/kg)</td>
<td>251.1 ± 21.6</td>
<td>252.2 ± 16.3</td>
<td>246.6 ± 35.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0 ± 0.8</td>
<td>1.0 ± 0.9</td>
<td>0.8 ± 0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>White blood cell (×10^9/L)</td>
<td>8.5 ± 4.0</td>
<td>8.4 ± 3.8</td>
<td>9.2 ± 4.9</td>
<td>0.34</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.1 ± 2.0</td>
<td>12.0 ± 2.0</td>
<td>12.3 ± 1.7</td>
<td>0.48</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0 ± 0.6</td>
<td>3.9 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.7 ± 0.5</td>
<td>8.7 ± 0.5</td>
<td>8.7 ± 0.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Phosphorous (mg/dL)</td>
<td>3.1 ± 0.9</td>
<td>3.1 ± 1.0</td>
<td>2.7 ± 0.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.0 ± 0.8</td>
<td>4.1 ± 0.7</td>
<td>3.6 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total CO₂ (mEq/L)</td>
<td>23.2 ± 5.0</td>
<td>23.2 ± 5.3</td>
<td>23.0 ± 3.8</td>
<td>0.59</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>155.3 ± 44.7</td>
<td>154.8 ± 41.8</td>
<td>157.4 ± 54.8</td>
<td>0.79</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.4</td>
<td>1.1 ± 0.6</td>
<td>0.003</td>
</tr>
<tr>
<td>AST (units/L)</td>
<td>35.0 ± 29.2</td>
<td>32.7 ± 27.2</td>
<td>43.7 ± 34.8</td>
<td>0.01</td>
</tr>
<tr>
<td>ALT (units/L)</td>
<td>20.2 ± 17.0</td>
<td>19.1 ± 17.7</td>
<td>24.5 ± 13.8</td>
<td>0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>28.3 ± 48.5</td>
<td>30.0 ± 50.3</td>
<td>21.9 ± 40.8</td>
<td>0.21</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>424.1 ± 164.2</td>
<td>424.7 ± 170.4</td>
<td>421.9 ± 139.9</td>
<td>0.85</td>
</tr>
<tr>
<td>Urine Na (mEq/L)</td>
<td>72.1 ± 49.3</td>
<td>71.8 ± 48.7</td>
<td>73.2 ± 52.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Urine K (mEq/L)</td>
<td>34.6 ± 22.5</td>
<td>34.2 ± 23.5</td>
<td>36.1 ± 18.8</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Cumulative amount of 3% saline volume (mL)

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>127.4 ± 107.6</td>
</tr>
<tr>
<td>6 hr</td>
<td>241.5 ± 115.4</td>
</tr>
<tr>
<td>24 hr</td>
<td>402.5 ± 257.3</td>
</tr>
<tr>
<td>48 hr</td>
<td>554 ± 347.9</td>
</tr>
</tbody>
</table>

Urine volume during 48 hr (mL)

<table>
<thead>
<tr>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,862 ± 2,830</td>
</tr>
</tbody>
</table>

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

AED, antiepileptic drug; ALT, alanine aminotransferase; AST, aspartate transaminase; BP, blood pressure; d/t, due to; ECF, extracellular fluid; Na, sodium; NSAID, nonsteroidal anti-inflammatory drug; SIAD, syndrome of inappropriate antidiuresis; SSRI, selective serotonin reuptake inhibitor.
using risk factors for overcorrection based on multivariable analysis as described in Supplementary Table 1 (available online). The NASK (hypoNatremia, Alcoholism, Severe symptoms, and hypoKalemia) score was calculated as the arithmetic sum of the points for each of these variables. The scores were as follows: chronic alcoholism, 7 points; severe symptoms of hyponatremia, 3 points; low serum potassium level of <3.0 mEq/L, 3 points; and initial sodium level of ≤110, 110–115, 115–120, and 120–125 mEq/L were scored as 7, 4, 2, and 0 points, respectively. NASK score was significantly associated with overcorrection (OR, 1.41; 95% CI, 1.24–1.61; p < 0.01) (Fig. 1). The receiver-operating characteristic curves for each risk factor and NASK score with regard to overcorrection are shown in Fig. 2. NASK score had a good discriminatory ability, with an AUROC curve of 0.757 (95% CI, 0.66–0.85; p < 0.01). The AUROC was 0.752 (95% CI, 0.67–0.84; p = 0.88), 0.691 (95% CI, 0.60–0.79; p = 0.27), 0.634 (95% CI, 0.55–0.72; p < 0.01), and 0.533 (95% CI, 0.49–0.58; p < 0.01) for the baseline serum sodium level, baseline serum potassium level, severity of hyponatremia symptoms, and chronic alcoholism, respectively. The AUROC curve of the NASK score was significantly bet-
Comparison of the predictive abilities of NASK and SHOR scores

We compared the predictive ability of NASK and SHOR1/SHOR2 scores for hyponatremia overcorrection using AUROC curve analysis (Supplementary Fig. 2, available online). The AUROC curve were 0.757 (95% CI, 0.66–0.85; p < 0.01), 0.722 (95% CI, 0.63–0.82; p = 0.46), 0.722 (95% CI, 0.63–0.82; p = 0.47) for NASK, SHOR1, and SHOR2 scores, respectively. The AUROC curve for NASK score was higher than those of SHOR1 and SHOR2 scores, although there were no significant differences between the values.

Cumulative hyponatremia overcorrection and predictive ability of NASK score by time

We subdivided the overcorrection group by time (within the first 6, 12, 24, and 48 hours) to evaluate whether hyponatremia overcorrection risk factors changed according to time. Overcorrection occurred in 5.1% (n = 9), 6.7% (n = 12), 18.5% (n = 33), and 20.8% (n = 37) of 178 patients within the first 6, 12, 24, and 48 hours, respectively. We analyzed the predictive ability of the four risk factors for overcorrection and NASK score by time based on age, sex, and hypertonic saline infusion method. The four identified risk factors (initial serum sodium level, chronic alcoholism, initial symptoms, and initial serum potassium level) and NASK score had significant predictive abilities for cumulative overcorrection within 6, 12, 24, and 48 hours, excluding initial potassium level within 6 hours and chronic alcohol-
ism within 48 hours (Fig. 3, Fig. 4; Supplementary Table 2, available online).

Urine output over 48 hours based on hyponatremia overcorrection risk factors

The 48 hours urine output was higher in chronic alcoholics than in non-alcoholics (5,688 mL vs. 3,809 mL, p = 0.08), although the difference was not significant. Furthermore, the urine output was higher in patients with severe symptoms than that of patients with moderate symptoms (5,231 mL vs. 3,419 mL, p < 0.01). Patients with severe hyponatremia had a higher urine output than in those with less severe hyponatremia (6,025, 4,158, 3,767, 3,378 mL for patients with serum sodium levels of ≤110, 110–115, 115–120, and ≥120 mEq/L, respectively; p = 0.02). Moreover, there was a negative correlation between hypokalemia and urine output based on linear regression analysis (β = -0.216, p < 0.01). NASK score also was positively associated with 48 hours urine output (β = 304.5, p < 0.001).

Discussion

In this post hoc analysis of a prospective randomized controlled study, we aimed to evaluate risk factors for hyponatremia overcorrection and to establish a novel scoring system for predicting overcorrection. We identified chronic alcoholism and severe symptoms of hyponatremia as well as lower baseline serum sodium and potassium levels as significant risk factors for hyponatremia overcorrection. The NASK score, an arithmetic sum of the points for each factor after converting the influence of each factor to integer scores, had a higher predictive ability for hyponatremia overcorrection than each factor. Chronic alcoholism, severe symptoms of hyponatremia, low baseline serum sodium levels, and NASK score were risk factors for overcorrection at any time within 48 hours. However, low baseline serum potassium was a risk factor for overcorrection only after 6 hours.

We found that chronic alcoholism, initial serum sodium level, severity of initial symptoms, and initial serum potassium level were significant patient baseline factors that affected the incidence of hyponatremia overcorrection. Alcohol suppresses the endogenous release of antidiuretic hormone (ADH) and occasionally causes free water diuresis when consumed. However, continuous alcohol consumption increases ADH levels, thereby causing water retention. Water and electrolyte retention are resolved
within 3 to 6 days after alcohol discontinuation in chronic alcoholics [28,29]. When chronic alcoholics are admitted for hyponatremia, they undergo a period of alcohol withdrawal. Therefore, chronic alcoholics have an increased overcorrection risk during hypertonic saline treatment due to increased diuresis as confirmed by our findings. Previous studies have revealed that lower initial sodium levels [14,17,18,21] and severe symptoms of hyponatremia [17,21] are risk factors for hyponatremia overcorrection, in line with our finding. Hyponatremia overcorrection mainly arises from hypertonic saline treatment or water diuresis. Hyponatremia causes a hypo-osmolar state, leading to a decreased release of ADH and an increase in free water excretion. Supplying hypertonic saline in patients with hyponatremia induces an increase in ADH level and a decrease in water clearance, which occur at a slower rate in patients with severe hyponatremia [30]. However, we cannot explain the mechanism underlying free water excretion using our data because we did not collect urine sodium and potassium levels, as well as osmolality during the 48-hour follow-up. Symptoms of hyponatremia were classified into two groups, and the initial hypertonic saline infusion rate was decided based on symptom severity. A higher amount of hypertonic saline was administered in patients with severe symptoms compared with that in patients with moderate symptoms (317 mL vs. 217 mL, p < 0.01; within the first 6 hours). In line with previous studies [14,17], we found that lower initial potassium levels were associated with hyponatremia overcorrection occurrence. In the Adrogue-Madias formula, replacing potassium plays a significant role in correcting hyponatremia [7,31]. The loss of sodium or potassium induces an osmolar shift to maintain the osmolar balance between the extracellular and intracellular spaces [32]. Potassium loss shifts sodium intracellularly, induces hyponatremia, and enhances ADH release, thereby worsening hyponatremia [33]. Moreover, potassium restriction reportedly increases free water clearance [34]. Therefore, hypokalemia can increase the incidence of hyponatremia overcorrection by increasing diuresis, as confirmed by our findings. Not only each factor but also the NASK score, which combines the scores of these factors, had a positive relationship with the 48 hours urine output.

In addition to the four risk factors identified in our study, previous studies have identified younger age, higher infusion volume, lower urine osmolality, and lower urine sodium levels as risk factors for hyponatremia overcorrection [14,21]. Volume overload and the presence of chest tumor are negatively associated with hyponatremia overcorrection [17]. Woodfine et al. [17] established a novel scoring system (the SHOR score) to predict hyponatremia overcorrection, with risk factors such as age, vomiting, somnolence, volume overload, initial serum sodium level, initial serum potassium level, urine osmolality, and the presence of chest tumor. Our study found the following risk factors for hyponatremia overcorrection: chronic alcoholism, initial serum sodium level, severity of initial symptoms, and initial serum potassium level. Moreover, we developed a scoring system (NASK score) to aid clinicians in quantitatively stratifying an individual’s risk for hyponatremia overcorrection. We compared the predictive ability of hyponatremia overcorrection between the NASK scoring system and the SHOR scoring system [17]. The AUROC curve for NASK score was greater than those for SHOR1 and SHOR2 scores but without statistical significance (Supplementary Fig. 2). Nevertheless, NASK score is easy to calculate as it requires only four factors, whereas SHOR scores require eight discrete factors. Furthermore, NASK score seems to predict hyponatremia overcorrection better than SHOR scores.

This study has several strengths. First, we obtained complete baseline characteristics as well as laboratory data on all patients because of the prospective nature of the original study. We also assessed the cumulative hyponatremia overcorrection rate and verified the predictive ability of each risk factor and the NASK score for the overcorrection rate. Second, in comparison with other retrospective studies, we set up the treatment protocol with hypertonic saline and serum sodium rerowering treatment following international guideline recommendations (RIB and SCI). This allowed us to correct the impact of the infusion method of hypertonic saline on treatment outcomes. Therefore, we were able to establish a more explainable scoring system using baseline characteristics. There are several studies or guidelines regarding the adequate amount of hypertonic saline and the required rate for effective hyponatremia treatment and hyponatremia overcorrection prevention. However, inadvertent hyponatremia overcorrection occurs because of unanticipated water diuresis, even when the recommended quantity of hypertonic saline is administrated at a recommended rate or when hypertonic saline administration is
stopped. Using this scoring system, physicians can predict hyponatremia overcorrection occurrence before hypertonic saline administration; patients with high NASK scores and increased diuresis can undergo careful monitoring of their status and laboratory data during treatment.

This study also has several limitations. First, due to the prospective design, our study population was smaller than those of other retrospective studies. Second, serum sodium levels were obtained from the three study sites using different measuring machines that were not calibrated. Variabilities in serum sodium assays using each device were inevitable due to the post hoc study design. Third, low sensitivity and positive predictive value were imperative, as the prevalence of chronic alcoholism was low. We additionally performed sensitivity analyses with the NSK (Na, symptoms of hyponatremia, and K) score (total, ≤13) and NK (Na and K) score (total, ≤10) for hyponatremia overcorrection models. The AUROC curves for NSK and NK scores were 0.745 (95% CI, 0.65–0.84; p < 0.01) and 0.721 (95% CI, 0.63–0.81; p < 0.01), respectively. Fourth, the findings were not validated in an external cohort of patients with hyponatremia. A validation study with a larger population should be conducted to confirm our study findings. Specifically, further studies should clarify whether treatment policies should be modified based on the finding that the NASK score influences hyponatremia overcorrection incidence and prognosis. Fifth, some studies have reported a relationship between the occurrences of hyponatremia overcorrection and ODS, which is a critical outcome of hyponatremia overcorrection [11,14,17,24,35,36]. However, there was no ODS in 20.8% of patients with hyponatremia overcorrection, although risk factors for overcorrection were identified to prevent ODS in patients with hyponatremia by reducing overcorrection occurrence. Hyponatremia overcorrection is considered a good laboratory outcome that predicts ODS occurrence because it can be monitored. Moreover, it is a correctable factor during hypertonic saline treatment.

In conclusion, hyponatremia overcorrection occurred in 20.8% of patients who underwent hypertonic saline treatment. The risk factors for hyponatremia overcorrection included chronic alcoholism and severe symptoms of hyponatremia as well as lower initial serum sodium and potassium levels. In patients undergoing symptomatic hyponatremia treatment, overcorrection might be quantitatively predicted using a novel risk score that is summarized by patient baseline information. External validation studies of the NASK score are required to clarify our results.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

The study was supported by grants from the National Research Foundation of Korea (No. 2021R1C1C1008966 and 2019R1A2C1085411).

**Authors’ contributions**

Conceptualization: HY, SHB, SK
Data curation: SHB, SK
Formal analysis: HY, SHB, SK
Funding acquisition: SHB, SK
Technical and material support: All authors
Visualization: HY, SHB, SK
Writing–original draft: HY, SHB, SK
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Role of bicarbonate and volume therapy in the prevention of acute kidney injury in rhabdomyolysis: a retrospective propensity score-matched cohort study

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Background: Although bicarbonate has traditionally been used to treat patients with rhabdomyolysis at high risk of acute kidney injury (AKI), it is unclear whether this is beneficial. This study compared bicarbonate therapy to non-bicarbonate therapy for the prevention of AKI and mortality in rhabdomyolysis patients.

Methods: In a propensity score-matched cohort study, patients with a creatine kinase (CK) level of >1,000 U/L during hospitalization were divided into bicarbonate and non-bicarbonate groups. Patients were subgrouped based on low-volume (<3 mL/kg/hr) or high-volume (≥3 mL/kg/hr) fluid resuscitation in the first 72 hours. Logistic regression analyses were used to identify the impacts of bicarbonate use and fluid resuscitation on AKI risk and need for dialysis. The Kaplan-Meier method was used to estimate survival. Volume overload and electrolyte imbalances were assessed.

Results: Among 4,077 patients, we assembled a cohort of 887 pairs of patients treated with and without bicarbonate. Bicarbonate group had a higher incidence of AKI, higher rate of dialysis dependency, higher 30-day mortality, and longer hospital stay than the non-bicarbonate group. Further, patients who received high-volume fluid therapy had worse renal outcomes and a higher mortality than those who received low-volume fluids regardless of bicarbonate use. Bicarbonate use, volume overload, and AKI were associated with higher mortality. Volume overload was significantly higher in the bicarbonate group than in the non-bicarbonate group.

Conclusion: Bicarbonate or high-volume fluid therapy for patients with rhabdomyolysis did not reduce AKI or improve mortality compared to non-bicarbonate or low-volume fluid therapy. Limited use of bicarbonate and adjustment of fluid volume may improve the short- and long-term outcomes of patients with rhabdomyolysis.

Keywords: Acute kidney injury, Rhabdomyolysis, Sodium bicarbonate

Received: April 20, 2021; Revised: September 30, 2021; Accepted: October 10, 2021
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Introduction

Acute kidney injury (AKI) occurs in 14% to 46% of patients with rhabdomyolysis [1]. When muscle cells are damaged, intracellular myoglobin is released into the circulation causing renal damage due to direct tubule injury, tubular obstruction, or renal vasoconstriction. Subsequently, a large amount of water is rapidly expelled into the extracellular fluid, causing a decreased glomerular filtration rate with worsening of AKI [2]. Life-threatening AKI leads to electrolyte and volume imbalances, which can cause arrhythmia or cardiac arrest, and renal replacement therapy is required. de Meijer et al. [3] reported that mortality in patients who developed AKI was twice as high as in those who did not. Therefore, for patients with severe rhabdomyolysis with a high risk of AKI and death, judicious and timely treatment remains essential.

The hypotheses that acidic urine worsens acute tubulonephropathy and that the risk of AKI is associated with dehydration were used to support bicarbonate therapy for patients with rhabdomyolysis, wherein bicarbonate inhibits myoglobin cast formation, and a large amount of fluid results in solute diuresis due to alkalization of the urine [4]. However, there are only a few studies, with no clear consensus, on whether the routine use of bicarbonate can prevent the development of AKI [5,6]. No randomized controlled clinical trials have compared bicarbonate therapy with fluid therapy alone. Indeed, some studies suggested that early, rather than late initiation of fluid therapy can help improve outcomes [7–9]; however, fluid type and target fluid volume, duration of therapy, monitoring parameters, target urine output, and the onset of initiation of fluid therapy used in these studies varied widely. Various studies have attempted to identify the optimal fluid therapy in various diseases or situations, such as renal transplant [10]. However, no prior study has investigated what fluid is optimal for the treatment of rhabdomyolysis despite the importance of fluid therapy in this condition. Thus, we investigated whether bicarbonate therapy could prevent AKI compared to fluid therapy alone and assessed the effect of fluid volume on patient outcomes. Further, we analyzed predictors of AKI, dialysis, and death in patients hospitalized for rhabdomyolysis.

Methods

Study design

We conducted a propensity score-matched cohort study in Seoul National University Bundang Hospital (a 1,334-bed, public, university-affiliated, teaching hospital). Patients with a creatine kinase (CK) level of >1,000 U/L during hospitalization were divided into two groups; patients who received fluid with bicarbonate and those who received fluid without bicarbonate. Patients were subgrouped based on low-volume (<3 mL/kg/hr) and high-volume (≥3 mL/kg/hr) fluid resuscitation in the first 72 hours of admission. Primary outcomes were the development of AKI, the incidence of dialysis, and mortality in the propensity score-matched cohort of patients exposed and unexposed to bicarbonate. The study protocol complied with the Declaration of Helsinki and received ethics approval from the Institutional Review Board of Seoul National University Bundang Hospital (No. B-1809/495-105); the need for informed consent was waived as the study did not infringe on patient privacy or health status.

Study population

We identified 6,492 hospitalized patients with a CK level of >1,000 U/L between March 2003 and August 2018. Based on preestablished exclusion criteria, we excluded children and adolescents under 18 years (n = 130) and patients with chronic kidney disease (CKD) undergoing renal replacement treatment (n = 684) or with cardiac enzyme elevation owing to myocardial infarction or cardiomyopathy (n = 1,601). Finally, 4,077 patients were included in this study. The study flow chart is presented in Fig. 1.

Data collection

All clinical records and laboratory data, including age, sex, length of hospital stay, inpatient vital signs, and laboratory values such as levels of serum CK, blood urea nitrogen (BUN), serum creatinine (Cr), sodium, potassium, serum bicarbonate measured as total carbon dioxide (TCO₂), calcium, and albumin, were gathered from electronic medical records. Shock was defined as an initial systolic blood pressure (BP) of <90 mmHg; additionally, a BUN/Cr ratio of >20
was used as a surrogate marker of prerenal status; that is, volume depletion. An increase of over 10% in body weight during hospitalization was regarded as volume overload. Mortality and date of death were determined from death certificates as well as the database of the Ministry of Interior and Safety of Korea.

Definitions and measurements

Onset of rhabdomyolysis was recorded as the first day of hospitalization of the patient and the occurrence of AKI was monitored throughout the hospitalization period. AKI was defined as a Cr level increase of ≥1.5 times or ≥0.3 mg/dL and staged according to the Clinical Practice Guideline for the Evaluation and Management of Kidney Disease: Improving Global Outcomes (KDIGO) Group. Stage 2 AKI was defined as an increase in the Cr to ≥2 times the baseline. Stage 3 AKI was defined as a Cr of ≥3.0 or more times the baseline, an increase in Cr of ≥4.0 mg/dL, or the initiation of renal replacement therapy regardless of previous KDIGO.
We used the preadmission Cr value as the baseline Cr level. If these values were not available, we used the lowest Cr level measured during hospitalization. We confirmed cases and dates of dialysis by evaluating the Korean Society of Nephrology end-stage renal disease registry.

**Statistical analyses**

Baseline characteristics were analyzed as frequencies and percentages for categorical variables and as means and standard deviation (SD) for continuous variables. Unadjusted associations between the covariates and the primary outcomes were analyzed using the chi-square test for categorical data and Student t test or Mann-Whitney U test for continuous data. To minimize selection bias, propensity score-matched logistic regression analysis was performed for the likelihood of bicarbonate use. A propensity score was calculated using the baseline covariates of age, sex, underlying comorbidities (diabetes, hypertension, heart failure, liver failure, and CKD), cause of rhabdomyolysis (e.g. statins), shock (systolic BP < 90 mmHg), BUN/Cr ratio of >20 as a surrogate marker for volume depletion, fluid amount level, initial serum TCO₂ level at admission, baseline Cr levels, and baseline CK levels (Supplementary Fig. 1, available online). The propensity score was estimated using logistic regression of the use of bicarbonate on the covariates. We used 1:1 nearest neighbor propensity score matching without replacement based on the propensity score estimated using logistic regression of the treatment on the covariates. Caliper width was set to 0.2 of the SD of the logit of the propensity score. Adequacy of balance for the covariates in the matched samples was assessed using the standardized mean difference between the two groups, with differences of <10% reflecting good balance. Multiple logistic regression analysis models were used to identify adjusted odds ratios (ORs) for AKI and dialysis. Restricted cubic spline analysis was performed to explore the nonlinear relationship between outcomes and both serum bicarbonate levels and fluid volume. Cutoff values were calculated from maximally selected log-rank statistics using the condMC method. Comparisons between prerenal (baseline BUN/Cr ratio > 20) and normal (BUN/Cr ratio ≤ 20) groups, and between shock (systolic BP < 90 mmHg) and normal (systolic BP ≥ 90 mmHg) groups were performed with high-volume fluid therapy and with the consequences of volume overload, AKI, dialysis, and death within 30 days as outcomes. In addition to examining the individual effects of bicarbonate and high-volume fluid therapy on the outcome variables, further analyses were conducted to determine additional risks caused by the interaction between the use of bicarbonate and amount of fluid provided using the Relative Excess Risk due to Interaction (RERI). In brief, the RERI metric reflects the additive interaction of two risk factors. If an interaction between risk factor A and risk factor B is present, the combined effect of A and B is greater or smaller than the sum of the individual effects of A and B. RERI = 0 means no interaction or exact additivity; RERI > 0 means a positive interaction or more than additivity; and RERI < 0 means a negative interaction. Besides RERI, interaction on an additive scale includes an attributable proportion (AP); the synergy index (S) was also computed. AP is the proportion of disease in the doubly exposed group that is attributable to the interaction and S is the ratio of the combined effect and the sum of the individual effects. In the absence of an additive interaction effect, AP equals 0 and S equals 1 [12]. Kaplan-Meier method was used to estimate survival and survival was compared between groups using a log-rank test. A Cox proportional hazards model was used to calculate hazard ratios (HRs) for AKI and death. Logistic and Cox regression models were adjusted based on relevant baseline covariates. To estimate the significance of differences in HRs for bicarbonate use, subgroup analysis was performed using multivariate Cox proportional hazard regression, and a p-value for the interaction was estimated. R version 3.6.3 (R Foundation for Statistical Computing Platform) or IBM SPSS Statistics for Windows, version 23 (R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses.

**Results**

**Participant characteristics**

Mean age of all study participants was 57.9 ± 20.8 years, and 66.7% of participants were male. Baseline Cr level was 1.0 ± 0.8 mg/L, and 1.2% of participants had CKD as a baseline comorbidity prior to hospitalization for rhabdomyolysis. Based on medical records, we classified the cause of rhabdomyolysis as medical or surgical, with 1,834 medical (45.0%) and 2,243 surgical cases (55.0%). Detailed causes
### Table 1. Selected baseline characteristics and outcomes according to bicarbonate use in propensity-matched patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total population</th>
<th>After propensity score matching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No bicarbonate</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>No. of patients</td>
<td>1,584</td>
<td>2,493</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>61.1 ± 17.8</td>
<td>60.5 ± 17.7</td>
</tr>
<tr>
<td>Male sex</td>
<td>1,077 (68.0)</td>
<td>1,644 (65.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.2 ± 6.3</td>
<td>23.9 ± 8.7</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>78 (4.9)</td>
<td>121 (4.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>92 (5.8)</td>
<td>150 (6.0)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>12 (0.8)</td>
<td>38 (1.5)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>0 (0)</td>
<td>3 (0.1)</td>
</tr>
<tr>
<td>Liver failure</td>
<td>1 (0.1)</td>
<td>20 (0.8)</td>
</tr>
<tr>
<td>Cause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td>869 (54.9)</td>
<td>1374 (55.1)</td>
</tr>
<tr>
<td>Medical</td>
<td>715 (45.1)</td>
<td>1,119 (44.9)</td>
</tr>
<tr>
<td>Baseline Cr (mg/dL)</td>
<td>0.9 ± 0.6</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>Baseline serum CK (IU/L)</td>
<td>1,330 (527–2,599)</td>
<td>1,133 (287–3,194)</td>
</tr>
<tr>
<td>Baseline serum TCO₂ (mEq/L)</td>
<td>22.4 ± 3.6</td>
<td>20.7 ± 5.0</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, number (%), or median (interquartile range). CK, creatine kinase; Cr, serum creatinine; NA, not applicable; TCO₂, total carbon dioxide.

### Table 2. Outcomes of the study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total population</th>
<th>After propensity score matching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No bicarbonate</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>1.4 ± 1.3</td>
<td>2.4 ± 2.0</td>
</tr>
<tr>
<td>At discharge</td>
<td>0.9 ± 0.7</td>
<td>1.3 ± 1.2</td>
</tr>
<tr>
<td>Follow-up</td>
<td>0.9 ± 0.7</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Serum CK (IU/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>1,945 (1,331–3,609)</td>
<td>3,125 (1,589–7,893)</td>
</tr>
<tr>
<td>At discharge</td>
<td>985 (279–1,563)</td>
<td>962 (204–2,277)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>77 (41–151)</td>
<td>62 (33–124)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI</td>
<td>730 (46.1)</td>
<td>1,974 (79.2)</td>
</tr>
<tr>
<td>AKI stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>854 (53.9)</td>
<td>519 (20.8)</td>
</tr>
<tr>
<td>1</td>
<td>409 (25.8)</td>
<td>617 (24.7)</td>
</tr>
<tr>
<td>2</td>
<td>175 (11.0)</td>
<td>433 (17.4)</td>
</tr>
<tr>
<td>3</td>
<td>146 (9.2)</td>
<td>924 (37.1)</td>
</tr>
<tr>
<td>Dialysis</td>
<td>25 (1.6)</td>
<td>473 (19.0)</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>22.0 ± 22.7</td>
<td>26.6 ± 54.4</td>
</tr>
<tr>
<td>30-Day mortality</td>
<td>96 (6.1)</td>
<td>659 (26.4)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>495 (31.3)</td>
<td>1,126 (45.2)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, median (interquartile range), or number (%). CK, creatine kinase; AKI, acute kidney injury.
of rhabdomyolysis are described in Supplementary Table 1 (available online). Bicarbonate-containing solution was administered to 2,493 patients (61.1%) during hospitalization. Patients from the bicarbonate group tended to be older and to have higher baseline and peak Cr levels (Table 1). Serum CK level at presentation and peak serum CK level during hospitalization were higher in the bicarbonate group than in the non-bicarbonate group.

**Bicarbonate use and outcomes**

A significantly higher incidence of AKI development was observed in the bicarbonate group than in the non-bicarbonate group (n = 1,974 [79.2%] vs. n = 730 [46.1%], p < 0.001) (Table 2; Supplementary Fig. 2A, available online).

Multivariate logistic regression analysis for AKI showed that the use of bicarbonate was independently associated with increased risk of AKI development, with an OR of 3.17 (95% confidence interval [CI], 2.62–3.85, p < 0.001).

In the propensity score-matched model, bicarbonate users still had a higher risk of AKI than the non-bicarbonate group (Supplementary Fig. 2B). Moreover, the bicarbonate group showed a statistically higher risk of dialysis dependency (n = 129 [14.5%] vs. n = 16 [1.8%], p < 0.001), extended hospital stay (28.1 ± 59.6 days vs. 22.1 ± 21.7 days, p = 0.005) (Table 2), and higher mortality than the non-bicarbonate group (Supplementary Table 2, available online). Using multivariate logistic regression analysis, use of bicarbonate (OR, 8.97; 95% CI, 5.05–15.91), medical cause of rhabdomyolysis (OR, 1.90; 95% CI, 1.28–2.81), use of diuretics (OR, 1.90; 95% CI, 1.28–2.81), baseline Cr level (OR, 3.44; 95% CI, 2.68–4.40), and medical cause (OR, 3.17; 95% CI, 2.62–3.85) were independently associated with increased risk of AKI development. Use of bicarbonate was also associated with increased risk of dialysis dependency (OR, 8.97; 95% CI, 5.05–15.91), medical cause of rhabdomyolysis (OR, 1.90; 95% CI, 1.28–2.81), and baseline Cr level (OR, 3.44; 95% CI, 2.68–4.40).

**Figure 2.** ORs for the development of AKI and the initiation of dialysis based on multivariate logistic regression of the propensity-matched cohort.

AKI, acute kidney injury; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; Cr, serum creatinine; DM, diabetes mellitus; HTN, hypertension; OR, odds ratio; PSM, propensity score matching.

*p < 0.05, **p < 0.01, ***p < 0.001.
of diuretics (OR, 1.89; 95% CI, 1.08–3.30), higher baseline Cr (OR, 1.81; 95% CI, 1.39–2.34), and high-volume fluid resuscitation (OR, 1.48; 95% CI, 1.00–2.19) were independent risk factors for the need for dialysis (Fig. 2). Kaplan-Meier curves estimating the probability of all-cause mortality showed a significantly higher risk in the bicarbonate group than in the non-bicarbonate group (Fig. 3). Kaplan-Meier curves relating bicarbonate use to all-cause mortality. (A) For the total population. (B) For the propensity-matched patients.

Medical cause (adjusted HR [aHR], 1.8; 95% CI, 1.57–2.21), volume overload (aHR, 1.47; 95% CI, 1.18–1.82), higher AKI stage (aHR, 1.23; 95% CI, 1.26–1.47), bicarbonate use (aHR, 1.33; 95% CI, 1.12–1.59), and higher baseline Cr level (aHR, 1.16; 95% CI, 1.05–1.29) were significant predictors of mortality in the multivariate Cox proportional regression analysis (Supplementary Fig. 3C, available online). Moreover, we observed that the hazards of AKI, dialysis, and mortality increased as peak serum bicarbonate, measured as the TCO₂, increased to above the normal range in restricted cube splines (Supplementary Fig. 4, available online).

Differences in the hazard ratio for the development of acute kidney injury according to use of bicarbonate in prespecified strata

Subgroup analysis suggested a stronger association between bicarbonate use and AKI in patients with a surgical cause of rhabdomyolysis (OR, 1.73; 95% CI, 1.50–1.73) than in patients with a medical cause (OR, 1.56; 95% CI, 1.35–1.81) (p for interaction < 0.001), although bicarbonate use was significantly associated with AKI in patients with either medically or surgically-induced rhabdomyolysis (Fig. 4). Irrespective of age, sex, underlying comorbidities except for CKD, fluid amount, baseline serum TCO₂, BUN/Cr ratio, or BP, the use of bicarbonate was associated with AKI across subgroups of patients (Fig. 4).

Fluid resuscitation and outcomes

Slightly more patients received low-volume fluid resuscitation than high-volume fluid resuscitation (53.7% and 46.3%, respectively). Patients administered a high volume of fluid had a higher incidence of AKI regardless of the use of bicarbonate (AKI: OR, 2.09; 95% CI, 1.82–2.41; p < 0.001; dialysis: OR, 2.05; 95% CI, 1.68–2.49; p < 0.001; AKI in the propensity score-matched cohort: OR, 1.40; 95% CI, 1.14–1.73; p = 0.001; and dialysis in the propensity score-matched cohort: OR, 1.28; 95% CI, 0.90–1.82; p = 0.163) (Fig. 5). Hazards of AKI, dialysis, and mortality increased as the amount of fluid received increased during the first 72 hours of treatment (Supplementary Fig. 4). According to the estimated cutoff value calculated using the maximally
Figure 4. Differences in HRs for the development of AKI with respect to the use of bicarbonate in prespecified strata.
AKI, acute kidney injury; BP, blood pressure; BUN/Cr, blood urea nitrogen to creatinine; CKD, chronic kidney disease; DM, diabetes mellitus; HTN, hypertension; HR, hazard ratio; TCO\textsubscript{2}, total carbon dioxide.

Figure 5. Rate of AKI, severe AKI, dialysis, and mortality according to the amount of fluid treatment for rhabdomyolysis. (A) Total population. (B) After propensity score matching. AKI, acute kidney injury.
selected log-rank statistics with the condMC method, over 5.5 mL/kg/hr of fluid for a period of 72 hours was associated with higher mortality (p < 0.001). The low-volume fluid group had a lower risk of mortality in Kaplan-Meier analysis, and the cutoff fluid rate of 5.5 mL/kg/hr maximized the difference in survival estimates (Supplementary Fig. 5, available online).

As expected, patients in the prerenal (baseline BUN/Cr ratio > 20) and shock (systolic BP < 90 mmHg) groups received high-volume fluid therapy and had higher rates of volume overload, AKI, dialysis, and death within 30 days. In subgroup analyses stratified by both BP status (normal BP vs. shock) and volume status (BUN/Cr ratio of ≤20 vs. >20), high-volume fluid therapy was associated with a significantly higher rate of AKI, dialysis, severe AKI, and death in 30 days in every subgroup except the shock group, indicating that high-volume fluid therapy itself has a negative effect on prognosis (Supplementary Fig. 5, available online).

### Interaction between use of bicarbonate and fluid amount

Given that the use of both bicarbonate and high-volume fluid therapies were associated with a poor prognosis, we evaluated additive risks using the RERI and found a significant additive interaction between bicarbonate and high-volume fluid therapy and had higher rates of volume overload, AKI, dialysis, and death within 30 days. In subgroup analyses stratified by both BP status (normal BP vs. shock) and volume status (BUN/Cr ratio of ≤20 vs. >20), high-volume fluid therapy was associated with a significantly higher rate of AKI, dialysis, severe AKI, and death in 30 days in every subgroup except the shock group, indicating that high-volume fluid therapy itself has a negative effect on prognosis (Supplementary Table 3, available online).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No bicarbonate</th>
<th>Bicarbonate</th>
<th>OR for bicarbonate group within strata of the fluid group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>299/309</td>
<td>418/156</td>
<td>1.62 (1.39–1.87)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.0 (reference)</td>
<td>1.62</td>
<td>1.62</td>
</tr>
<tr>
<td>High-volume fluid</td>
<td>152/127</td>
<td>253/60</td>
<td>1.81 (1.53–2.14)</td>
</tr>
<tr>
<td>OR for the fluid group within strata of the bicarbonate group</td>
<td>1.14</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

Measure of interaction on additive scale (95% CI): relative excess risk due to interaction, 1.56 (0.86–2.27); attributable proportion due to interaction, 0.56 (0.42–0.71); and synergistic index, 8.65 (2.02–37.04).

CI, confidence interval; OR, odds ratio.

With/without acute kidney injury.

### Discussion

In this retrospective, propensity score-matched cohort study involving patients with rhabdomyolysis, we concluded that bicarbonate therapy increased AKI risk, the need for dialysis, and mortality. Additional hypervolemic treatment also appeared to be deleterious as it was associated with volume overload and resulted in poor renal outcomes. Early fluid resuscitation and bicarbonate therapy had been advocated since a 1984 study of seven participants with crush injuries reported treatment by the induction of
alkaline solute diuresis [13]; since then, however, results concerning the outcomes of bicarbonate treatment for the prevention of AKI have been conflicting [5,14–17]. In a previous retrospective study of more than 2,000 patients with traumatic rhabdomyolysis, bicarbonate with mannitol did not prevent AKI, the need for dialysis, or decrease mortality. Thus, the authors concluded that the use of bicarbonate with mannitol should be reevaluated [6]. Several meta-analyses have noted the lack of prospective controlled trials comparing bicarbonate therapy with fluid therapy alone for preventing rhabdomyolysis-induced AKI [8,9].

In our study, higher rates of AKI in the bicarbonate group might have resulted from several factors. First, clinicians may have used bicarbonate therapy a priori in patients with presumptive high-risk factors with worse expected disease courses because its use was at the discretion of the treating physicians. We used propensity score matching by adjusting for underlying disease, sex, volume and BP status, baseline Cr level, and baseline CK level in an attempt to correct for this; however, the bicarbonate group still presented with a higher AKI incidence, need for dialysis, and mortality. Based on a review of the literature for other studies with higher AKI rates in the bicarbonate group than in the non-bicarbonate group, we found one study that reported that of 157 patients, 16.5% had AKI and the incidence of AKI was significantly higher in those patients who received bicarbonate therapy; however, the authors speculated that bicarbonate administration was not the cause of AKI but the result of receiving more aggressive therapy [18]. However, this study was not designed to identify the difference between bicarbonate and non-bicarbonate groups; thus, causal relations could not be assessed. Second, bicarbonate therapy may potentially have detrimental effects. The effects of volume and sodium overload are not disputable [19]. Consequences of bicarbonate therapy are hypervolemia, hyperosmolarity, and hypernatremia. Bicarbonate infusion may induce hyperlactatemia. In contrast to the beneficial use of bicarbonate for bicarbonate-losing metabolic acidosis, such as metabolic acidosis caused by diarrhea or renal tubular acidosis, the use of bicarbonate for lactic acidosis has been disproved [19]. Furthermore, a hyperosmolar state caused by bicarbonate infusion may lead to paradoxical intracellular acidosis [8,20]. Additionally, serum ionized calcium level is reduced by bicarbonate infusion, and hypocalcemia is associated with reduced left ventricular contractility [21]. These reactions may offset any beneficial effect of alkalization of urine, and bicarbonate therapy may contribute to the development of AKI. We found that hypernatremia, volume overload, and other electrolyte imbalances were more prominent in the bicarbonate group than in the non-bicarbonate group, although the rates of hypocalcemia were not significantly different between the bicarbonate and non-bicarbonate groups, and lactate levels were not measured.

Hypervolemic treatment may also contribute to the development of AKI [2]. The primary mechanism of rhabdomyolysis-induced AKI is renal vasoconstriction; thus, early intravenous fluid treatment has been used to restore blood flow and glomerular filtration to protect the kidneys. Surprisingly, previous studies have shown that a greater degree of kidney dysfunction results from venous congestion transmitted to the renal venous compartment than from lack of arterial perfusion [22,23]. Therefore, aggressive volume treatment to maintain renal flow may contribute to peripheral overload, resulting in renal congestion and AKI. We demonstrated that patients with volume overload were at increased risk of developing AKI. Moreover, volume overload was an independent risk factor for mortality in our study. Our results are consistent with those of a previous study that reported that volume overload increases the risk of AKI and mortality, independent of the severity of acute illness, indicating that prevention of volume overload is crucial in managing critically ill patients [24]. We stratified high-volume fluid therapy and bicarbonate therapy using RERI analysis, and each variable independently and synergistically had detrimental effects on the development of AKI. We suggest that <5.5 mL/kg/hr fluid resuscitation should be used to prevent AKI and mortality in patients with rhabdomyolysis, and volume overload, hourly urine output, and central venous pressure should be monitored periodically. Nevertheless, bicarbonate use may have a role in severe acidic patients with rhabdomyolysis. Despite the lack of relevant clinical data supporting its effectiveness, theoretical evidence still exists [25–27].

Although the risk of AKI was higher for medically-caused rhabdomyolysis than surgically-induced rhabdomyolysis, the association between bicarbonate use and AKI was higher in patients with rhabdomyolysis with a surgical cause. In terms of AKI, bicarbonate use appeared harmful in patients who were postsurgical, hypothermic, or im-
mobilized, whereas bicarbonate use appeared beneficial in cases of crush, seizure, hyperthermia, and malignant neuroleptic syndrome. However, given the large numerical differences in causal subcategories in our cohort, further subanalyses are required to elucidate important subgroup effects. Therefore, we propose a large-scale cohort study wherein patients have rhabdomyolysis of homogeneous etiology.

Our study had several strengths. We used propensity score-matched analyses to reduce selection bias and potential baseline differences between bicarbonate and non-bicarbonate groups. AKI was defined and staged according to standard definitions proposed by the KDIGO Group [11]; therefore, the study results are likely generalizable to patients at other institutions. More than 4,000 patients were enrolled, and after propensity score matching, over 3,000 patients were included in the analyses, which to the best of our knowledge is the largest cohort of rhabdomyolysis patients analyzed in a single study.

Our study also has several limitations. First, it was not specifically designed to illustrate the initiation and maintenance of bicarbonate therapy, bicarbonate dosage, or target serum and urine pH levels. We did not tailor the infusion of bicarbonate according to serum or urine pH levels. Second, information on the type of fluid was not stratified. Different fluid mixtures such as isotonic saline and dextrose fluids were used in our study in the real-world setting. However, the amount of fluid was adjusted by body weight to set individual target volumes. Third, although we separated medical and surgical causes of rhabdomyolysis, numerous heterogeneous causes of rhabdomyolysis were included. Fourth, although we performed propensity score matching to avoid potential bias in the use of bicarbonate in patients with predicted worse outcomes, such as severe acidemia, volume depletion, or state of shock, there may have been confounding effects due to complex and unintended treatment settings. Future research should prospectively evaluate the need for bicarbonate infusion and the amount of fluid therapy without these limitations.

In conclusion, we found that the use of bicarbonate and high-volume fluid treatment that resulted in volume overload were not beneficial but rather harmful to a certain subset of patients with rhabdomyolysis, as reflected by increased AKI risk, the need for dialysis, and increased mortality.

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

**Funding**
This work was supported by a grant from the Seoul National University Bundang Hospital Research Fund (grant no. 02-2019-034).

**Acknowledgments**
We would like to acknowledge So Yeon Ahn, Associate Professor at the Department of Medical Research Collaborating Center, Seoul National University Bundang Hospital, for statistical counseling. We thank Yunseo Lee, a student at Seoul National University Medical School, for his assistance in data acquisition and cleaning.

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Data curation, Formal analysis, Visualization: HWK
Funding acquisition: HWK, SK, YL, JHO
Investigation, Software: HWK, SK, JHO, NHK
Methodology: HWK, SK
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References


Estimating baseline creatinine values to define acute kidney injury in critically ill pediatric patients

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Background: Acute kidney injury (AKI) is a common complication in critically ill children. However, the common lack of baseline serum creatinine values affects AKI diagnosis and staging. Several approaches for estimating baseline creatinine values in those patients were evaluated.

Methods: This single-center retrospective study enrolled pediatric patients with documented serum creatinine measurements within 3 months before admission and more than two serum creatinine measurements within 7 days after admission to the pediatric intensive care unit of a tertiary care children’s hospital between January 2016 and April 2020. Four different approaches for estimating AKI using serum creatinine measurements were compared: 1) back-calculation using age-adjusted normal reference glomerular filtration rates, 2) age-adjusted normal reference serum creatinine values, 3) minimum values measured within 7 days after admission, and 4) initial values upon admission.

Results: The approach using minimum values showed the best agreement with the measured baseline value, with the largest intraclass correlation coefficient (0.623), smallest bias (–0.04), and narrowest limit of agreement interval (1.032). For AKI diagnosis and staging, the minimum values were 80.8% and 76.1% accurate, respectively. The other estimated baseline values underestimated AKI and showed poor agreement with baseline values before admission, with a misclassification rate of up to 42% (p < 0.001).

Conclusion: Minimum values of serum creatinine measured within 7 days after hospital admission showed the best agreement with creatinine measured within 3 months before admission, indicating the possibility of using it as a baseline when baseline data are unavailable. Further large-scale studies are required to accurately diagnose AKI in critically ill children.

Keywords: Acute kidney injury, Creatinine, Critical illness, Pediatrics

Introduction

Acute kidney injury (AKI) is a common problem associated with increased adverse outcomes and high mortality in critically ill pediatric patients [1–4]. AKI in children is currently diagnosed using standardized diagnostic criteria derived from those for adults [5–10], most of which use changes in baseline serum creatinine values (SCr-base) or the glomerular filtration rate (GFR) for diagnosing and staging AKI. Ideally, SCr-base accurately reflects a patient’s steady-state kidney function before the onset of AKI [11]. However, many patients do not have preadmission SCr-base values in

Received: May 29, 2021; Revised: August 27, 2021; Accepted: September 10, 2021
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their records, which complicates AKI diagnosis and staging [12–14]. In the absence of consensus-based standardized methods for replacing missing SCr-base data, several values have been suggested for estimating a surrogate SCr-base value, including the initial admission SCr values, minimum SCr values during hospitalization, dynamic SCr values during a 7-day or 48-hour window, or back-calculation by imputing an estimated GFR (eGFR) of 75 mL/min/1.73 m² with the Modification of Diet in Renal Disease (MDRD) equation or the Chronic Kidney Disease Epidemiology Collaboration formula [13,15–20]. However, those substitutes have mainly been studied and validated in adult populations. Few studies have evaluated the various estimation approaches in critically ill children. Therefore, it is difficult to determine which SCr-base surrogate should be used to evaluate AKI and related clinical outcomes in critically ill pediatric patients.

This study evaluated several approaches for calculating an estimated SCr-base (eSCr-base) value, including methods commonly used in adult patients and those specified and adjusted for use in pediatric patients. The eSCr-base values calculated using different approaches were compared with a reference SCr-base value measured 3 months or less before admission (mSCr-base). The value of the different estimations in diagnosing and staging AKI was also compared.

**Methods**

**Study subjects**

This study was a single-center, retrospective cohort study. All critically ill children consecutively admitted to a 14-bed multidisciplinary pediatric intensive care unit (PICU) in Asan Medical Center Children’s Hospital, Seoul, Korea between January 2016 and April 2020 were screened. Patients aged 1 month to 18 years with available mSCr-base data, defined as the lowest value measured 3 months or less before PICU admission, and at least two documented SCr measurements within the first 7 days of PICU admission were enrolled. Exclusion criteria were as follows: age of <1 month or >18 years, no available mSCr-base value, known kidney anomalies, preexisting chronic renal failure, dialysis used before PICU admission, PICU stay of <24 hours, and do-not-resuscitate orders (Fig. 1). This study was approved by the Institutional Review Board of Asan Medical Center (No. 2020-0878), which waived the requirement for informed consent because the study was retrospective. This study was conducted in accordance with the principles of the 1964 Declaration of Helsinki.

**Figure 1. Flow chart for inclusion and exclusion of the study population.**

mSCr-base, measured SCr-base (within 3 months prior to admission); PICU, pediatric intensive care unit; SCr, serum creatinine.
ki and later amendments. It also conforms to the Strengthening the Reporting of Observational Guidelines statement for reporting observational studies.

**Data collection**

We retrospectively reviewed the electronic medical records of all included patients and collected data on their baseline demographic characteristics, underlying disorders, reasons for PICU admission, initiation of continuous renal replacement therapy (CRRT), duration of PICU stay, 28-day mortality rate, and laboratory findings, including SCr values. We evaluated four different methods for calculating an eSCR-base value: 1) back-calculation of SCr values using the age-adjusted normal reference value for eGFR (SCr-eGFR) [21–23] (Supplementary Table 1, available online) 2) normal reference values adopted from the pediatric Sequential Organ Dysfunction Assessment (pSOFA) (SCr-ref) [24] (Supplementary Table 1); 3) minimum SCr value measured within 7 days after PICU admission (SCr-min); and 4) initial SCr values measured at PICU admission (SCr-adm). Imputation of SCr-base by back-calculation was performed using the original Schwartz formula because it was derived from SCr measured using the Jaffe method, which is used in our institution [22,25–28]. To evaluate disease severity and organ dysfunction, the Pediatric Risk of Mortality III and pSOFA scores were calculated using the worst documented values within the first 24 hours of PICU admission [24,29].

**Study design**

The primary outcome was agreement between mSCR-base and the eSCR-base values calculated using the different methods. For this purpose, reliability analyses were performed to assess the intraclass correlation coefficients (ICCs). In addition, descriptive statistics were used to calculate the bias (mean difference) and limit of agreement (LOA), and the results are depicted as scatterplots. The secondary outcome was the performance of the various eSCR-base values in AKI diagnosis and staging. For that purpose, we compared the AKI diagnoses and stages determined using the various eSCR-base values with those made using mSCR-base.

**Definitions**

AKI was defined as an absolute increase in SCr of 0.3 mg/dL within 48 hours or a >50% relative increase in SCr compared with “SCr-base” during the first 7 days of PICU admission (higher than stage 1 of the Kidney Disease: Improving Global Outcomes [KDIGO] 2012 criteria) [10]. AKI was diagnosed and classified according to the definition of the KDIGO workgroup, which considers the maximum increase in SCr during the first 7 days of PICU admission. The urine output criteria were not used because of data uncertainty.

**Statistical analyses**

The data were analyzed using IBM SPSS for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables with a normal distribution are reported as means and standard deviations (SDs), and those with skewed distributions are reported as medians and interquartile ranges (IQRs). Normality was determined using the Kolmogorov-Smirnov test. Categorical variables are expressed as numbers and proportions. The agreement between eSCR-base and mSCR-base was assessed using a reliability analysis with ICCs. The bias and LOA (defined as the bias ± 1.96 × SD), were calculated using descriptive statistics. The performance of the various eSCR-base values in AKI diagnosis and staging was compared with the results with mSCR-base using chi-square testing in a trend analysis. Based on crosstables of the mSCR-base and eSCR-base results, we calculated and report the sensitivity, specificity, positive predictive value (PPV = true positive/[true positive + false positive]), negative predictive value (NPV = true negative/[true negative + false negative]), positive likelihood ratio (PLR = sensitivity/1 – specificity), negative likelihood ratio (NLR = 1 – sensitivity/specíficity), misclassification rate, and percent positive agreement. Kappa statistics with 95% confidence intervals (CIs) are used to report the agreement levels. The misclassification rate was evaluated using McNemar test. For all analyses, variables with a two-sided p-value of <0.05 were considered statistically significant.
Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>710</td>
</tr>
<tr>
<td>Male sex</td>
<td>383 (53.9)</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>23.0 (7.0–88.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.0 (5.7–21.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>81.0 (63.0–118.2)</td>
</tr>
<tr>
<td>Duration of PICU stay (day)</td>
<td>7 (3–16)</td>
</tr>
<tr>
<td>Hospital stay (day)</td>
<td>37 (19–82)</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>200 (28.2)</td>
</tr>
<tr>
<td>Hemato-oncologic</td>
<td>158 (22.3)</td>
</tr>
<tr>
<td>Gastrointestinal/hepatic</td>
<td>121 (17.1)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>83 (11.7)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>49 (6.9)</td>
</tr>
<tr>
<td>Genetic</td>
<td>44 (6.2)</td>
</tr>
<tr>
<td>Endocrinologic</td>
<td>24 (3.4)</td>
</tr>
<tr>
<td>Nephrology</td>
<td>11 (1.6)</td>
</tr>
<tr>
<td>None</td>
<td>19 (2.7)</td>
</tr>
<tr>
<td>Cause of PICU admission</td>
<td></td>
</tr>
<tr>
<td>Respiratory problems</td>
<td>281 (39.6)</td>
</tr>
<tr>
<td>Gastrointestinal/hepatic problems</td>
<td>133 (18.7)</td>
</tr>
<tr>
<td>Cardiac problems</td>
<td>103 (14.5)</td>
</tr>
<tr>
<td>Shock</td>
<td>64 (9.0)</td>
</tr>
<tr>
<td>Neurological problems</td>
<td>48 (6.8)</td>
</tr>
<tr>
<td>Hemato-oncologic problems</td>
<td>30 (4.2)</td>
</tr>
<tr>
<td>Nephrological problems</td>
<td>24 (3.4)</td>
</tr>
<tr>
<td>Post-cardiopulmonary arrest</td>
<td>12 (1.7)</td>
</tr>
<tr>
<td>Others</td>
<td>15 (2.1)</td>
</tr>
<tr>
<td>CRRT within 7 days of PICU admission</td>
<td>48 (6.8)</td>
</tr>
<tr>
<td>Presence of AKI</td>
<td>417 (58.7)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>166 (39.8)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>112 (26.9)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>139 (33.3)</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>192 (27.0)</td>
</tr>
<tr>
<td>28-Day mortality</td>
<td>49 (6.9)</td>
</tr>
<tr>
<td>mSCr-base</td>
<td>0.19 (0.17–0.31)</td>
</tr>
<tr>
<td>SCR-eGFR</td>
<td>0.49 (0.40–0.66)</td>
</tr>
<tr>
<td>SCR-ref</td>
<td>0.40 (0.30–0.70)</td>
</tr>
<tr>
<td>SCR-min</td>
<td>0.21 (0.17–0.34)</td>
</tr>
<tr>
<td>SCR-adm</td>
<td>0.32 (0.22–0.53)</td>
</tr>
<tr>
<td>PRISM III score</td>
<td>9.0 (5.3–14.0)</td>
</tr>
<tr>
<td>pSOFA score</td>
<td>6.0 (4.0–8.0)</td>
</tr>
</tbody>
</table>

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

AKI, acute kidney injury; CRRT, continuous renal replacement therapy; eGFR, estimated glomerular filtration rate; mSCr-base, measured SCr-base (within 3 months prior to admission); PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality; pSOFA, pediatric Sequential Organ Failure Assessment; SCR, serum creatinine; SCR-adm, initial SCR value measured at PICU admission; SCR-eGFR, back-calculation of SCR values using age-adjusted normal reference value of eGFR; SCR-min, minimum SCR value measured within 7 days after PICU admission; SCR-ref, normal reference SCR values adopted from the pediatric Sequential Organ Dysfunction Assessment score.

Table 2. The ICCs, bias (mean difference), and LOAs between mSCr-base and SCR-eGFR, SCR-ref, SCR-min, and SCR-adm

<table>
<thead>
<tr>
<th>Estimation method</th>
<th>ICC</th>
<th>Bias</th>
<th>LOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR-eGFR</td>
<td>0.35</td>
<td>-0.27</td>
<td>1.13</td>
</tr>
<tr>
<td>SCR-ref</td>
<td>0.34</td>
<td>-0.25</td>
<td>1.19</td>
</tr>
<tr>
<td>SCR-min</td>
<td>0.62</td>
<td>-0.04</td>
<td>1.03</td>
</tr>
<tr>
<td>SCR-adm</td>
<td>0.57</td>
<td>-0.22</td>
<td>1.68</td>
</tr>
</tbody>
</table>

eGFR, estimated glomerular filtration rate; ICC, intraclass correlation coefficient; LOA, limits of agreement; SCR, serum creatinine; SCR-adm, initial SCR value measured at pediatric intensive care unit admission; SCR-eGFR, back-calculation of SCR values using age-adjusted normal reference value of eGFR; SCR-min, minimum SCR value measured within 7 days after pediatric intensive care unit admission; SCR-ref, normal reference SCR values adopted from the pediatric Sequential Organ Dysfunction Assessment score.

Results

Baseline characteristics of the study population

A total of 710 patients were included. The median (IQR) age and body weight were 23.0 months (7.0–88.0 months) and 10.0 kg (5.7–21.0 kg), respectively. Cardiac (28.2%) and hemato-oncological disorders (22.3%) were the most frequently encountered underlying disorders. Respiratory problems (39.6%) were the most common indication for PICU admission (Table 1). Forty-eight patients (6.8%) required CRRT within the first 7 days of PICU admission. The overall 28-day mortality rate was 6.9%. Of all patients, 27% were malnourished.

Comparison of mSCr-base and eSCr-base

The ICCs between mSCr-base and SCR-eGFR, SCR-ref, SCR-min, and SCR-adm were 0.351, 0.337, 0.623, and 0.571, respectively (Table 2). Fig. 2 shows the degree of agreement between mSCr-base and each eSCr-base value. According to the corresponding measurements of agreement, the largest bias was found between mSCr-base and SCR-eGFR (-0.21). SCR-adm showed the worst agreement with mSCr-base and the widest LOA interval (1.683), whereas SCR-min showed the best agreement, with the smallest bias (-0.04) and narrowest LOA interval (1.032) (Table 2).
Evaluation of estimated serum creatinine-based performance

Acute kidney injury diagnosis based on different estimated serum creatinine-based values
The incidence of AKI calculated using mSCr-base was 58.7%, whereas it was overestimated as 63.5% by SCr-min. All other eSCr-base values (SCr-eGFR, SCr-ref, and SCr-adm) underestimated the incidence of AKI (as 19.1%, 22.1%, and 23.8%, respectively). Table 3 presents the sensitivity, specificity, PPV, NPV, PLR, NLR, misclassification rate, percent positive agreement, McNemar test results, and kappa statistics of the various eSCr-base values compared with mSCr-base. Among the eSCr-base values, SCr-min showed the best agreement for AKI diagnosis, with a positive percent agreement of 80.8% and kappa statistic of 0.598 (95% CI, 0.537–0.659).
Acute kidney injury staging and associated 28-day mortality rate

Using mScr-base, 23.4%, 15.8%, and 19.6% of AKI cases were classified as stages 1, 2, and 3, respectively. In the comparison between mScr-base and the various eScr-base values, SCr-min showed the best positive percent agreement of 76.1%, with a kappa statistic of 0.510 (95% CI, 0.463–0.557) for AKI staging (Table 4). The 28-day mortality rate showed a statistically significant tendency to increase along with the AKI stage calculated using mScr-base and all the eScr-base values (p < 0.001). The 28-day mortality data sorted by AKI stage based on SCr-min showed the best agreement with those sorted by mScr-base, with a positive percent agreement of 64.5% and a kappa statistic of 0.522 (95% CI, 0.474–0.569).

Discussion

In this study, we evaluated four different methods for calculating a surrogate SCr-base value for critically ill pediatric patients and found overall poor agreement with mScr-base.

No definite consensus-based diagnostic criteria for AKI are available for children; several standardized criteria have been adopted from those used in adults, including the pediatric Risk Injury Failure Loss End-stage Renal Disease (pRIFLE) criteria, the Acute Kidney Injury Network criteria, and the KDIGO criteria [5,7,8,10,30]. Most of those criteria require a baseline kidney function, represented by mScr-.
base or GFR, to make an AKI diagnosis. However, mSCr-base is missing for up to 50% of patients, which makes the accurate evaluation of AKI and associated clinical outcomes difficult [12,13,31]. For patients without mSCr-base data, several previous studies in adults have evaluated different estimation methods [13,15,16,18,32], but the pediatric and adult populations differ in several important, relevant ways. The most widely used approach in the adult population is the imputation method with the MDRD equation, which assumes an eGFR of 75 mL/min/1.73 m² [13,16,33]. However, the MDRD equation is inappropriate in children because the normal reference eGFR differs by age, and the age- and growth-dependent reference ranges for SCr and eGFR values in pediatric patients are wide [21,23,34,35]. In this study, we used the age-adjusted normal reference values for eGFR and SCr [21–24] in our SCr-eGFR and SCr-ref, calculations, respectively. Nonetheless, SCr-eGFR and SCr-ref showed poor agreement with mSCr-base. The finding that mSCr-base was smaller than both SCr-eGFR and SCr-ref can be explained using various factors that depend on the conditions of individual patients. Most of the patients included in this study had underlying disorders, most commonly cardiac problems. In addition, 27.0% had severe malnourishment, which could be related to decreased muscle mass. Consequently, assuming that the renal function of critically ill pediatric patients admitted to a PICU is equivalent to that of “normal healthy” children may be inappropriate.

Although the definition of mSCr-base used here, an SCr value measured within 3 months before hospitalization, is the most widely accepted, the use of mean, most recent, or nadir outpatient SCr values at time intervals of up to 730 days before hospitalization has been suggested in adult population studies [11,15,36]. However, in children, the normal reference values can change significantly over time, so using older data could lead to AKI evaluation inaccuracies. Furthermore, the use of data from >3 months of hospitalization could mistakenly suggest chronic renal failure. Therefore, we used SCr values measured within 3 months before hospitalization as mSCr-base.

SCr-min was another eSCr-base method used in this study. Although a strong consensus exists that the lowest SCr value should be used for the SCr-min calculation, the duration within which the lowest SCr value is determined varies among the relevant studies, ranging from the whole hospitalization period to 2 weeks or 3 days after PICU admission [11,13,16,17,31,32,37]. In this study, SCr-min was defined as the lowest SCr value measured within 7 days after PICU admission for the following reasons. First, that time period is consistent with the diagnostic criteria provided by pRIFLE and KDIGO. Second, from a clinical standpoint, a longer time window could impede the prompt evaluation and management of AKI. Third, the condition of pediatric patients admitted to a PICU with temporary AKI caused by dehydration or acute deterioration, often improves dramatically with treatment.

Among the eSCr-base calculation methods tested here, SCr-min showed the best agreement with mSCr-base (i.e., the actual SCr-based value), with the largest ICC, smallest bias, and best percent agreement in AKI diagnosis, staging, and the associated 28-day mortality rate. However, it slightly overestimated the incidence of AKI. The eSCr-base values calculated using the other methods underestimated the incidence of AKI; thus, the diagnosis and staging of AKI based on those calculations were inaccurate, with large misclassification rates.

In adult populations, researchers have suggested several approaches for SCr-base imputation and reported various degrees of agreement between mSCr-base and their eSCr-base calculations. However, the best technique to use remains controversial; no definite universal baseline SCr or eSCr-base method has reached consensus-level support. In addition, some investigators have expressed significant disagreement consistent with our results and suggested the use of recorded SCr values whenever possible [15,32,38].

Critically ill patients in intensive care unit settings differ from the normal healthy population in many important ways that can affect their SCr values, renal function, and AKI evaluation, such as chronic illness, impaired nutritional status, decreased muscle mass, fluid imbalance, and the use of various medications [31]. Likewise, individual characteristics and clinical situations might need to be considered to accurately estimate SCr-base and achieve good agreement with the actual mSCr-base. In this study, SCr-min showed the best agreement with mSCr-base, which suggests that considering individual traits might provide a better estimate of a patient’s steady-state than using a value assumed to represent “normal healthy” children.

On the other hand, SCr itself has several drawbacks as a marker of renal function. It is affected by age, sex, ethnicity, dietary factors, and muscle mass. In addition, it is actively
secreted by the proximal tubule, leading to an overestima-
tion of GFR. There is also a time lag between significant
renal injury and the elevation of SCr levels. Cystatin C was
recently introduced as an alternative endogenous marker
of renal function because it is freely filtered by the renal
glomerulus, does not have non-renal elimination or glo-
merular secretion, and is independent of muscle mass.
Consequently, cystatin C measurement could provide some
advantages in evaluating AKI, especially in critically ill chil-
dren [39,40]. Large-scale, well-designed studies of pediatric
patients are needed to develop pediatric-specific defini-
tions, diagnostic tools, and staging criteria for AKI that use
proper markers, including cystatin C.

This study has some limitations. First, it was a single-cen-
ter retrospective study. Due to the characteristics of the
study population, the results might have limited generaliz-
ability. Second, in back calculating SCr from eGFR, we used
the original Schwartz equation (instead of the revised bed-
side Schwartz equation) because it was derived from SCr
measured using the Jaffe method, which is used to measure
SCr in our institution. However, the kinetic Jaffe assay can
be affected by factors such as albumin, glucose, and bil-
rubin in ways that can result in the overestimation of SCr
[25,27,41].

Third, mSCr-base was defined as the lowest value within 3
months before PICU admission. All the SCr values included
were measured regardless of the patients’ status and whether
they were drawn in the emergency room or at outpatient
clinics. Those values might not fully represent the patients’
best normal condition. Fourth, only SCr-based criteria were
used for AKI diagnosis and classification. However, a recent
report emphasizes that changes in urine output can be
helpful for detecting AKI that might be missed when using
only SCr criteria [2].

Despite those limitations, this study has several strengths.
Its main advantage is that, to the best of our knowledge, it is
the first to evaluate different eSCr-base methods in critically
ill pediatric patients. In addition to considering methods
widely studied and used in adult populations, we examined
the characteristics of the children and used age-adjusted
normal reference SCr and eGFR values. We further evalu-
ated not only the agreement between each eSCr-base and
mSCr-base, but also the clinical performance of each
eSCr-base and mSCr-base in AKI diagnosis and staging to
determine which method could be most useful in clinical
practice. Because this was a single-center study, unified di-
agnosis and staging using the same criteria and general in-
tensive care were applied consistently throughout the study
period, which reduced the number of confounding factors.

In conclusion, the diagnosis and staging of AKI are greatly
affected by the SCr-base value used; therefore, the careful
selection of an appropriate SCr-base is important. In this
study, SCr-min showed the best agreement with mSCr-base
in both the actual SCr value calculated and the diagnosis
and staging of AKI. However, further large-scale studies are
required to establish definite, accurate diagnostic and stag-
ing criteria for AKI in critically ill pediatric patients.

Conflicts of interest

No potential conflicts of interest relevant to this article are
reported.

Acknowledgments

We thank all our colleagues involved in the care of
COVID-19 patients for their intellectual generosity. This
study did not receive any specific grants from funding agen-
cies in the public, commercial, or not-for-profit sectors.

Authors’ contributions

Conceptualization: SJP, WKJ
Data curation: YJL, WKJ
Formal analysis: WKJ
Intellectual contributions: YSP
Writing–original draft: YJL, WKJ
Writing–review & editing: All authors
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Recalibration and validation of the Charlson Comorbidity Index in acute kidney injury patients underwent continuous renal replacement therapy

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Background: Comorbid conditions impact the survival of patients with severe acute kidney injury (AKI) who require continuous renal replacement therapy (CRRT). The weights assigned to comorbidities in predicting survival vary based on type of index, disease, and advances in management of comorbidities. We developed a modified Charlson Comorbidity Index (CCI) for use in patients with AKI requiring CRRT (mCCI-CRRT) and improved the accuracy of risk stratification for mortality.

Methods: A total of 828 patients who received CRRT between 2008 and 2013, from three university hospital cohorts was included to develop the comorbidity score. The weights of the comorbidities were recalibrated using a Cox proportional hazards model adjusted for demographic and clinical information. The modified index was validated in a university hospital cohort (n = 919) using the data of patients treated from 2009 to 2015.

Results: Weights for dementia, peptic ulcer disease, any tumor, and metastatic solid tumor were used to recalibrate the mCCI-CRRT. Use of these calibrated weights achieved a 35.4% (95% confidence interval [CI], 22.1%–48.1%) higher performance than unadjusted CCI in reclassification based on continuous net reclassification improvement in logistic regression adjusted for age and sex. After additionally adjusting for hemoglobin and albumin, consistent results were found in risk reclassification, which improved by 35.9% (95% CI, 23.3%–48.5%).

Conclusion: The mCCI-CRRT stratifies risk of mortality in AKI patients who require CRRT more accurately than does the original CCI, suggesting that it could serve as a preferred index for use in clinical practice.

Keywords: Acute kidney injury, Charlson Comorbidity Index, Continuous renal replacement therapy, Mortality, Risk assessment
Despite advances in critical care medicine over the past decades, treatment outcomes for patients admitted to the intensive care unit (ICU) with acute kidney injury (AKI) remain poor [1,2]. Since continuous renal replacement therapy (CRRT), developed by Kramer in 1977, has become an indispensable treatment option for critical care, the mortality rate of severe AKI patients who require CRRT has remained very high over the past 40 years, likely due to the increased severity of underlying disease. Since the number and severity of underlying comorbid diseases are among the main causes of such an increase [1–3], predicting prognosis using an appropriate scoring system is important in treating critically ill AKI patients as well as for providing and allocating medical resources.

The Charlson Comorbidity Index (CCI) is the most widely used tool to evaluate the effects of comorbid diseases on patient prognosis, and its use has been validated in various acute/chronic conditions, including kidney diseases [4]. However, since the CCI was created originally as an index for general ward-admitted patients with various comorbid diseases, it is questionable whether each disease covered by the index exerts the same impact in severe AKI patients requiring CRRT. Since the index was developed 30 years ago, there is an inherent disadvantage that the effects of the diseases addressed might have changed with treatment technology. Recently, various tools such as the Davies index and the Index of Coexistent Disease score [5–7] have been created to evaluate the burden of coexistent chronic diseases. Nevertheless, the CCI remains the most widely used and clinician-friendly scoring system [4]. Although a series of studies has recalibrated and validated CCI for patients undergoing peritoneal dialysis, hemodialysis, and renal transplantation, research regarding CRRT patients is lacking [8–10]. Therefore, we recalibrated the weight of comorbidities on mortality in severe AKI patients requiring CRRT by modifying the original CCI (mCCI) and validated the performance of the new mCCI index compared with that of the original CCI.

We collected data from 858 AKI patients aged ≥18 years who received CRRT from Seoul National University Hospital (n = 439), Seoul National University Boramae Medical Center (n = 218), or Dongguk University Ilsan Hospital (n = 201) between 2008 and 2013, as the development cohort. We excluded patients who had incomplete data regarding hemoglobin and albumin levels (n = 30). Therefore, the following demographic and clinical information were obtained for 828 AKI patients: sex, age, hemoglobin and albumin at baseline, 28-day mortality, and the 15 comorbidities constituting the CCI (peripheral vascular disease, dementia, myocardial infarction, congestive heart failure, cerebrovascular disease, hemiplegia, diabetes, diabetes with end organ damage, chronic pulmonary disease, connective tissue disease, peptic ulcer disease, any tumor, metastatic solid tumor, mild liver disease, and moderate to severe liver disease), defined based on the International Classification of Disease, the 10th Revision (ICD-10). Although there is a criterion for classifying moderate/severe renal disease in the original CCI appendix, scoring for underlying kidney disease was excluded. This is because our study population was composed of CRRT patients, who are those with severe AKI. These data were confirmed by trained clinicians at each medical center. This study is in compliance with the Declaration of Helsinki and received full approval from the Institutional Review Boards of Seoul National University Hospital (No. H-1404-028-568), Seoul National University Boramae Medical Center (No. 26-2014-15), Yonsei University Severance Hospital (No. 4-2021-0082), and Dongguk University Ilsan Hospital (No. DUIH 2018-12-010-012). Informed consent was waived for this study because retrospective data were used.

A total of 1,144 patients who received CRRT at Yonsei University Severance Hospital participated in the validation cohort between 2009 and 2015. We excluded 225 patients with missing information regarding albumin (n = 216) and comorbidities (n = 9).
albumin, hemoglobin, and CCI score of 919 patients was collected.

Statistical analyses

Baseline characteristics in the development and validation cohorts were described using mean and standard deviation for continuous variables and frequency and percentile for categorical variables. The primary outcome of this study was 28-day all-cause mortality, which was observed in each hospitalization. To estimate the calibrated weights of 15 comorbidities in AKI patients, Cox proportional hazard models were used after being stratified by age (<50, 50–59, and ≥60 years) and treatment center in the development cohort and adjusted for sex, albumin, hemoglobin, and the 15 comorbidities. We tested the proportional hazard assumption using the Schoenfeld test. After estimating the hazard ratios (HRs) of the comorbidities in the survival model, the modified CCI of CRRT patients (mCCI-CRRT) was calculated using each significant HR among the CCI diseases divided by the lowest significant HR. The comorbidity score of the mCCI-CRRT for each patient was the sum of the rounded weights. We obtained Kaplan-Meier curves stratified into three categories (<2, 2–4, ≥5) for the original CCI and the mCCI-CRRT scores in the development and validation cohorts to compare the performances. The index discriminations between CCI and mCCI-CRRT were assessed by C-statistics and continuous net reclassification improvement (cNRI). The C-statistic was the area under the receiver operator curve between sensitivity and 1-specificity. To overcome the disadvantages of C-statistics, such as difficulty in clinical interpretation and unclear decision threshold, cNRI, which is the sum of the proportion in event prediction of the event group (cNRIevent) and the non-event prediction of the non-event group (cNRInon-event) was used [11]. Improvement in the mortality prediction of mCCI-CRRT compared to the age-adjusted and original CCI was estimated using a logistic regression model adjusted for sex and age in model 1 and additionally adjusted for hemoglobin and albumin in model 2. All analyses were conducted using R software, version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and p < 0.05 was considered statistically significant.

Results

Baseline characteristics of both the development and validation cohorts are presented in Table 1. In the development cohort, most participants were in the ≥60-year age group at initiation of CRRT therapy, and 61.0% of patients were male. Similar distributions were observed regarding age and sex in the validation cohort. The average follow-up duration and 28-day mortality rate during hospitalization were 14.45 ± 11.96 days and 61.5% (n = 509) in the development cohort, respectively, and 18.92 ± 11.25 days and 58.8% (n = 540) in the validation cohort. In the development cohort, most subjects (82.6%) had one or more comorbidities. Among the 15 comorbidities, tumor was the most prevalent (24.4%), followed by diabetes without end organ damage (19.6%), moderate to severe liver disease (18.1%), and congestive heart failure (18.0%). Additionally, 34.5% of the patients in the validation cohort had diabetes without end organ damage, followed by any tumor (31.9%), diabetes with end organ damage (24.4%), and congestive heart disease (16.5%).

Table 2 lists the β coefficients, adjusted HR, and weights for each comorbidity in the Cox proportional hazard model. We confirmed a relationship between time and residuals in proportional hazard assumption using the Schoenfeld test (p = 0.76). The original CCI calculated the adjusted relative risk of each item for 1-year mortality. A relative risk of <1.2 was excluded from the weight calculation. Relative risk from 1.2 to <1.5 was set as a weight of 1, from 1.5 to <2.5 as a weight of 2, from 2.5 to <3.5 as a weight of 3, and relative risk ≥3.5 was set as a weight of 6 [4]. Unlike the original CCI, weighting of the mCCI-CRRT was derived by dividing the significant HR of each item by the significant smallest adjusted HR (peptic ulcer disease). Metastatic solid tumor scored 5 points and was the strongest predictor of mortality among the comorbidities, followed by a score of 3 for any tumor (including leukemia and lymphoma) and a score of 1 for dementia and peptic ulcer disease.

The total scores of the original and recalibrated weights were allocated to each patient in the development cohort. The median CCI and mCCI-CRRT in the development cohort were 2 and 0, respectively (Fig. 1). We categorized the CCI and mCCI-CRRT into three score categories using the same cutoff values (<2, 2–4, ≥5) to compare the probability of survival using CCI and mCCI-CRRT. Fig. 2 illustrates the Kaplan-Meier curves for the CCI (Fig. 2A, C) and mCCI-CRRT.
CI-CRRT (Fig. 2B, D) differentiated by the three risk groups in both cohorts. When the survival probability was classified by CCI in the development (Fig. 2A) and validation (Fig. 2C) cohorts, survival curves for the ≥5 and 2 to 4 groups intersected and overlapped, while group differences based on the log-rank test were significant in the validation cohort. However, survival curves of mCCI-CRRT (Fig. 2B) showed differences in survival probability according to the classification of risk scores in the development cohort (p < 0.01). In addition, survival probability analysis showed a decrease in mCCI-CRRT (Fig. 2D) as risk increased, with significant between-group differences in the validation cohort.

We estimated the mortality prediction of mCCI-CRRT compared to those of CCI and age-adjusted CCI in the validation cohort using C-statistics (Table 3). Significant difference was observed in the comparison between CCI (0.54; 95% confidence interval [CI], 0.50–0.58) and mCCI-CRRT (0.59; 95% CI, 0.55–0.62) (p = 0.01). However, the C-statistics difference in the age-adjusted CCI (0.58; 95% CI, 0.54–0.61) compared with that of the mCCI-CRRT was not significant (p = 0.60). Our calibrated weights in the mCCI-CRRT achieved a 35.4% (95% CI, 22.1%–48.1%) higher performance than

### Table 1. Descriptive characteristics in the development and validation cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Development cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>828</td>
<td>919</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>111 (13.4)</td>
<td>162 (17.6)</td>
</tr>
<tr>
<td>50–59</td>
<td>132 (15.9)</td>
<td>172 (18.7)</td>
</tr>
<tr>
<td>≥60</td>
<td>585 (70.7)</td>
<td>585 (63.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>505 (61.0)</td>
<td>567 (61.7)</td>
</tr>
<tr>
<td>Female</td>
<td>323 (39.0)</td>
<td>352 (38.3)</td>
</tr>
<tr>
<td>28-Day mortality</td>
<td>509 (61.5)</td>
<td>540 (58.8)</td>
</tr>
<tr>
<td>Follow-up duration (day)</td>
<td>14.45 ± 11.96</td>
<td>18.92 ± 11.25</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.86 ± 2.25</td>
<td>9.66 ± 2.26</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.68 ± 0.55</td>
<td>2.71 ± 0.52</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No comorbidity</td>
<td>144 (17.4)</td>
<td>140 (15.2)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>23 (2.8)</td>
<td>36 (3.9)</td>
</tr>
<tr>
<td>Dementia</td>
<td>32 (3.9)</td>
<td>33 (3.6)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>98 (11.8)</td>
<td>86 (9.4)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>149 (18.0)</td>
<td>152 (16.5)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>74 (8.9)</td>
<td>91 (9.9)</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>44 (5.3)</td>
<td>20 (2.2)</td>
</tr>
<tr>
<td>Diabetes, without end organ damage</td>
<td>162 (19.6)</td>
<td>317 (34.5)</td>
</tr>
<tr>
<td>Diabetes, with end organ damage</td>
<td>134 (16.2)</td>
<td>224 (24.4)</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>31 (3.7)</td>
<td>66 (7.2)</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>26 (3.1)</td>
<td>22 (2.4)</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>49 (5.9)</td>
<td>105 (11.4)</td>
</tr>
<tr>
<td>Any tumor</td>
<td>202 (24.4)</td>
<td>293 (31.9)</td>
</tr>
<tr>
<td>Metastatic solid tumor</td>
<td>25 (3.0)</td>
<td>89 (9.7)</td>
</tr>
<tr>
<td>Mild liver disease</td>
<td>69 (8.3)</td>
<td>119 (12.9)</td>
</tr>
<tr>
<td>Moderate to severe liver disease</td>
<td>150 (18.1)</td>
<td>76 (8.3)</td>
</tr>
<tr>
<td>Charlson Comorbidity Index score</td>
<td>3.4 ± 2.35</td>
<td>3.16 ± 2.28</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>28.16 ± 7.69</td>
<td>27.47 ± 7.65</td>
</tr>
<tr>
<td>SOFA score</td>
<td>12.83 ± 3.41</td>
<td>13.33 ± 3.59</td>
</tr>
</tbody>
</table>

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

APACHE II, Acute Physiology And Chronic Health Evaluation II; SOFA, sequential organ failure assessment.
did CCI in reclassification, based on cNRI in logistic regression adjusted for age and sex. After additionally adjusting for hemoglobin and albumin, consistent results were found in risk reclassification improvement by 35.9% (95% CI, 23.3%–48.5%). In comparison with age-adjusted CCI, mCCI-CRRT significantly improved the net risk reclassification of mortality by 22.9% (95% CI, 9.9%–35.8%) in model 1 and by 15.2% (95% CI, 2.1%–28.2%) in model 2.

### Table 2. Weights of mCCI-CRRT based on Cox proportional hazard model compared with original CCI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coef</th>
<th>SE</th>
<th>HR</th>
<th>p-value</th>
<th>CCI</th>
<th>mCCI-CRRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>0.07</td>
<td>0.09</td>
<td>1.08</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.44</td>
<td>0.09</td>
<td>0.65</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.03</td>
<td>0.02</td>
<td>1.03</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>-0.14</td>
<td>0.29</td>
<td>0.87</td>
<td>0.62</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dementia</td>
<td>-0.58</td>
<td>0.27</td>
<td>0.56</td>
<td>0.03</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>-0.06</td>
<td>0.17</td>
<td>0.94</td>
<td>0.72</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>-0.16</td>
<td>0.15</td>
<td>0.85</td>
<td>0.29</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>-0.17</td>
<td>0.16</td>
<td>0.84</td>
<td>0.28</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>-0.46</td>
<td>0.24</td>
<td>0.63</td>
<td>0.06</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes, without end organ damage</td>
<td>-0.12</td>
<td>0.12</td>
<td>0.89</td>
<td>0.31</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes, with end organ damage</td>
<td>-0.19</td>
<td>0.13</td>
<td>0.83</td>
<td>0.17</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>0.26</td>
<td>0.2</td>
<td>1.30</td>
<td>0.20</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>0.14</td>
<td>0.25</td>
<td>1.15</td>
<td>0.57</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>-0.68</td>
<td>0.22</td>
<td>0.51</td>
<td>0.001</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Any tumor</td>
<td>0.40</td>
<td>0.11</td>
<td>1.50</td>
<td>0.001</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Metastatic solid tumor</td>
<td>0.87</td>
<td>0.24</td>
<td>2.38</td>
<td>0.001</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mild liver disease</td>
<td>0.00</td>
<td>0.17</td>
<td>1.00</td>
<td>0.99</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Moderate to severe liver disease</td>
<td>0.14</td>
<td>0.12</td>
<td>1.15</td>
<td>0.24</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

CCI, Charlson Comorbidity Index; Coef, coefficient; HR, hazard ratio; mCCI-CRRT, modified CCI-continuous renal replacement therapy; SE, standard error.

### Figure 1. Distribution of the CCI (A) and mCCI-CRRT (B) in the development cohort.

CCI, Charlson Comorbidity Index; mCCI-CRRT, modified CCI-continuous renal replacement therapy.
In this study, we modified the original CCI using the AKI-CRRT cohort database of three hospitals in Korea to provide better risk stratification in AKI patients requiring CRRT. Furthermore, we validated the accuracy of the modified index by comparing its performance with that of the original index using data of independent hospitals for the development cohort. We found that mCCI-CRRT exhibited better risk stratification performance for mortality in incident AKI-CRRT patients compared with that provided by the original CCI.

In patients with severe AKI requiring CRRT, the effect of comorbidities on outcome is considerable and warrants development of a comorbidity index [12,13]. However, for the original CCI, the scoring system was created with a development cohort of patients admitted to a general ward with various disease entities, while validation was performed on
breast cancer patients [4]. Therefore, it might not be appropriate to apply the original CCI in AKI patients requiring CRRT. To our knowledge, there have been few attempts to recalibrate the CCI of patients requiring CRRT. Therefore, this study is meaningful in that it used a large sample of incident AKI-CRRT patients and validated the index using an independent observational cohort.

The disease weights in the original CCI showed many changes when using the mCCI-CRRT. When considering only HR without statistical significance, the more prevalent causes of septic AKI (metastatic solid tumor, any tumor, connective tissue disease, chronic pulmonary disease, and moderate to severe liver disease) showed higher HR values. In contrast, HRs tended to be <1.0 in common causes of ischemic AKI, hypovolemic AKI, and postoperative AKI (peptic ulcer disease, dementia, hemiplegia, cerebrovascular disease, congestive heart failure, and myocardial infarction) [14].

Metastatic solid tumors show the highest weight, even in mCCI-CRRT [15–18]. Although target therapy and immunotherapy, which did not exist in the 1980s when the original CCI was developed, are used widely and new anticancer drugs are being developed, the mortality and risk of sepsis and septic AKI in cancer patients remain high. This highest weight also might be due to cancer treatment-related immune dysfunction and secondary infections [19,20].

Although the difference was not statistically significant, the weight of connective disease in mCCI-CRRT increased from 1 to 2 compared with the original CCI. Recently, biologics such as tumor necrosis factor-α or interleukin-6 blocking monoclonal antibodies, which did not exist 25 years ago, have been used for various autoimmune diseases and connective tissue disorders [21]. The disturbance of the immune system following the use of these biologics is believed to cause sepsis more frequently and is more difficult to treat in patients with connective tissue disease [22,23]. On the other hand, the weights of diabetes and hemiplegia were lower in the mCCI-CRRT group than in the original CCI group. Since CCI was first developed in 1977, the guidelines for diabetes control have become more sophisticated, and the target serum glucose level has been lowered, which might decrease the risk of mortality due to diabetes. In this study, peptic ulcer disease showed a statistically significant hazard ratio. Previous studies have shown that the incidence of stress-induced ulcers was high in critically ill patients receiving ICU care [24–26]. In one observational retrospective study, 90-day mortality was significantly higher in a bleeding group among patients admitted to the ICU, but there was no difference in mortality at <28 days compared to the non-bleeding group [24]. In another study, critically important gastrointestinal (GI) bleeding was not significantly related to 90-day mortality in patients who received ICU care for more than 7 days, and 90-day mortality was increased by comorbidities such as liver disease, renal replacement therapy, and coagulopathy [27]. Finally, based on a recent randomized trial comparing proton pump inhibitor and placebo groups in critically ill patients admitted to the ICU, 90-day mortality and the number of clinically important events did not show significant difference between the two groups [28]. These studies show that GI bleeding had relatively little influence on mortality in critically ill patients compared to other comorbidities, which is consistent with the results of this study.

In both the original CCI and mCCI-CRRT, there was no

| Table 3. Model performance of the mCCI-CRRT in the validation cohort |
|----------------|----------------|----------------|----------------|
| Variable      | C-statistics  | cNRI   | cNRI   |
| CCI vs. mCCI-CRRT |               |       |       |
| CCI           | 0.54 (0.50–0.58) | 35.4 (22.1–48.1) | 35.9 (23.3–48.5) |
| mCCI-CRRT     | 0.59 (0.55–0.62) | 0.01  | 0.001 |
| p-value       |               |       |       |
| Age-adjusted CCI vs. mCCI-CRRT |           |       |       |
| Age-adjusted CCI | 0.58 (0.54–0.61) | 22.9 (9.9–35.8) | 15.2 (2.1–28.2) |
| mCCI-CRRT     | 0.59 (0.55–0.62) | 0.6   | 0.001 |
| p-value       |               |       | 0.02  |

CCI, Charlson Comorbidity Index; cNRI, continuous net reclassification improvement; mCCI-CRRT, modified CCI-continuous renal replacement therapy.

*Adjusted for age and sex; †adjusted for age, sex, hemoglobin, and albumin.
There was a statistically significant difference in mortality between men and women, as shown in Table 2. Previous studies have found similar lack of association. Zettersten et al. [29] reported no difference in mortality between men and women in a retrospective cohort analysis of approximately 9,000 patients admitted to a Swedish university hospital ICU between 2006 and 2016. Sakr et al. [30] reported that the overall ICU mortality rate did not differ significantly between males and females in 3,902 patients admitted to one of 24 participating medical and/or surgical ICUs between April 2006 and September 2006.

Our study has some limitations. First, risk stratification for underlying chronic diseases was performed without considering the differences in acute antecedent factors such as myocardial infarction, bleeding, or sepsis that cause AKI. In this situation, the acute medical condition requiring CRRT is severe, and the contribution of underlying chronic diseases can be underestimated. Second, this dataset only included serum hemoglobin and albumin levels among many laboratory measures. Thus, we could not adjust for other variables such as baseline serum creatinine, quantitative proteinuria, or prothrombin time for recalibrating the weights of comorbidities. In addition, since there were no patients with acquired immunodeficiency syndrome in either cohort, this condition was not taken into consideration. Third, this is a scoring system created from a cohort composed of a single racial group, and it might not be generalizable or applicable to other racial groups and populations. Additional validation using disease-specific cohorts and other national data is necessary. Fourth, since the underlying comorbidity information is based on the ICD-10 code registered in the diagnosis window of electronic health records, specific relative risk of mortality could be underestimated. Finally, since information on CRRT dose, which is an important factor for CRRT patients, was not collected, there might be a difference in CRRT dose between the development and validation cohorts. However, since the sample size of each cohort was large and the same CRRT protocol was used, a large difference in CRRT dose between the two cohorts was less likely. In addition, our study focused on pre-existing conditions, i.e., underlying comorbidities prior to CRRT initiation. Therefore, we did not consider post-CRRT factors.

A major strength of our study is that the new index was created based on a relatively large cohort from a multi-center ICU, and it was validated with an independent, similarly sized cohort, which is quite large in terms of CRRT-related studies. Lastly, the mCCI-CRRT demonstrated superior performance in a head-to-head comparison with the original CCI.

In conclusion, we suggest use of the new mCCI-CRRT scoring system that offers superior risk stratification for mortality in incident AKI-CRRT patients compared with the original CCI. In addition, this could be a preferred scoring system in clinical practice and statistical analysis in epidemiological research. As treatment options for various diseases develop, the effects of individual chronic diseases on mortality will change. Therefore, this edition of the mCCI-CRRT is not final, and it will require periodic updates to remain current and fit the trends over time.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2021R111A3052012).

Authors’ contributions

Conceptualization: JPL, JYP
Data curation: Jinwoo L, Jangwook L, JTP, CYJ, YCK
Methodology: JJ, DKK, SJS
Writing–original draft: Jinwoo L, JJ, Jangwook L
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Background: The coronavirus disease 2019 (COVID-19) vaccine is not readily available in many countries where dosing interval is spaced more than ideal. Patients with chronic kidney disease, especially those on maintenance hemodialysis, have a tendency for a reduced immune response. This study was undertaken to demonstrate the distinct humoral immune response to the viral vector COVID-19 vaccine in patients with kidney failure receiving maintenance hemodialysis.

Methods: The study was carried out with two cohorts: 1) patients receiving maintenance hemodialysis and 2) healthcare workers from the same dialysis center as controls, each group with 72 subjects. Participants received a dose of Covishield ChAdOx1 nCoV-19 coronavirus vaccine. The humoral immunological response was determined using electrochemiluminescence immunoassay which quantitatively measures antibodies to the severe acute respiratory syndrome coronavirus 2 spike protein receptor-binding domain.

Results: All study subjects in the control group developed a humoral response (antibody titer of ≥0.8 U/mL), while only 64 of 72 in the dialysis group (88.9%) were responders. Age (ρ = –0.234, p = 0.04) and sodium level (ρ = 0.237, p = 0.04) correlated with low antibody titer in bivariate analysis. In multivariate analysis, only age (odds ratio, 1.10; 95% confidence interval, 1.01–1.22; p = 0.045) was associated with nonresponders.

Conclusion: Our study demonstrated a weak antibody response of hemodialysis patients to the viral vector COVID-19 vaccine. Older age was associated with nonresponders. Evaluation of both humoral and cellular immunity after the second vaccine dose and serial antibody titers can help determine the need for booster shots.

Keywords: Antibody formation, Secondary immunization, Chronic kidney failure, COVID-19, Hemodialysis

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has severely affected countries worldwide. Vaccination of the majority of the population is an effective method to end the pandemic. However, vaccine availability remains unfeasible in many parts of the world, especially in developing nations. Although in many countries, including India, the replication-defective viral vector vaccine, ChAdOx1 nCoV-19 (Oxford-AstraZeneca), was administered initially as two doses 4 weeks apart, it was later spaced to 12 to 16 weeks for better coverage of the population with at least a single dose and due to improved efficacy with the increased interval between doses [1]. The delay of the second dose
might be appropriate for the general population; however, patients with chronic kidney disease (CKD), especially those receiving maintenance hemodialysis, tend to have a reduced immune response, as evidenced with the hepatitis B virus vaccine \[2\] and other vaccinations \[3,4\].

Furthermore, these patients suffer from high morbidity and mortality due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) \[5,6\] and are at high risk of contracting the infection due to continuous visits to medical facilities and exposure to other people, even during peak times of the COVID-19 pandemic \[7\]. This study was undertaken to demonstrate the distinct humoral immune response in patients with kidney failure receiving maintenance hemodialysis. The antibody titers against the SARS-CoV-2 spike (S) protein were evaluated in maintenance hemodialysis patients and compared with those in healthcare workers in the same center as controls.

**Methods**

**Study design**

**Study participants**

This study was conducted in our hemodialysis center with two cohorts: 1) patients receiving maintenance hemodialysis and 2) healthcare workers from the same dialysis center as controls. Participants received a dose of Covishield ChAdOx1 nCoV-19 coronavirus vaccine (recombinant) developed by AstraZeneca (Cambridge, United Kingdom), and manufactured by Serum Institute of India Pvt Ltd. (Pune, India) from COVID-19 vaccination centers deputed by the government. All patients received the standard dose (0.5 mL) of the vaccine containing $5 \times 10^{10}$ viral particles \[8\]. A total of 141 patients was receiving maintenance hemodialysis in our center. At the time of the study, 20 patients had been vaccinated for approximately 6 to 7 weeks and were waiting for their scheduled second dose at 12 to 16 weeks, and 72 patients had been vaccinated 4 weeks prior. Twenty-one patients had a history of COVID-19 infection, 22 were not vaccinated at the time of the study, and six refused to get vaccinated. Participant selection is shown in Fig. 1.

Seventy-two patients included in the study were receiving maintenance hemodialysis in our center and had been vaccinated with the first dose 4 weeks prior. Subjects who were younger than 18 years, had a history of COVID-19 infection, were vaccinated previously at 6 weeks or more, or had an acute illness at the time of the study were excluded. In addition, 72 healthcare workers from the same dialysis center were enrolled as controls for the study.

![Study participant selection](https://example.com/study-diagram)

**Figure 1. Study participant selection.** All participants, including the controls, provided blood samples in the 5th week after vaccination. COVID-19, coronavirus disease 2019.
Antibody measurement

The humoral immunological response was determined by measuring antibody titers using electrochemiluminescence immunoassay with Elecsys Anti-SARS-CoV-2 S on a Cobas 6000 analyzer e 601 module (Roche Diagnostics, Rotkreuz, Swiss) [9]. This test is an immunoassay for in vitro quantitative determination of antibodies (including immunoglobulin G [IgG]) to the SARS-CoV-2 S protein receptor-binding domain (RBD) in human serum and plasma. In previous studies, although full-length and fragments of S protein induced specific antibodies with neutralizing activity, the RBD was suggested as a major target for eliciting highly potent neutralizing antibodies with protective efficacy [10]. This test has a linear range of 0.4 to 250 U/mL. Titers of <0.8 U/mL were considered nonreactive and ≥0.8 U/mL reactive.

Data and sample collection

Informed consent was obtained from the participants including controls for drawing blood samples and participation in the study. Demographic details, medical history, and adverse events following vaccination of the patients and controls were collected with a proforma. Venous blood samples were taken from the patients during their dialysis visits before the initiation of the session.

Although the SARS-CoV-2 antibodies against S antigen and its subunits can be detected as early as 1 to 3 weeks after an infection or vaccination, the test used attains maximum sensitivity by the 5th week [9]. Therefore, all samples were collected between 28 and 35 days after the first dose of the vaccine for both patients and controls. Body mass index (BMI) was defined as dry weight in kilograms divided by the square of the height in meters.

Statistical analyses

Statistical analysis was performed using the IBM SPSS ver. 23.0 (IBM Corp., Armonk, NY, USA). All categorical variables were presented as frequency and percentage and continuous variables as either mean (standard deviation) or median (interquartile range). The shape of the data distribution of antibody titers was assessed using the histogram/Shapiro-Wilk test. The chi-square test and Fisher exact test were used to determine the difference between the dialysis and control groups based on proportion values, and an independent sample t test or Mann-Whitney U test was used to determine the difference among the groups based on continuous values. Spearman correlation was used to determine the relationship between antibody titers and demographic and laboratory parameters. Multivariate logistic regression was used to determine the most important predictors of nonreactive antibody titers. BMI was classified based on Asian BMI classification guidelines. A p-value of <0.05 was considered statistically significant.

Ethics statement

The Institutional Ethics Committee in our hospital requires approval only for original research with trial/experimental interventions (procedure/drugs) administered patients (approval No. DHR Reg.No.EC/NEW/INST/2020/484). These patients were not treated with any trial/experimental therapy. All procedures followed the guidance of the Declaration of Helsinki. Written informed consent was obtained from the patients for participation in the study and for performing investigations.

Results

The dialysis and control groups each included 72 participants. The majority of patients in the dialysis group were males (80.6%) and in the control group females (66.7%). The average age in the dialysis group was higher (46.14 ± 8.89 years) than in the control group (40.35 ± 6.65 years). Mean BMI was lower in the dialysis group (20.87 ± 4.26 kg/m²).

The majority of patients (n = 48) had hypertension (66.7%), 20 (27.8%) had diabetes mellitus, four (5.6%) had dilated cardiomyopathy, four (5.6%) had a history of cerebrovascular accident, and two (2.8%) had hypothyroidism. Among patients in the dialysis group, 36 (50.0%) had hepatitis C virus (HCV) infection at the time of the study, and 26 (36.1%) were receiving treatment. On average, the dialysis duration was 8.65 ± 6.68 months; four patients (5.6%) had permanent catheters, and the remaining patients had arteriovenous fistula for hemodialysis access. None of the study participants were on or received immunosuppressive agents at least 3 months prior to the study.

Diabetic kidney disease was the most common native kidney disease, observed in 18 patients (25.0%), followed by chronic glomerulonephritis in 14 (19.4%), chronic in-
terstitial nephritis in six (8.3%), obstructive uropathy in five (6.9%), hypertensive nephrosclerosis in three (4.2%), and autosomal dominant polycystic kidney disease in two (2.8%). Native kidney disease was not known in 24 (33.3%) patients because many patients in India present with advanced stages of CKD. Among control subjects, four (5.6%) had diabetes mellitus, and two (2.8%) had hypertension. Demographic details, clinical characteristics, and laboratory results of the study subjects are shown in Table 1.

Lower humoral response in the dialysis group

All study subjects in the control group developed a positive humoral response defined based on a value of ≥0.8 U/mL compared with only 64 of 72 (88.9%) in the dialysis group (Table 1). All eight patients with nonreactive antibody titers were older (greater than the mean age of the dialysis group), four were HCV infected, and two had diabetes mellitus.

Adverse events

Fever and myalgia were significantly more common in the control subjects (Table 2). Other adverse events such as pain at the local site, pruritus, or abdominal pain were observed in only a few patients. Serious adverse events were not observed in any study subjects.

Factors affecting antibody titers

In the dialysis group, age negatively correlated with antibody titer (Table 3); titer was lower with increasing age (Spearman correlation $\rho = -0.234$, $p = 0.04$). Dialysis dose $(\text{Kt/V})$ did not correlate with antibody titer ($\rho = 0.085$, $p = 0.75$). Similarly, hemoglobin ($\rho = 0.003$, $p = 0.98$) and albumin levels ($\rho = 0.147$, $p = 0.22$) did not correlate with titer. Bivariate analysis of laboratory results, sex, age, and BMI with antibody titers is shown in Table 3.

In the dialysis group, age and sodium level correlated with antibody titer in bivariate analysis; titers were lower in patients with higher age (Spearman correlation $\rho = -0.234$, $p = 0.048$) and lower sodium level ($\rho = 0.237$, $p = 0.045$). Statistically significant correlation was not observed between antibody titers and other lab parameters.

Fisher exact test ($p > 0.05$) showed no significant difference in the proportion of reactive titer group subjects and nonreactive titer group subjects regarding HCV infection, diabetes mellitus, BMI, dialysis duration, lymphocyte count, and adverse events such as fever and myalgia (Table 4).

A multivariate logistic regression model was used to construct the statistical model to predict the risk of nonreactive antibody titers using the most significant two factors correlating with low antibody titers identified based on bivariate analysis, age, and sodium levels (Table 5). Only age ($p = 0.045$) was a statistically significant independent variable associated with nonreactive antibody titers.

Discussion

The compromised immunity in hemodialysis patients is evident by a poor immune response to vaccinations such as hepatitis B [1], pneumococcus [2], or influenza [3]. In the present study, the humoral immune response to COVID-19 vaccination (Covishield) in hemodialysis patients compared with a cohort of healthcare workers in the same center was investigated.

Covishield is a recombinant, monovalent vaccine composed of a replication-deficient chimpanzee adenovirus (ChAdOx1) vector that encodes the S glycoprotein of SARS-CoV-2. The antigen is expressed locally, stimulating neutralizing antibodies and cellular immune responses. The initial schedule of Covishield ChAdOx1 nCoV-19 coronavirus vaccine (recombinant) was two doses of 0.5 mL each, 4 weeks apart, which was later spaced to 12 to 16 weeks for better coverage of a larger population and due to improved efficacy in clinical trials with increased spacing. Due to a short supply of vaccines, a policy of initially vaccinating a larger cohort with a single dose might provide better overall population protection than vaccinating half the number of individuals with two doses in the short term [1].

In our study, the antibody titers were significantly lower in the dialysis group, including eight patients (11.1%) with absent antibody titer (<0.8 U/mL). This weaker antibody response was comparable with results obtained in studies with messenger RNA vaccines [11,12], in which approximately 10% of dialysis patients did not have an antibody response. Simon et al. [13] studied vaccine response in 86 dialysis patients and found 17 (19.8%) were nonresponders even after the second dose, which was almost double than reported in our study. The study by Simon et al. [13] excluded patients who tested positive for antibodies before
Table 1. Demographic details, clinical characteristics, and laboratory parameters of the study subjects

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dialysis group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (80.6)</td>
<td>24 (33.3)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (19.4)</td>
<td>48 (66.7)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.14 ± 8.89</td>
<td>40.35 ± 6.65</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.87 ± 4.26</td>
<td>23.12 ± 3.35</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20 (27.8)</td>
<td>4 (5.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>48 (66.7)</td>
<td>2 (2.8)</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>4 (5.6)</td>
<td>-</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>4 (5.6)</td>
<td>-</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>2 (2.8)</td>
<td>-</td>
</tr>
<tr>
<td>HCV infection</td>
<td>36 (50.0)</td>
<td>-</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>145.81 ± 15.10</td>
<td>127.56 ± 6.20</td>
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<tr>
<td>Diastolic blood pressure</td>
<td>94.77 ± 10.63</td>
<td>79.12 ± 4.21</td>
</tr>
<tr>
<td>Native kidney disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic kidney disease</td>
<td>18 (25.0)</td>
<td>-</td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>14 (19.4)</td>
<td>-</td>
</tr>
<tr>
<td>Chronic interstitial nephritis</td>
<td>6 (8.3)</td>
<td>-</td>
</tr>
<tr>
<td>Obstructive uropathy</td>
<td>5 (6.9)</td>
<td>-</td>
</tr>
<tr>
<td>Hypertensive nephrosclerosis</td>
<td>3 (4.2)</td>
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</tr>
<tr>
<td>ADPKD</td>
<td>2 (2.8)</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>24 (33.3)</td>
<td>-</td>
</tr>
<tr>
<td>Dialysis access</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriovenous fistula</td>
<td>68 (94.4)</td>
<td>-</td>
</tr>
<tr>
<td>Permanent catheter</td>
<td>4 (5.6)</td>
<td>-</td>
</tr>
<tr>
<td>Dialysis adequacy (Kt/V)</td>
<td>1.16 ± 0.17</td>
<td>-</td>
</tr>
<tr>
<td>Dialysis duration (mo)</td>
<td>8.65 ± 6.68</td>
<td>-</td>
</tr>
<tr>
<td>Laboratory parameters</td>
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</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.07 ± 1.33</td>
<td>12.69 ± 1.27</td>
</tr>
<tr>
<td>White blood cell count (cell/mm³)</td>
<td>6,944.4 ± 2,770.4</td>
<td>8,204.2 ± 1,991.0</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>67.85 ± 10.54</td>
<td>58.11 ± 9.13</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>22.49 ± 14.16</td>
<td>31.49 ± 6.72</td>
</tr>
<tr>
<td>Neutrophil-to-lymphocyte ratio</td>
<td>3 (2.09–5.26)</td>
<td>1.79 (1.38–2.57)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>7.15 ± 3.29</td>
<td>0.88 ± 0.17</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>133.5 ± 5.2</td>
<td>136.5 ± 4.0</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.49 ± 1.05</td>
<td>4.27 ± 0.36</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.03 ± 0.70</td>
<td>4.05 ± 0.37</td>
</tr>
<tr>
<td>Antibody titer (U/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive titer, ≥0.8</td>
<td>64 (88.9)</td>
<td>72 (100)</td>
</tr>
<tr>
<td>Nonreactive titer, &lt;0.8</td>
<td>8 (11.1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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

ADPKD, autosomal dominant polycystic kidney disease; HCV, hepatitis C virus.
vaccination, thereby eliminating subjects who developed antibodies due to subclinical infections. In contrast, all participants in the control group developed a humoral response, with the majority (89.9%) having titer of ≥250 U/mL. This comparison clearly demonstrated a weaker antibody response in dialysis patients, indicating this group was susceptible to higher morbidity and mortality following infection.

Overall, the age of dialysis subjects enrolled in the present study was relatively younger (46.14 ± 8.89 years) compared with that of CKD patients. Most of the older patients in our center were vaccinated much earlier than 4 weeks because only older individuals were eligible for the initial round of vaccinations in India, hence, were not eligible for the study. In addition, fewer older patients were receiving hemodialysis in the dialysis unit because many older end-stage renal disease patients refuse dialysis, and even those scheduled for maintenance hemodialysis typically do not receive dialysis regularly and are lost to follow-up due to economic burden on the family.

Age, sex, and BMI were distributed unequally between the control and dialysis groups; the majority of subjects were female in the control group and male in the dialysis group. Furthermore, the patients in the control group were younger on average and had higher BMI than subjects in

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**Table 2. Adverse events due to vaccination in dialysis and control groups**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Dialysis group (n = 72)</th>
<th>Control group (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>12 (16.7)</td>
<td>32 (44.4)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>6 (8.3)</td>
<td>32 (44.4)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>2 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pain at the local site</td>
<td>4 (5.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0 (0)</td>
<td>2 (2.8)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

---

**Table 3. Spearman rank correlation between pathological parameters, sex, age, BMI, and antibody titers**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dialysis group</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.131</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.234</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.023</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.003</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>White blood cell count</td>
<td>0.144</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.016</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>-0.117</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>NLR</td>
<td>0.123</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.057</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0.237</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.147</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Dialysis dose (Kt/V)</td>
<td>0.085</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 4. Comparison of antibody titer levels in HCV infection, diabetes mellitus, and BMI**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Responder</th>
<th>Nonresponder</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32 (50.0)</td>
<td>4 (50.0)</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Negative</td>
<td>32 (50.0)</td>
<td>4 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18 (28.1)</td>
<td>2 (25.0)</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Negative</td>
<td>46 (71.9)</td>
<td>6 (75.0)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>22 (16.2)</td>
<td>2 (25.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Normal</td>
<td>57 (41.9)</td>
<td>6 (75.0)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>47 (34.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>10 (7.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; HCV, hepatitis C virus.

---

**Table 5. Factors associated with nonreactive antibody titers among the dialysis patients based on multivariate logistic regression analysis**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.11 (1.01–1.22)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.76 (0.71–1.08)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

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BMI, body mass index; HCV, hepatitis C virus.

*World Health Organization’s Asian BMI classification.
the dialysis group.

A multivariate analysis was performed to account these confounding factors. Among the variables, only age affected the antibody titers with statistical significance, which was in agreement with various studies [13–16] showing similar relatively poor antibody responses in older age groups. This reinforces the importance of the age factor in the humoral response.

Bivariate analysis showed sodium to correlate with low antibody titer (\( \rho = -0.237, p = 0.04 \)) but was not significant in multivariate analysis including age and sodium level as variables. The correlation in bivariate analysis indicated the probable presence of cofounders. Hemodilution due to volume overload could have caused pseudo-low antibody titers because samples were collected before the dialysis session. Most patients in the study were presumed to have achieved dry weight because only subjects receiving hemodialysis for more than 2 months were enrolled in the study. However, volume status was assessed based on clinical examination, and objective methods such as body composition monitors were not used. Furthermore, hyponatremia in dialysis patients can be due to factors other than fluid overload, such as malnutrition and inflammation [17,18]. A detailed assessment is needed of nutritional status and inflammatory markers not tested in the present study, which are study’s limitations.

A statistically significant association was not observed between antibody levels and sex, BMI, dialysis duration, dialysis dose, hemoglobin, albumin, total count, lymphocyte count, urea, liver function tests, HCV infection, diabetes mellitus, and adverse events due to vaccines. Grupper et al. [15] demonstrated a correlation between lymphocyte count and the humoral response given their role in adaptive immunity, which was not observed here; however, the authors also did not find an association with BMI, dialysis duration, dialysis dose, or albumin level.

Although the seroconversion rate may be considered relatively good for dialysis patients, it is subpar when compared to normal subjects. These patients are exposed repeatedly to other patients and healthcare workers due to their regular visits for dialysis and have high morbidity and mortality due to comorbidities.

Baseline antibody titers were not measured in the study participants. Although previously infected patients were excluded from our study, seroconversion due to subclinical infections or minimal symptoms might not have been evident. However, this confounding factor was at least partly addressed by selecting control subjects from the same hemodialysis center.

Neutralization assays such as the plaque reduction neutralization test (PRNT) are the gold standard for serological testing and determining immune protection against COVID-19 [19]. PRNT has several limitations rendering it unsuitable for large-scale studies. The assay used in the present study, Elecsys Anti-SARS-CoV-2 S, is not a neutralization test but has a good correlation with pseudovirus neutralization assay (safer and more versatile than neutralization assay), with a positive agreement of 92.3% (95% confidence interval [CI], 63.97–99.81) [20]. This assay shows a positive predictive agreement of 96.6% (95% CI, 93.35–98.51) and a negative predictive agreement of 99.98% (95% CI, 99.91–100) [21].

SARS-CoV-2 antibodies against S protein and its subunits can be detected within 1 to 3 weeks after infection [22,23]. However, we tested in the 5th week after vaccination because our test attains maximum sensitivity by that period [9].

The upper limit of detection of Elecsys Anti-SARS-CoV-2 S assay is 250 U/mL, a limitation of our study. A higher upper limit of the assay would be preferable because a better difference in adaptive humoral response between dialysis and the control groups could have been identified. Similarly, the complete humoral response profile after the second dose of the COVID-19 vaccine will provide relevant information but will require a larger sample and control cohorts. This was a single-center study with a limited number of patients, and complete humoral response was not the focus; however, differences were apparent.

Lack of follow-up of the patient titers is another limitation of our study. Even in normal subjects with adequate antibody responses, titers have been shown to decline over time [24]. In some studies, SARS-CoV-2 IgG titers decline substantially in dialysis patients within 3 months after diagnosis [25]. Therefore, these patients should be followed up with titers to monitor the level of immunity, which can help determine the need and timing of booster doses.

In addition to antibody production in response to SARS-CoV-2 infection, host cellular immunity plays an essential role in impeding virus replication and expansion at various stages of COVID-19 disease [26]. CD4 and CD8 T
cells recognize multiple regions of the N protein of SARS-CoV-2 in previously exposed patients. T cells showed strong cross-reactivity to the N protein of SARS-CoV-2 in patients who recovered from SARS (the disease associated with SARS-CoV infection) 17 years after the outbreak in 2003 [27]. Furthermore, patients who had no detectable antibody at 6 months after COVID-19 infection were found to have SARS-CoV-2 antigen-specific T-cell responses [28]. Our study did not attempt to determine the T-cell immune response, which might contribute to protection from COVID-19.

In conclusion, our study results demonstrated a weak antibody response of hemodialysis patients to the viral vector COVID-19 vaccine, and older age was associated with nonresponders. Evaluation of both humoral and cellular immunity after the second dose of COVID-19 vaccine and serial follow-up of antibody titers can help determine the need for booster shots.

Conflicts of interest

All authors have no conflicts of interest to declare.

Authors’ contributions

Conceptualization: AR, NR
Data curation: NR, RD
Formal analysis: MR, RD
Investigation: NR, AP
Methodology: AR, NR
Project administration: NR, JA
Visualization: NR
Writing-original draft: NR
Writing-review & editing: all authors
All authors read and approved the final manuscript.

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References


Introduction

Cardiovascular (CV) disease is a major cause of mortality in end-stage kidney disease (ESKD) patients [1]. Both atherosclerosis and arteriosclerosis contribute to arterial disease in these patients by causing extensive media and intima calcification [2]. Inappropriate calcification of vessel walls can be measured by diverse noninvasive methods, and computed tomography (CT) is the gold standard for quantification of vascular calcification (VC) [3]. Abdominal
Aortic calcification can be used to assess both atherosclerosis and arteriosclerosis [4]. We previously demonstrated that the aortic calcification index (ACI), an estimate of abdominal aortic calcification, is a significant predictor of all-cause death and CV events in ESKD patients [5,6].

Red blood cell distribution width (RDW) is an index of erythrocyte size heterogeneity [7]. In addition to its role as a marker for differential diagnosis of anemia, RDW has emerged as an independent marker for predicting various medical conditions. We previously reported that increased RDW after initiation of dialysis independently predicts CV events and deaths in ESKD patients [8]. In addition, RDW has been reported to have a prognostic role in patients with various stages of chronic kidney disease (CKD), including ESKD [9,10]. However, the interaction between serum RDW and VC has not been investigated in clinical studies. We hypothesized that higher RDW at baseline would augment the risk of all-cause mortality and CV events associated with VC. This study evaluated the clinical impact of VC and RDW in ESKD patients starting dialysis.

Methods

Study population

In our center, we perform CT of the abdomen at the initiation of dialysis to evaluate for the presence of acquired cystic lesions and malignancy in the kidney. Among 713 adult ESKD patients (age, ≥20 years) starting maintenance dialysis (incident dialysis patients, including emergency dialysis) between January 2006 and July 2017, 131 patients who had not undergone noncontrast abdomen CT at the initiation of dialysis, who had undergone a major surgical procedure, or had incomplete medical records were excluded. A total of 582 patients who started maintenance dialysis and who underwent a noncontrast abdominal CT scan at the initiation of dialysis between January 2006 and July 2017 were included (Fig. 1).

The study was approved by the Institutional Review Board of The Catholic University of Korea (No. OC19OISI0172) and was conducted according to the principles of the Declaration of Helsinki. This was a retrospective study of anonymized patient data and therefore the need for written consent was waived.

Assessment of abdominal aortic calcification

The abdominal aorta was examined by noncontrast CT on consecutive, sequential 8-mm slices. The proportion of the aortic circumference covered by the most extensive arteriosclerotic portion was quantified in cross-section and expressed as the ACI [11]. Semiquantitative measurement

![Figure 1. A flow diagram of study population.](image-url)

ACI, aortic calcification index; ESKD, end-stage kidney disease; CT, computed tomography; RDW, red blood cell distribution width.
of the ACI was conducted independently by two observers and was reproducible for the patients examined.

**Data collection and definitions**

Demographic characteristics including age, sex, smoking status, body mass index (BMI), dialysis modalities, comorbidities, laboratory data, and treatment characteristics were compared. Blood samples were obtained from all patients at the initiation of dialysis. BMI was defined as the patient’s weight in kilograms divided by their height in meters squared (kg/m²). Estimated glomerular filtration rate (eGFR) was calculated using the following dialysis-specific equation recommended by Shafi et al. \[12\]: eGFR (mL/min/1.73 m²) = 2.4 × blood urea nitrogen⁰.⁹⁸⁴ × creatinine⁻¹.₈⁶⁴. Serum levels of creatinine, blood urea nitrogen, albumin, alkaline phosphatase, calcium, phosphorous, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, intact parathyroid hormone (PTH), iron, transferrin saturation, ferritin, vitamin B12, folate, and high sensitivity C-reactive protein (hs-CRP) were measured by laboratory tests, and hemoglobin content, RDW, leukocyte count, and platelet count were also determined. History of medication use such as erythropoiesis-stimulating agents, iron replacement medications, renin-angiotensin-aldosterone system (RAAS) blockers, beta-blockers, calcium channel blockers, calcium-based phosphorus-binders, non-calcium-based phosphorus-binders, statins, and vitamin D analogues was recorded.

Patients were divided into four groups (low ACI-low RDW, low ACI-high RDW, high ACI-low RDW, and high ACI-high RDW) according to median ACI (17.12) and RDW (14.3) values.

**Echocardiography**

In our center, we perform echocardiography at the initiation of dialysis to evaluate cardiac function. Two-dimensional echocardiographic measurements were performed by trained sonographers according to the recommendations of the American Society of Echocardiography \[13\]. Left ventricular (LV) end-systolic volume, LV end-diastolic volume (LVEDV), LV posterior wall thickness, interventricular septal thickness (IVST), and LV internal dimension (LVID) were assessed. LV ejection fraction and left atrial (LA) diameter were measured by biplane Simpson’s rule. LV mass index was calculated according to the Devereux formula and indexed to body surface area \[14\]. LV diastolic dysfunction was estimated by measuring mitral inflow velocities and myocardial velocities using pulsed-wave Doppler imaging. Peak velocity of early filling (E), peak velocity of atrial filling (A), and ratio of E to A waves (E/A ratio) were also recorded. Septal mitral annular early peak velocity (E’) and E/E’ ratio were determined by tissue Doppler imaging.

**Outcome measures**

The primary study endpoint was the composite of patient mortality and CV events. CV events were defined as the incidence of coronary heart disease (angina pectoris or myocardial infarction), heart failure, cerebrovascular disease (transient ischemic attack, cerebral infarction, or cerebral hemorrhage), peripheral vascular disease, or pulmonary vascular disease.

**Statistical analysis**

Baseline characteristics of the study cohort stratified by ACI and RDW are expressed as means ± standard deviations or medians with interquartile ranges (25th–75th percentile) for variables with a skewed distribution. Categorical data are expressed as numbers with percentages. Continuous variables were compared using the Kruskal-Wallis test or a one-way analysis of variance as appropriate. Categorical data were compared using the chi-squared test. Survival distributions of the different groups were estimated using Kaplan-Meier curves and compared using log-rank tests. Cox regression analysis was used to identify prognostic variables contributing to all-cause mortality and CV events. Univariate analysis was followed by multivariate analysis using the forward method. Variables with statistical significance were included in multivariate analysis. Estimated standard error of the coefficient (β) was used to establish the 95% confidence interval (CI) of the hazard ratio (HR). An interaction model was conducted in the context of Cox regression analyses using the forward method. In an interaction model, a third variable influences the relationship between an independent and dependent variable \[15\]. In this study, to determine if ACI mediated the relationship
between RDW and the primary study endpoint, or to determine if RDW mediated the relationship between ACI and the study endpoint, a two-way interaction term between ACI and RDW was included in the model. Correlations among variables were assessed by Spearman correlation analysis. A p-value less than 0.05 denoted statistical significance. Statistical analyses were performed using PASW Statistics version 18.0 (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

In total, 582 patients were included in the analysis (low ACI-low RDW, n = 154; low ACI-high RDW, n = 138; high ACI-low RDW, n = 135; and high ACI-high RDW, n = 155). Table 1 compares patient characteristics among the four groups. Four hundred seventeen patients (71.6%) were hemodialysis patients, and 165 (28.4%) were peritoneal dialysis patients. Patients in the high ACI groups were older, more likely to have diabetes mellitus and CV disease, and have higher eGFR, serum leukocyte counts, and calcium and folate levels at the initiation of dialysis than patients in the low ACI groups. The cause of ESKD, BMI, serum albumin, phosphorus, hemoglobin, triglyceride, intact PTH, hs-CRP, and iron levels, in addition to RDW, platelet count, and ACI differed significantly among the four groups. Patients in the high ACI groups used significantly less calcium-based and non-calcium-based phosphorus-binders and vitamin D analogues and significantly more beta-blockers than those in the low ACI groups. Levels of serum urea nitrogen, alkaline phosphatase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, transferrin saturation, ferritin, and vitamin B12, in addition to dialysis modality, sex, smoking percentage, and the use of erythropoiesis-stimulating agents, iron replacement medications, RAAS blockers, calcium channel blockers, and statins did not differ among the four groups.

Echocardiographic measurements

Table 2 compares echocardiographic measurements among the four groups. A total of 318 patients underwent echocardiography at dialysis initiation. LVID in systole, LVID in diastole, LA diameter, E/A ratio, and E/E’ ratio differed significantly between the four groups. Patients in the high ACI groups had a significantly lower LVID in systole, LVID in diastole, and LVEDV, as well as a significantly higher IVST at diastole, LA diameter, and E/E’ ratio, than patients in the low ACI groups.

Cardiovascular events and deaths

A total of 165 CV events (28.4%) and 124 deaths (21.4%) occurred during the follow-up (median duration, 3.1 years; range, 1.5–5.5 years). Kaplan-Meier analysis showed that cumulative event-free survival was significantly lower in the high ACI groups than in the low ACI groups (p < 0.001) (Fig. 2). There was a significant difference in cumulative event-free survival between the low ACI-high RDW group and the low ACI-low RDW group (p = 0.03), whereas cumulative event-free survival was not significantly different between the high ACI-low RDW group and high ACI-high RDW group (p = 0.99).

Predictors of cardiovascular events and death

Factors associated with CV events and death were compared using the low ACI-low RDW group as the reference (Table 3). In univariate analysis, the risk of CV events and death was higher in the low ACI-high RDW, high ACI-low RDW, and high ACI-high RDW groups than the low ACI-low RDW group. Moreover, multivariate analysis adjusted for older age, diabetes mellitus, history of CV disease, eGFR, hemoglobin level, ACI value per se, RDW value per se, albumin, hs-CRP, and total cholesterol levels, and use of non-calcium-based phosphorus-binders verified that the HR was increased in the low ACI-high RDW (adjusted HR, 1.66; 95% CI, 1.04–2.66; p = 0.03) and high ACI-low RDW (adjusted HR, 1.95; 95% CI, 1.21–3.14; p = 0.006) groups and was highest in the high ACI-high RDW group (adjusted HR, 2.23; 95% CI, 1.42–3.52; p = 0.001). In addition, there was a significant interaction between ACI and RDW for the composite endpoint (p for interaction = 0.005).

In subgroup analysis of patients with baseline echocardiographic data (n = 318), the high ACI-high RDW group (adjusted HR, 3.34; 95% CI, 1.62–6.89; p = 0.001) exhibited the highest HR in multivariate analysis after adjusting for older age, diabetes mellitus, history of CV disease, ACI,
Table 1. Baseline demographic and laboratory data of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low ACI-low RDW (n = 154)</th>
<th>Low ACI-high RDW (n = 138)</th>
<th>High ACI-low RDW (n = 135)</th>
<th>High ACI-high RDW (n = 155)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>154</td>
<td>138</td>
<td>135</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50.3 ± 12.7</td>
<td>53.6 ± 12.5</td>
<td>67.0 ± 10.0</td>
<td>68.8 ± 10.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>92 (15.8)</td>
<td>86 (14.8)</td>
<td>69 (11.9)</td>
<td>82 (14.1)</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 4.3</td>
<td>23.3 ± 3.8</td>
<td>24.6 ± 3.8</td>
<td>23.2 ± 3.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoking (%)a</td>
<td>40 (7.0)</td>
<td>35 (6.1)</td>
<td>30 (5.2)</td>
<td>29 (5.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>83 (14.3)</td>
<td>58 (10.0)</td>
<td>96 (16.5)</td>
<td>96 (16.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of CV disease (%)</td>
<td>19 (3.3)</td>
<td>14 (2.4)</td>
<td>41 (7.0)</td>
<td>41 (7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cause of ESKD (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysis modality</td>
<td>Hemodialysis</td>
<td>99 (17.0)</td>
<td>104 (17.9)</td>
<td>115 (19.8)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Peritoneal dialysis</td>
<td>55 (9.5)</td>
<td>39 (6.7)</td>
<td>31 (5.3)</td>
<td>40 (6.9)</td>
</tr>
<tr>
<td></td>
<td>eGFR (mL/min/1.73 m²)</td>
<td>4.2 (2.7–6.7)</td>
<td>3.9 (2.5–6.4)</td>
<td>6.1 (4.0–9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Creatinine (mg/dL)</td>
<td>6.9 (5.4–9.4)</td>
<td>7.2 (5.2–10.3)</td>
<td>5.5 (4.3–6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Urea nitrogen (mg/dL)</td>
<td>77.5 (58–99)</td>
<td>84.9 (59–98)</td>
<td>71.7 (54–89)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/dL)</td>
<td>3.6 (3.1–4.0)</td>
<td>3.4 (2.9–3.9)</td>
<td>3.4 (2.9–3.9)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>ALP (U/L)</td>
<td>101 (77–182)</td>
<td>108 (82–189)</td>
<td>119 (82–167)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Calcium (mg/dL)</td>
<td>7.9 (7.2–8.6)</td>
<td>7.7 (7.2–8.3)</td>
<td>8.2 (7.6–8.7)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Phosphorus (mg/dL)</td>
<td>5.5 (4.5–7.2)</td>
<td>5.8 (4.5–7.3)</td>
<td>5.0 (4.2–6.2)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin (g/dL)</td>
<td>8.9 (7.7–9.9)</td>
<td>8.8 (7.6–9.7)</td>
<td>9.3 (8.5–10.2)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>RDW (%)</td>
<td>13.2 (12.7–13.6)</td>
<td>15.1 (14.5–16.1)</td>
<td>13.2 (12.9–13.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Leukocyte count (×10³/μL)</td>
<td>7.5 (5.8–9.8)</td>
<td>7.4 (5.8–9.9)</td>
<td>7.4 (5.7–9.7)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Platelet count (×10³/μL)</td>
<td>203 (159–261)</td>
<td>182 (135–247)</td>
<td>206 (166–258)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol (mg/dL)</td>
<td>170 (137–199)</td>
<td>158 (125–201)</td>
<td>153 (121–198)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Triglyceride (mg/dL)</td>
<td>139 (111–203)</td>
<td>122 (92–172)</td>
<td>142 (104–181)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol (mg/dL)</td>
<td>37 (29–48)</td>
<td>38 (30–49)</td>
<td>36 (27–45)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol (mg/dL)</td>
<td>107 (84–134)</td>
<td>98 (71–128)</td>
<td>94 (77–127)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Intact PTH (pg/mL)</td>
<td>273 (169–439)</td>
<td>257 (141–421)</td>
<td>198 (121–316)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>hs-CRP (mg/L)</td>
<td>2.8 (0.9–18.3)</td>
<td>3.5 (1.1–16.0)</td>
<td>3.0 (0.9–17.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Iron (μg/dL)</td>
<td>57 (40–88)</td>
<td>57 (36–84)</td>
<td>56 (39–74)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Transferrin saturation (%)</td>
<td>26.5 (19–41)</td>
<td>25.6 (17–38)</td>
<td>25.1 (18–38)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Ferritin (ng/mL)</td>
<td>187 (98–326)</td>
<td>180 (66–339)</td>
<td>194 (105–339)</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td>ACIb</td>
<td>3.2 ± 5.67</td>
<td>3.8 ± 6.06</td>
<td>33.9 ± 12.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Vitamin B12 (pg/mL)</td>
<td>756 (512–1,009)</td>
<td>811 (517–1,087)</td>
<td>807 (576–1,123)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Folate (ng/mL)</td>
<td>7.8 (6–17)</td>
<td>8.2 (5–19)</td>
<td>9.9 (6–23)</td>
<td>13.9 (7–25)</td>
</tr>
<tr>
<td></td>
<td>ESA</td>
<td>142 (24.4)</td>
<td>135 (23.2)</td>
<td>128 (22.0)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Iron replacement</td>
<td>135 (23.2)</td>
<td>116 (19.9)</td>
<td>112 (19.6)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Non-Ca-based P-binders</td>
<td>110 (18.9)</td>
<td>101 (17.4)</td>
<td>75 (12.9)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Ca-based P-binders</td>
<td>41 (7.0)</td>
<td>47 (8.1)</td>
<td>21 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Vitamin D analogues</td>
<td>56 (9.6)</td>
<td>46 (7.9)</td>
<td>36 (6.2)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>RAAS blockers</td>
<td>118 (20.3)</td>
<td>95 (16.3)</td>
<td>97 (16.7)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Beta-blockers</td>
<td>108 (18.6)</td>
<td>102 (17.5)</td>
<td>97 (16.7)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Statins</td>
<td>84 (14.4)</td>
<td>63 (10.8)</td>
<td>80 (13.7)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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

ACI, aortic calcification index; ALP, alkaline phosphatase; Ca, calcium; CV, cardiovascular; eGFR, estimated glomerular filtration rate; ESA, erythropoietin stimulating agent; ESKD, end-stage kidney disease; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; P, phosphorus; PTH, parathyroid hormone; RAAS, renin-angiotensin-aldosterone system; RDW, red blood cell distribution width.

"n = 575. *Mean value despite skewed distribution since median value and interquartile range derive ‘zero’ in low ACI groups"
Table 2. Comparison of echocardiographic measurements of groups based on ACI and RDW

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low ACI-low RDW (n = 73)</th>
<th>Low ACI-high RDW (n = 81)</th>
<th>High ACI-low RDW (n = 80)</th>
<th>High ACI-high RDW (n = 84)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass index (g/m²)</td>
<td>56 (47–66)</td>
<td>56 (44–70)</td>
<td>60 (50–70)</td>
<td>60 (48–72)</td>
<td>0.31</td>
</tr>
<tr>
<td>LVID in diastole (mm)</td>
<td>49 (47–53)</td>
<td>51 (45–55)</td>
<td>48 (43–51)</td>
<td>49 (44–52)</td>
<td>0.03</td>
</tr>
<tr>
<td>LVID in systole (mm)</td>
<td>35 (30–38)</td>
<td>33 (30–39)</td>
<td>31 (27–34)</td>
<td>34 (29–37)</td>
<td>0.02</td>
</tr>
<tr>
<td>PW thickness in end-diastole (mm)</td>
<td>11 (10–12)</td>
<td>11 (10–12)</td>
<td>12 (11–12)</td>
<td>12 (11–12)</td>
<td>0.07</td>
</tr>
<tr>
<td>PW thickness in end-systole (mm)</td>
<td>15 (14–17)</td>
<td>15 (14–16)</td>
<td>15 (14–16)</td>
<td>15 (14–17)</td>
<td>0.54</td>
</tr>
<tr>
<td>LV end diastolic volume (mm³)</td>
<td>102 (89–139)</td>
<td>103 (79–132)</td>
<td>96 (78–107)</td>
<td>97 (78–117)</td>
<td>0.02</td>
</tr>
<tr>
<td>LV end systolic volume (mm³)</td>
<td>40 (31–69)</td>
<td>45 (29–59)</td>
<td>36 (28–50)</td>
<td>41 (32–56)</td>
<td>0.09</td>
</tr>
<tr>
<td>IVST at end-diastole (mm)</td>
<td>12 (11–12)</td>
<td>11 (10–12)</td>
<td>12 (11–13)</td>
<td>12 (11–13)</td>
<td>0.008</td>
</tr>
<tr>
<td>IVST at end-systole (mm)</td>
<td>15 (14–16)</td>
<td>15 (13–16)</td>
<td>15 (14–16)</td>
<td>15 (14–16)</td>
<td>0.44</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>60 (54–64)</td>
<td>61 (55–64)</td>
<td>61 (56–65)</td>
<td>58 (49–64)</td>
<td>0.081</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>40 (34–44)</td>
<td>40 (35–44)</td>
<td>42 (40–45)</td>
<td>43 (40–47)</td>
<td>0.001</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>0.87 (0.7–1.2)</td>
<td>0.90 (0.6–1.1)</td>
<td>0.94 (0.6–0.8)</td>
<td>0.88 (0.6–1.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>10.5 (8–14)</td>
<td>11.1 (9–14)</td>
<td>14.5 (12–17)</td>
<td>14.1 (11–19)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range).
ACI, aortic calcification index; E/A ratio, ratio of peak velocity of early filling (E) to peak velocity of atrial filling (A); E/E’ ratio, ratio of peak velocity of early filling (E) to early diastolic mitral annular early peak velocity (E’); IVST, interventricular septal thickness at end-diastole; LA, left atrium; LV, left ventricle; LVID, left ventricular internal dimension; PW, left ventricular posterior wall; RDW, red blood cell distribution width.

Figure 2. Comparison of event-free survival rate for all-cause mortality and cardiovascular events according to the ACI and serum RDW value.
ACI, aortic calcification index; RDW, red blood cell distribution width.
The interaction between ACI and RDW for the composite endpoint was also statistically significant (p for interaction = 0.001).

## Correlation between aortic calcification index and red blood cell distribution width

In Spearman correlation analysis, ACI and RDW were not significantly correlated (r = 0.068 and p = 0.100) (Fig. 3).

## Discussion

This study showed that a high serum RDW and ACI interacted to increase the risk of CV events and all-cause death in ESKD patients. Using the low ACI-low RDW group as the reference, there was a significant trend of increasing CV events and deaths from the low ACI-low RDW group to the high ACI-high RDW group. This finding was independent of other powerful predictors such as age, traditional CV risk factors, and laboratory parameters associated with mineral metabolism disorders. In patients for whom echocardiographic data were available at the initiation of dialysis,

### Table 3. Cox regression analysis of predictors of all-cause mortality and CV events

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.12 (0.85–1.47)</td>
<td>0.44</td>
</tr>
<tr>
<td>Age, &gt;60 yr</td>
<td>2.41 (1.82–3.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.90 (0.66–1.23)</td>
<td>0.50</td>
</tr>
<tr>
<td>HD vs. PD</td>
<td>1.02 (0.77–1.36)</td>
<td>0.90</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.99 (0.97–1.03)</td>
<td>0.94</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.81 (1.35–2.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of CV disease</td>
<td>1.97 (1.45–2.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR</td>
<td>1.03 (1.01–1.06)</td>
<td>0.009</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1.09 (1.01–1.18)</td>
<td>0.03</td>
</tr>
<tr>
<td>ACI</td>
<td>1.02 (1.02–1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.72 (0.57–0.91)</td>
<td>0.007</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.00 (1.00–1.01)</td>
<td>0.04</td>
</tr>
<tr>
<td>RAAS blockers</td>
<td>0.86 (0.63–1.17)</td>
<td>0.33</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>1.01 (0.76–1.35)</td>
<td>0.95</td>
</tr>
<tr>
<td>Statins</td>
<td>1.03 (0.79–1.36)</td>
<td>0.82</td>
</tr>
<tr>
<td>Non-Ca-based P-binders</td>
<td>0.44 (0.29–0.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D analogues</td>
<td>0.75 (0.55–1.02)</td>
<td>0.07</td>
</tr>
<tr>
<td>Iron</td>
<td>0.99 (0.99–1.00)</td>
<td>0.08</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>0.99 (0.99–1.00)</td>
<td>0.40</td>
</tr>
<tr>
<td>Ferritin</td>
<td>1.00 (0.99–1.00)</td>
<td>0.63</td>
</tr>
<tr>
<td>RDW</td>
<td>1.09 (1.01–1.18)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.99 (0.99–1.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>1.00 (0.99–1.00)</td>
<td>0.39</td>
</tr>
<tr>
<td>Folate</td>
<td>1.00 (0.99–1.00)</td>
<td>0.77</td>
</tr>
<tr>
<td>Low ACI-low RDW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ACI-high RDW</td>
<td>1.64 (1.03–2.59)</td>
<td>0.04</td>
</tr>
<tr>
<td>High ACI-low RDW</td>
<td>3.39 (2.21–5.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High ACI-high RDW</td>
<td>3.46 (2.26–5.30)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ACI, aortic calcification index; Ca, calcium; CI, confidence interval; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HD, hemodialysis; HR, hazard ratio; hs-CRP, high sensitivity C-reactive protein; P, phosphorus; PD, peritoneal dialysis; RAAS, renin-angiotensin-aldosterone system; RDW, red blood cell distribution width.
Table 4. Cox regression analysis of factors associated with CV events and death among patients with echocardiographic data available

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th></th>
<th></th>
<th>Multivariate analysis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, &gt;60 yr</td>
<td>2.17 (1.42–3.32)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.96 (1.27–3.04)</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of CV disease</td>
<td>1.83 (1.15–2.93)</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR</td>
<td>1.03 (0.99–1.07)</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1.09 (0.98–1.21)</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACI</td>
<td>1.02 (1.01–1.03)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>0.65 (0.46–0.91)</td>
<td>0.01</td>
<td>0.52 (0.36–0.76)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.00 (0.99–1.01)</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Ca-based P-binders</td>
<td>0.39 (0.23–0.66)</td>
<td>&lt;0.001</td>
<td>0.40 (0.23–0.69)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass index</td>
<td>1.01 (0.99–1.01)</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV ejection fraction</td>
<td>0.98 (0.97–0.99)</td>
<td>0.03</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E/E’ ratio</td>
<td>1.02 (0.99–1.06)</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D analogues</td>
<td>0.69 (0.45–1.05)</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>1.00 (0.99–1.01)</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>1.01 (0.99–1.02)</td>
<td>0.24</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>1.00 (0.99–1.00)</td>
<td>0.51</td>
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<tr>
<td>RDW</td>
<td>1.05 (0.93–1.19)</td>
<td>0.42</td>
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<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.99 (0.99–0.99)</td>
<td>0.008</td>
<td>0.99 (0.99–0.99)</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>1.00 (0.99–1.00)</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>1.00 (0.99–1.01)</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ACI-low RDW</td>
<td>1.71 (0.80–3.66)</td>
<td>0.17</td>
<td>1.70 (0.79–3.66)</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ACI-high RDW</td>
<td>3.50 (1.70–7.18)</td>
<td>0.001</td>
<td>2.66 (1.27–5.57)</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ACI-low RDW</td>
<td>4.01 (1.97–8.16)</td>
<td>&lt;0.001</td>
<td>3.34 (1.62–6.89)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACI, aortic calcification index; Ca, calcium; CI, confidence interval; CV, cardiovascular; E/E’, ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (E’); eGFR, estimated glomerular filtration rate; HR, hazard ratio; hs-CRP, high sensitivity C-reactive protein; LV, left ventricle; P, phosphorous; RDW, red blood cell distribution width.

There was also a consistent trend toward an increase in the composite endpoint in the high ACI-high RDW group. Thus, patients with a high ACI and high RDW had the greatest risk of CV events and all-cause death.

The degree of red blood cell volume heterogeneity can be measured as a percentage known as the RDW [7]. RDW has been used for differential diagnosis of anemia. Elevated RDW suggests profound deregulation of erythrocyte homeostasis, reflecting malnutrition, chronic inflammatory status, and erythropoietin resistance [7,16,17]. In addition, several studies have demonstrated the clinical usefulness of RDW in various medical conditions. High RDW is associated with higher mortality rates in patients with congestive heart failure, stroke, peripheral artery disease, and acute kidney injury who are treated with continuous renal replacement therapy [18–21]. Furthermore, in patients with
stage 3–5 CKD, a high RDW is associated with all-cause mortality and CV disease [22]. In hemodialysis patients, a higher RDW is a stronger predictor of mortality than traditional laboratory markers of anemia such as hemoglobin, ferritin, and transferrin saturation [10]. The findings of the current study are consistent with previous studies, namely that an elevated RDW was associated with increased risk of CV events and death.

CV disease is the leading cause of death in patients with ESKD [23], and the extent of arterial calcification is regarded as the predominant determinant of CV disease and mortality [24]. VC is a characteristic marker of coronary atherosclerotic burden [25], and the extent of VC can be assessed using various imaging modalities, with CT being the gold standard for quantifying VC. Previous studies reported a relationship between VC and CT-based abdominal aortic calcification and demonstrated that quantification of CT-based abdominal aortic calcification provided information about the risk of CV events [26]. Advanced age, extended dialysis vintage, diabetes mellitus, elevated calcium, phosphorus, and lipid levels, and inflammation are risk factors for VC [25,27–31]. This study showed that patients in the high-ACI groups were older, had a higher prevalence of diabetes mellitus and CV disease, and had a higher calcium level and leukocyte count at the initiation of dialysis than patients in the low-ACI groups. VC is also correlated with LV diastolic dysfunction in ESKD patients [5]. We found a significant relationship between LV diastolic stiffness and limited diastolic filling in the high ACI groups based on a higher LA diameter and E/E’ ratio, and LVEDV was lower in the high ACI groups than in the low ACI groups.

In this study, the correlation between ACI and RDW was not statistically significant. To the best of our knowledge, no prior study has reported a significant correlation between VC and RDW in ESKD patients. Although there are differences in the study population between our study and previous studies, RDW and VC have also been reported to show a relationship in other patient populations. Several recent studies revealed a relationship between elevated RDW and the burden of coronary atherosclerosis, where elevated RDW was related to a higher coronary artery calcification score [32]. Another study demonstrated a positive correlation between RDW and the coronary artery calcification score after adjustment for age, sex, BMI, diabetes mellitus, glomerular filtration rate, and high-density lipo-protein cholesterol level [33]. Oleksiak et al. [34] found that an elevated RDW and coronary calcium score both independently increased the coronary atherosclerotic burden in patients diagnosed with coronary artery disease. RDW also predicted the outcomes of patients with coronary artery disease. RDW was a clinically useful prognostic factor for all-cause mortality in patients with coronary artery disease [35]. Additionally, an incremental relationship was found between increased RDW and the risk of death in patients with coronary disease [36]. Although the pathophysiological mechanism underlying the relationship between RDW and CV outcomes is not fully understood, it has been suggested that greater variation in erythrocyte volume increases blood viscosity and impairs blood flow, leading to vascular occlusion [37]. Another explanation involves oxidative stress [38]; high oxidative stress can decrease the erythrocyte lifespan and suppress the response of erythropoietin to anemia, which ultimately increases RDW [7]. Such anisocytosis disrupts blood flow through the microcirculation, resulting in tissue hypoxia and a concomitant increase in CV events [7,37]. In the current study, increased RDW at the initiation of dialysis had an additive effect on the risk of CV events and mortality in both the low ACI and high ACI groups, even after adjusting for traditional CV risk factors. The prognostic significance of RDW was also evident after adjustment for echocardiographic parameters. Therefore, it is reasonable to speculate that a high RDW contributes to increased CV events and death in ESKD patients irrespective of the degree of VC at the start of dialysis. The exact mechanism by which RDW augments VC-associated risk is not known. An elevated RDW reflects several conditions, such as impaired glycemic control, systemic inflammation, oxidative stress, impaired iron metabolism, hemodynamic overload, tissue hypoxia, endothelial dysfunction, and malnutrition [22]; a higher RDW may therefore reflect the presence of the conditions listed above that confer a higher risk of VC.

When we compared survival between the low RDW and high RDW groups, survival was not statistically significant by Kaplan-Meier analysis (data not shown, p = 0.98). Additionally, RDW was statistically significant only in univariate regression analysis, and not in multivariate analysis. Similarly, in our previous study [8], a higher RDW at the start of dialysis was not associated with mortality and CV events. However, we found that a progressive increase in RDW was
associated with increased mortality and CV events in ESKD patients. Those with a higher increase in baseline RDW and RDW over the follow-up period had lower event-free survival rate than those with less of an increase in baseline RDW and RDW [8]. A large cohort study of 109,674 hemodialysis patients reported that a higher RDW was linked to higher mortality [10]. The reason for RDW alone not being a statistically significant risk factor in the current study and our previous study [8] may be due to the relatively small sample sizes of these studies. Nevertheless, the HR increased incrementally in the low ACI-high RDW group, high ACI-low RDW group, and high ACI-high RDW group in this study. Large-scale studies are needed to determine whether the effect of ACI is more important than that of RDW and to further investigate the association between RDW, mortality, and CV events.

In the current study, the interaction between ACI and RDW was statistically significant in the entire patient population and in patients with echocardiography data. Since the correlation between ACI and RDW was not significant, this means that RDW and ACI do not interact as correlating factors. Our results indicate that besides the effect of ACI, the risk of CV events and all-cause mortality in ESKD patients is augmented by high RDW. Because patients with ESKD have a high mortality rate compared to the general population [39], our study suggests that the additive effect of RDW on ACI requires further consideration. Monitoring serial changes in ACI and RDW may be helpful to further study the interaction between these two factors.

This study had several limitations. First, the retrospective design limited robust analysis of the data. Second, data were collected from a single center, which limits the generalizability of the results. Third, the pathophysiological link underlying the interaction between high RDW and VC is unclear. However, demographics, laboratory variables, medications, and echocardiographic parameters were all included in the analysis, which strengthens the clinical implications of our findings of an interaction between RDW and VC.

In conclusion, we found that high serum RDW augmented the risk of CV events and death associated with VC in ESKD patients starting dialysis. High RDW increased the risks in all groups, including the low ACI and high ACI groups, and the risk was highest in the high ACI-high RDW group. This finding was consistent after adjusting for factors associated with anemia, nutritional status, inflammation, mineral metabolism disorder, and traditional CV risk factors. Therefore, the combination of RDW, which is a convenient and cost-effective index marker, and ACI, which reflects the degree of VC as assessed by abdominal CT, can be used to stratify risk of future CV disease and mortality in ESKD patients.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Conceptualization: HEY
Formal analysis: DWK, HEY
Investigation: All authors
Writing–original draft: DWK, HEY
Writing–review & editing: DWK, HEY
All authors read and approved the final manuscript.

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**References**


System of integrating biosignals during hemodialysis: the CONTINUAL (Continuous mOnitoriNg viTal slgN dUring hemodiALysis) registry

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Background: Appropriate monitoring of intradialytic biosignals is essential to minimize adverse outcomes because intradialytic hypotension and arrhythmia are associated with cardiovascular risk in hemodialysis patients. However, a continuous monitoring system for intradialytic biosignals has not yet been developed.

Methods: This study investigated a cloud system that hosted a prospective, open-source registry to monitor and collect intradialytic biosignals, which was named the CONTINUAL (Continuous mOnitoriNg viTal slgN dUring hemodiALysis) registry. This registry was based on real-time multimodal data acquisition, such as blood pressure, heart rate, electrocardiogram, and photoplethysmogram results.

Results: We analyzed session information from this system for the initial 8 months, including data for some cases with hemodynamic complications such as intradialytic hypotension and arrhythmia.

Conclusion: This biosignal registry provides valuable data that can be applied to conduct epidemiological surveys on hemodynamic complications during hemodialysis and develop artificial intelligence models that predict biosignal changes which can improve patient outcomes.

Keywords: Biosignal, Cardiac arrhythmias, Hypertension, Hypotension, Renal dialysis

Introduction

End-stage kidney disease is an increasing burden for global health care, such that approximately 2.6 million patients are receiving dialysis worldwide and this number is expected to more than double in 2030 [1]. Approximately
80% to 90% of end-stage kidney disease patients receive hemodialysis and the rest undergo peritoneal dialysis or transplantation [2,3]. Hemodialysis frequently leads to hemodynamic instability and autonomic imbalances, which predispose patients to intradialytic complications, such as hypotension, hypertension, and arrhythmia [4]. These hemodynamic events ultimately lead to cardiovascular death, which is the most common cause of death after starting hemodialysis, and accounts for more than 40% of deaths [5,6]. According to the United States Renal Data System database, among hemodialysis patients, arrhythmia is responsible for up to 60% and 20% of cardiovascular and all-cause deaths, respectively [7,8]. Accordingly, appropriate monitoring of hemodynamic complications during hemodialysis is essential to prevent adverse outcomes.

A biosignal is a physiological sign, such as blood pressure (BP), heart rate (HR), electrocardiogram (ECG) results, cardiac output, central venous pressure, heart rhythm, electroencephalogram, electrolytes, sympathetic nerve activity, and respiratory rhythms [9,10]. Monitoring biosignals increases the awareness of their clinical importance because they can serve as indicators for unpredictable events during routine or urgent practice. Hemodialysis per se changes the biosignals of patients with or sometimes without symptoms, and thus monitoring changes in biosignals may allow for tracing or predicting hemodynamic complications during hemodialysis [11–13]. Some studies have traced intradialytic biosignals such as BP and ECG, and the risk of hemodynamic complications and relevant outcomes could be identified in detail by monitoring these signals [8,14–16]. Nevertheless, intradialytic biosignals other than BP have been underutilized because systems that coordinate detection and storage have not been established in most centers.

To improve care quality and patient outcomes during hemodialysis, a system that integrates and utilizes biosignals should be incorporated into clinical practice. Regarding ECG, devices such as implantable loop recorders [8,14,15], Holter ECGs [16], and adhesive single-lead patches [17] could be applied during hemodialysis, but the clinical accessibility and applicability have not been validated. Herein, we developed system that integrated conventional monitoring of BP, HR, ECG, and photoplethysmogram with peripheral oxygen saturation (SpO₂), which was used to provide information to the cloud-based CONTINUAL (Continuous mOnitorNg viTal sIgN dUring hemodiALysis) registry. This registry can be utilized in future studies to apply intradialytic biosignals in epidemiological surveys on hemodynamic complications and to develop artificial intelligence models with biosignals to predict relevant cardiovascular risks.

Methods

Ethical considerations

This study protocol was approved by the Institutional Review Board (IRB) of the Seoul National University Hospital in Seoul, Republic of Korea (No. 2005-018-1121) and was conducted in accordance with the principles of the Declaration of Helsinki. The registry did not include personal information such as name and unique identification information. The requirement to obtain informed consent from the patients was waived by the IRB.

Aim and study design

The system was established in the hemodialysis facility of the Seoul National University Hospital, which has maintained the biosignal registry since September 2020. Two categories of datasets were collected and could be merged, including hemodialysis-setting data from electronic medical records and real-time biosignal data from bedside monitoring. The former data included hemodialysis dates, times to start and end, the target value of the blood filtration rate and ultrafiltration, and the components and temperature of the dialysates and anticoagulants. The information was stored in Microsoft Excel format (Fig. 1A).

Study population

The registry consisted of adult patients (aged ≥18 years) who received vital sign monitoring with the developed system regardless of the reason for hemodialysis.

Data collection

Bedside monitors (Solar 8000i; GE Healthcare, Waukesha, WI, USA) produced the biosignals, including BP, HR, ECG, and SpO₂ by photoplethysmogram. For three-lead values
for all patients, two electrodes were placed below the right and left clavicles, and the other electrode was placed on the left lower chest. The bandpass filter for ECG ranged from 0.05 to 40 Hz. Values of changes in the ST segment, either elevation or depression, were also measured. Waveform biosignals such as ECG and photoplethysmogram were sampled at 500 Hz and updated every 2 seconds. Fig. 1A shows the overall system integration and delivery of intradialytic biosignals to the cloud, wherein the Vital Recorder program was applied [18]. Fig. 1B shows a repre-

**Figure 1.** System and registry. (A) Schematic representation of the system platform from monitoring to storage. (B) Representative image of the bedside equipment.

AC, anticoagulants; BFR, blood flow rate; CONTINUAL, Continuous mOnitoring hemodiALysis; ECG, electrocardiogram; HD, hemodialysis; HIS, hospital information system; HR, heart rate; SpO\textsubscript{2}, peripheral oxygen saturation; Temp, temperature; UF, ultrafiltration.
sentative image of the bedside system equipment which provided time-synchronized data to facilitate integrated biosignal analysis. Data recording was initiated once the connection between the monitors and the Vital Recorder was established. The connection started to work when the HR and SpO\textsubscript{2} input values were recorded more than 5 times within 1 minute. The data were continuously backed up to the intranet-attached storage. The recordings and transfers automatically ended after 10 minutes if the biosignal inputs stopped. Subsequently, clinicians could monitor the real-time biosignals via the screen of any accessible computer. After acquiring the hemodialysis and biosignal data, these were merged based on unique identifiers such as date, time, and the bed number for the hemodialysis session.

Safety issue

The research team regularly inspects the system and the registry every month.

Statistical analysis

All statistical analyses were performed using IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Data are presented as percentages for categorical parameters. Means ± standard deviations or medians (interquartile ranges) were used for continuous parameters according to the normal distribution.

Results

Baseline characteristics during the initial 8-month period

Data were collected for cases during an initial 8-month period and data for approximately 300 sessions per month continue to be collected. A total of 2,243 hemodialysis sessions were collected from 612 patients between September 2020 and April 2021. The mean age was 64 ± 15 years old, and 1,279 (57.0%) were male. Comorbidities of permanent and paroxysmal atrial fibrillation were noted in 11.4% and 5.8% of cases, respectively. The hemodialysis time per session was 3.7 ± 0.9 hours. The initial blood flow rate and target ultrafiltration were 220 ± 38 mL/min and 1.7 ± 1.0 L, respectively. More than 60% of patients used nafamostat mesylate as an anticoagulant. Additional information is presented in Table 1.

### Hemodynamic complications

Four representative cases with hemodynamic complications are presented to support the need for continuous monitoring of biosignals, and the present system and relevant registry are proposed as an approach to address this unmet need.

The first case was an 80-year-old male patient who was admitted due to aspiration pneumonia. He had been on hemodialysis for 6 years due to diabetic nephropathy. His comorbidities included hypertension, immune thrombocytopenia.

#### Table 1. Baseline characteristics of the hemodialysis sessions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>2,243</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64.1 ± 14.6</td>
</tr>
<tr>
<td>Male sex</td>
<td>1,279 (57.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m\textsuperscript{2})</td>
<td>23.7 ± 6.2</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2,166 (96.6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1,770 (78.9)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>361 (16.1)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>599 (26.7)</td>
</tr>
<tr>
<td>Stroke</td>
<td>354 (15.8)</td>
</tr>
<tr>
<td>Permanent atrial fibrillation</td>
<td>256 (11.4)</td>
</tr>
<tr>
<td>Paroxysmal atrial fibrillation</td>
<td>130 (5.8)</td>
</tr>
<tr>
<td>Hemodialysis time (hr)</td>
<td>4.0 (3.5–4.0)</td>
</tr>
<tr>
<td>Blood flow rate (mL/min)</td>
<td>219.9 ± 37.6</td>
</tr>
<tr>
<td>Ultrafiltration (L)</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>Dialysate findings</td>
<td></td>
</tr>
<tr>
<td>Dialysate sodium (mmol/L)</td>
<td>137.8 ± 1.6</td>
</tr>
<tr>
<td>Dialysate potassium (mmol/L)</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Dialysate bicarbonate (mmol/L)</td>
<td>33.7 ± 1.4</td>
</tr>
<tr>
<td>Dialysate temperature (°C)</td>
<td>36.5 ± 0.6</td>
</tr>
<tr>
<td>Use of anticoagulant</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>713 (31.8)</td>
</tr>
<tr>
<td>Nafamostat mesilate</td>
<td>1,357 (60.5)</td>
</tr>
<tr>
<td>None</td>
<td>173 (7.7)</td>
</tr>
<tr>
<td>Access</td>
<td></td>
</tr>
<tr>
<td>Arteriovenous fistula</td>
<td>1,072 (47.8)</td>
</tr>
<tr>
<td>Arteriovenous graft</td>
<td>128 (5.7)</td>
</tr>
<tr>
<td>Catheter</td>
<td>1,043 (46.5)</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%).
topenia, and two-vessel coronary artery disease. The initial systolic and diastolic BPs were 175 mmHg and 72 mmHg, respectively, with an HR of 81 per minute. He had a normal sinus ECG rhythm. After starting hemodialysis, his BP gradually decreased to 130/56 mmHg at 34 minutes and 98/54 mmHg at 97 minutes. The patient reported no symptoms, and BP then increased up to 176/72 mmHg at 130 minutes without prompting any medical action (Fig. 2). The National Kidney Foundation’s Kidney Disease Outcomes Quality Initiative guidelines define intradialytic hypotension as a decrease in either systolic BP of ≥20 mmHg or mean arterial pressure of ≥10 mmHg [19]. This case is a clear example of intradialytic hypotension, but physicians were not notified because his BP recovered. Nevertheless, recurrent hypotensive events, even without symptoms and with recovery, could be associated with a high risk of cardiovascular death and thus should be continuously monitored.

The second case was a 52-year-old male patient who received a kidney transplant because of progressive immunoglobulin A nephropathy and underwent radical nephrectomy of the graft 10 years later because of kidney cancer. Accordingly, the patient underwent hemodialysis thrice weekly for 7 years. His comorbidities included hypertension, hypothyroidism, and left ventricular hypertrophy. On initiation of hemodialysis, sinus tachycardia was noted, and the systolic and diastolic BPs were 156 mmHg and 120 mmHg, respectively. Forty-six minutes after starting hemodialysis, the ratio of R to S on ECG was ≥1 [20], followed by depression of the ST segment and high amplitude of the R wave (Fig. 3). His BP increased over time, but he did not have any symptoms, such as chest pain or dyspnea. This case indicates that the system could be used to identify subclinical cardiac ischemia.

The third case was a 66-year-old female patient who had been on hemodialysis for 9 years because of drug-induced nephrotoxicity. Her comorbidities included hypertension and paroxysmal atrial fibrillation. She was admitted to the ward because of fungal pneumonia. Before hemodialysis, she had a normal sinus ECG rhythm, and HR was 98 beats per minute. The initial systolic and diastolic BP values were 140 mmHg and 82 mmHg, respectively. Paroxysmal atrial fibrillation occurred at 60 minutes, followed by a drop in systolic and diastolic BPs to 90 mmHg and 63 mmHg, respectively (Fig. 4). This case indicates that a preceding arrhythmia during hemodialysis can affect the risk of hypotension.

The fourth case was a 68-year-old female patient. She was on hemodialysis for 7 years due to diabetic nephropathy. Her comorbidities included hypertension, paroxysmal atrial fibrillation, and three-vessel coronary artery disease. She

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**Figure 2.** Case with subclinical intradialytic hypotension (IDH). (A) Biosignal changes with time of dialysis as monitored through the system. Sweep rate = 25 mm/sec. Voltage (vertical axis) against time (horizontal axis) = –1.5 to 2.5 mV. (B) Blood pressure (BP) and heart rate (HR) during hemodialysis. DBP, diastolic BP; SBP, systolic BP.
had received a percutaneous coronary artery intervention procedure on the proximal, middle left anterior descending, and right coronary arteries 3 years before. She was admitted due to left foot necrosis and underwent an amputation below the knee. At 60 minutes after starting hemodialysis, she had nonsustained ventricular tachycardia (Fig. 5) for her ECG rhythm, and this arrhythmia was repeated in subsequent hemodialysis sessions. Currently, limited data for the prognostic significance of incidentally detected arrhythmias in hemodialysis patients are limited. Therefore, monitoring intradialytic arrhythmia may be helpful for identifying patients at risk of sudden complications.

**Figure 3. Case with intradialytic hypertension.** (A) Biosignal changes with time of dialysis as monitored through the system. Sweep rate = 25 mm/sec. Voltage (vertical axis) against time (horizontal axis) = –2.5 to 2.5 mV. (B) Blood pressure (BP) and heart rate (HR) during hemodialysis. An R/S ratio equal to or greater than 1 suggests the presence of potential pathology in heart. DBP, diastolic BP; SBP, systolic BP.

**Figure 4. Case with paroxysmal atrial fibrillation (Af), followed by intradialytic hypotension (IDH).** (A) Biosignal changes with time of dialysis as monitored through the system. Sweep rate = 25 mm/sec. Voltage (vertical axis) against time (horizontal axis) = –0.5 to 1.5 mV. (B) Blood pressure (BP) and heart rate (HR) during hemodialysis. DBP, diastolic BP; SBP, systolic BP.
Discussion

Biosignal monitoring may be essential for detecting and preventing intradialytic complications. We developed an intradialytic biosignal-integrating system, which continuously updated the CONTINUAL registry. This approach can overcome some inherent limitations of current technology, such as providing real-time monitoring and storage of data. In this report, we also provided representative cases with hemodynamic complications, all of which required prompt prediction, prevention, and treatment. This CONTINUAL registry will be used in the future for predicting patient risks and preventive hemodialysis services based on both handcrafted and artificial intelligence models.

An approach that supports continuous and real-time monitoring of biosignals during hemodialysis is needed, and it will be more feasible if the system is noninvasive. This will allow clinicians to dynamically track changes in the patient statuses during hemodialysis more closely than with sporadic measurements conducted at most centers [21]. As shown in previous cases, a threshold number of sessions could provide sufficient data to predict hemodynamic complications during hemodialysis, some of which can have asymptomatic features. Hemodialysis can induce significant alterations in the hemodynamics of the circulatory system, which imposes a cardiac burden [22]. The burden will manifest as arrhythmias, silent or evident myocardial ischemia, and reversible or irreversible cardiac dysfunction [23]. Currently, hemodialysis machines do not collect biosignal datasets, and thus, some biosignals are missed. This missing data may reflect the cardiovascular risk of hemodialysis patients.

The practical goal of using this system is to utilize the biosignal registry for developing predictive models and to enhance decisions for complication risks. The large quantity of biosignals necessitates advanced or novel analytics that range from collection to interpretation [24]. Machine learning, including deep learning, is a rapidly developing branch of artificial intelligence that has shown promise for use in clinics [13,25]. A major limitation in utilizing biosignals for artificial intelligence-based clinical purposes is the lack of data storage [26]. This system supports intranet-attached storage to facilitate future utilization. The availability of a large, readily accessible registry with biosignals can shorten the time of model training [26]. We are currently conducting several projects with the help of machine learning using this CONTINUAL system.

Some limitations should be considered before fully utilizing this system and registry. The connection with the bedside monitor could be momentarily lost because of vio-
lent movement or arbitrary removal of the connector. This may result in loss of the significant biosignals and relevant intradialytic events and thus provide insufficient information to medical doctors or in developing models. The registry currently consists of data from hemodialysis patients hospitalized in a tertiary hospital, whose characteristics and risks of hemodynamic complications could differ from those admitted to general hospitals.

In summary, we developed an integrated system of intradialytic biosignals that included BP, HR ECG, and photoplethysmogram with SpO₂. This system-derived biosignal registry will facilitate epidemiological surveys on hemodynamic complications, enhance artificial intelligence models for predicting risks, and thus improve patient outcomes.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Authors’ contributions**

Conceptualization: SSH
Data curation: S Kim, DY, HCL, CWJ
Formal analysis: S Kim, DY, S Kwon, SRL, KK
Investigation: S Kim, DY, S Kwon, SRL, KK, YCK, DKK, KHO, KWJ, YSK
Writing—original draft: S Kim, SSH
Writing—review & editing: All authors
All authors read and approved the final manuscript.

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**References**

significant arrhythmias are common in hemodialysis patients and related to dialytic cycle. *Kidney Int* 2018;93:941–951.


Elderly kidney transplant recipients have favorable outcomes but increased infection-related mortality

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**Background:** The number of elderly patients with end-stage kidney disease has been increasing, but the outcomes of kidney transplants (KT) remain poorly understood in elderly patients. Therefore, we evaluated the clinical outcomes of elderly KT recipients and analyzed the impact of elderly donors.

**Methods:** This retrospective cohort study included patients who underwent KT between 2000 and 2019. KT recipients were divided into four groups according to a combination of recipient and donor age (≥60 or <60 years); elderly recipients: old-to-old (n = 46) and young-to-old (n = 83); young recipients: old-to-young (n = 98) and young-to-young (n = 796). We compared the risks of mortality, graft failure, and acute rejection between groups using Cox regression analysis.

**Results:** The incidence of delayed graft function, graft failure, and acute rejection was not different among groups. Annual mean tacrolimus trough level was not lower in elderly recipients than young recipients during 10-year follow-up. Mortality was significantly higher in elderly recipients (p = 0.001), particularly infection-related mortality (p < 0.001). In multivariable Cox regression analysis, old-to-old and young-to-old groups had increased risk of mortality (adjusted hazard ratio [aHR], 2.89; 95% confidence interval [CI], 1.14–7.32; p = 0.03; aHR, 3.06; 95% CI, 1.51–6.20; p = 0.002). However, graft failure and acute rejection risks were not increased in elderly recipients.

**Conclusion:** In elderly recipients, graft survival and acute rejection-free survival were not inferior to those of young recipients. However, mortality, especially risk of infection-related death, was increased in elderly recipients. Thus, low immunosuppression intensity might help decrease mortality in elderly recipients.

**Keywords:** Aged, Graft rejection, Graft survival, Kidney transplantation, Mortality, Transplant donors, Transplant recipients

Received: September 14, 2021; Revised: October 29, 2021; Accepted: November 16, 2021

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Introduction

The prevalence and related burden of end-stage kidney disease (ESKD) is rapidly increasing globally [1]. South Korea is a developed country with a rapidly aging population [2,3]. Moreover, the number of patients with ESKD is increasing rapidly due to the increased burden of metabolic diseases, such as diabetes and hypertension, and economic development that improves the accessibility of renal replacement therapy (RRT) [1,4]. According to the United States Renal Data System report, South Korea has the fourth-highest incidence and the third-highest prevalence of ESKD in the world [5]. Furthermore, among ESKD patients in South Korea, the proportion of elderly patients has steadily increased from 36.0% in 2010 to 51.9% in 2019 [6].

Kidney transplant (KT) is the treatment of choice with the best prognosis for patients with ESKD [7,8]. Previous studies have demonstrated that the prognosis of KT is better than that of dialysis in elderly patients with ESKD [9–11]. Moreover, KT from elderly living donors to elderly recipients showed better graft survival than standard criteria deceased donor or expanded criteria deceased donor KT [12]. However, few studies have analyzed prognosis considering the ages of donors and recipients together, particularly Asians. Thus, this study investigated patient and graft outcomes of KT in elderly patients based on the combination of ages of the recipient and donor.

Methods

Patients

Patients aged >18 years who underwent KT at Kyungpook National University Hospital in Daegu, Korea between January 2000 and December 2019 were retrospectively analyzed. Retransplantation patients were also included. Old patients were defined as those 60 years or older, and we divided KT recipients into four groups according to combination of ages of recipient and donor, as follows (Fig. 1):

1. Elderly recipients: old-to-old, defined as both donor and recipient aged ≥60 years; young-to-old, defined as donor aged <60 years and recipient aged ≥60 years.
2. Young recipients: old-to-young, defined as donors aged ≥60 years and recipients aged <60 years; young-to-young, defined as both donor and recipient aged <60 years.

Data collection

Patient data were retrospectively collected from electronic medical records. The demographics of the recipients include age, sex, body mass index (BMI), primary renal diseases, comorbid diseases, pretransplant dialysis vintage,

![Figure 1. Flow diagram of the study.](www.krcp-ksn.org)
pretransplant desensitization, number of human leukocyte antigen (HLA) mismatches, and type of immunosuppressants. Serial follow-up serum creatinine and tacrolimus trough levels were also collected, as well as demographic characteristics of donors, including age, sex, and BMI. The estimated glomerular filtration rate (eGFR) was calculated using the MDRD equation (derived from the Modification of Diet in Renal Disease study) [13]. Delayed graft function was diagnosed in KT recipients who underwent dialysis during the first week after transplant because of poor graft function [8]. Finally, annual mean tacrolimus trough level was calculated as the average value of all measured tacrolimus trough levels yearly after transplant.

**Immunosuppressive treatment**

Induction and maintenance immunosuppressive agents were used according to our center’s protocol. The detailed protocol is described in our previous studies [8,14]. Briefly, intravenous basiliximab was used as induction immunosuppression in normal-risk patients, and anti-thymocyte globulin was used for high-risk patients. For maintenance immunosuppression, standard triple immunosuppressive therapy (calcineurin inhibitor, mycophenolate, and corticosteroid) was applied. The target tacrolimus trough level was 5–10 ng/mL until 3 months after transplant, then it was tapered to 3–7 ng/mL. The target cyclosporine trough level was 200–300 ng/mL during the 3 months after transplant, after which it was tapered to 50–150 ng/mL for the maintenance period. Mycophenolate mofetil was administered daily at a fixed dose of 1.0 to 2.0 g. For corticosteroid treatment, 500 mg intravenous methylprednisolone was administered at the time of transplant and was tapered to 5 mg per day oral prednisolone within six months after transplant.

**Outcomes**

The study outcomes were patient survival, death-censored graft survival, and biopsy-proven acute rejection (BPAR)-free survival. Patient survival was defined as the time from KT to death from all causes. Death-censored graft survival was defined as the time from transplant to the restart of RRT. In cases of patient death with a functioning graft, graft survival was censored at the time of death. BPAR was diagnosed based on the Banff 07 classification [15] and was subdivided according to the time of diagnosis after transplant (early, 1 year or less after transplant; late, more than 1 year after transplant) and type of rejection.

**Statistical analysis**

Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as number (percentage). Analysis of variance or Student t test was used to determine differences between continuous variables. Pearson chi-square test or Fisher exact test was used to determine differences among categorical variables. Patient survival, death-censored graft survival, and BPAR-free survival were analyzed using Kaplan-Meier analysis with log-rank tests. Multivariable Cox proportional hazard regression analyses were conducted for survival analyses. The results of Cox regression analysis were presented as hazard ratio (HR) with 95% confidence interval (CI). To avoid confounding effects, variables that were significantly different at baseline (p < 0.05; comorbid diabetes, BMI of recipient and donor, tacrolimus use, and number of HLA mismatches) or were associated with clinical outcomes of transplant (sex, transplant type, immunosuppressants given) were entered in the multivariable Cox regression analysis. Here, Model 1 was adjusted for sex and diabetes; Model 2 for Model 1 variables plus recipient BMI, donor BMI, and transplant type; Model 3 was adjusted for Model 2 variables as well as tacrolimus use, number of HLA mismatches, anti-thymocyte globulin use, and maintenance mycophenolate use. Statistical analyses were conducted using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and R (version 3.6.2; The R Foundation for Statistical Computing, Vienna, Austria). The p-values of <0.05 were considered statistically significant.

**Ethics statement**

The Institutional Review Board of Kyungpook National University Hospital reviewed and approved the study protocol (No. 2021-07-069). The requirement for informed consent was waived because the study was conducted as a retrospective review of electronic medical records. All patient information was anonymized and deidentified before analyses. This study was conducted according to the 2000 Dec-

**Results**

**Baseline characteristics**

Of the total 1,023 KT recipients examined during the study period, 129 (12.6%) were ≥60-years-old at the time of transplant. Among these elderly recipients, 46 (4.5% of the total) received KT from old donors (age, ≥60 years), which is defined as the old-to-old group; 83 elderly recipients (8.1% of the total) were included in the young-to-old group. There were 893 KT recipients (87.4%) aged <60 years at the time of transplant; of them, 98 (9.6% of the total) received KT from old donors (age, ≥60 years) and were defined as the old-to-young group. There were 796 cases (77.8% of the total) in which both donor and recipient were aged <60 years, defined as the young-to-young group and set as the reference. The median follow-up duration for all patients was 69.2 months (interquartile range [IQR], 39.8–115.9 months); that for old-to-old was 52.0 months (IQR, 38.0–77.0 months), that for young-to-old was 57.7 months (IQR, 32.9–86.2 months), that for old-to-young was 55.8 months (IQR, 29.8–81.9 months), and that of the young-to-young groups was 73.4 months (IQR, 44.8–131.6 months). Baseline characteristics of each patient group are shown in Table 1. The mean age was 64.8 ± 3.5 years in the old-to-old group, 63.8 ± 3.2 years in the young-to-old group, 45.1 ± 3.2 years in the old-to-young group, and 43.6 ± 10.2 years in the young-to-young group. The proportion of males was higher among elderly recipients (old-to-old and young-to-old) compared with young recipients (old-to-young and young-to-young) (p = 0.007). More than half of the elderly recipients had ESKD due to diabetes, while more than half of the young recipients had ESKD due to glomerulonephritis (p < 0.001). Comorbid diabetes was more frequent in the elderly recipients compared with the young recipients (p < 0.001). Pretransplant dialysis vintage and pretransplant desensitization rates did not differ among groups. The mean ages of donors were 66.7 ± 4.9 years in old-to-old, 43.7 ± 12.0 years in young-to-old, 46.2 ± 3.7 years in old-to-young, and 41.9 ± 12.3 years in young-to-young (p < 0.001). Pretransplant donor serum creatinine levels were not different among groups. The proportion of living or deceased donors and type of induction immunosuppression did not differ among groups.

**Tacrolimus immunosuppression after transplant**

The majority of KT recipients (83.9%, 858 of 1,023) were on immunosuppressive therapy, including tacrolimus. Therefore, we compared mean tacrolimus trough levels to identify degree of immunosuppression after transplant (Fig. 2). The mean tacrolimus trough level was significantly lower for the old-to-old group than the young-to-young group at one year after transplant. However, there were no differences in annual mean tacrolimus trough level among groups at 2, 3, 4, 5, and 10 years after transplant.

**Outcomes and graft function**

Patient and graft outcomes are summarized in Table 2. The incidence of delayed graft function, graft failure, and BPAR including early and late events was not different among groups. The proportion of KT recipients whose serum creatinine level was higher than 1.5 mg/dL at 1 year after transplant was higher in the old-to-old and old-to-young groups. The proportions of patients who were hospitalized to treat infection did not differ among groups. The incidence of patient death was 13.0% (6 of 46) in the old-to-old group and 16.9% (14 of 83) in the young-to-old group. However, young recipients showed lower mortality (5.1% [5 of 98; old-to-young] and 6.0% [48 of 796; young-to-young]) (p = 0.001). Infection-related mortality was higher among elderly recipients (p < 0.001). The incidence of cardiovascular and cancer-related deaths was not different among the four groups. Supplementary Table 1 (available online) shows detailed information about infection-related death, the most common cause of which is pneumonia.

In subgroup analysis according to donor type, elderly recipients showed higher all-cause mortality and infection-related mortality than young recipients in both living donor KT and deceased donor KT groups (all ps < 0.05; Supplementary Table 2, available online).

We increased the cutoff age of the elderly group to 65 years and compared outcomes among the four groups (Supplementary Table 3, available online). The number of patients in the old-to-old group was 16, in the young-to-old group was 33, and in the old-to-young group was 48. The results were similar to those of the analysis with an
Table 1. Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Old-to-old</th>
<th>Young-to-old</th>
<th>Old-to-young</th>
<th>Young-to-young</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>46</td>
<td>83</td>
<td>98</td>
<td>796</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>64.8 ± 3.5</td>
<td>63.8 ± 3.2</td>
<td>45.1 ± 3.2</td>
<td>43.6 ± 10.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>34 (73.9)</td>
<td>63 (75.9)</td>
<td>54 (55.1)</td>
<td>483 (60.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 2.5</td>
<td>23.3 ± 3.0</td>
<td>23.0 ± 4.6</td>
<td>22.2 ± 3.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Primary renal disease</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>25 (54.3)</td>
<td>46 (55.4)</td>
<td>28 (28.6)</td>
<td>173 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (6.5)</td>
<td>9 (10.8)</td>
<td>3 (3.1)</td>
<td>54 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>14 (30.4)</td>
<td>22 (26.5)</td>
<td>57 (58.2)</td>
<td>492 (61.8)</td>
<td></td>
</tr>
<tr>
<td>Comorbid disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>42 (91.3)</td>
<td>73 (88.0)</td>
<td>76 (77.6)</td>
<td>640 (80.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Diabetes</td>
<td>31 (67.4)</td>
<td>49 (59.0)</td>
<td>31 (31.6)</td>
<td>195 (24.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CVA</td>
<td>1 (2.2)</td>
<td>3 (3.6)</td>
<td>4 (4.1)</td>
<td>16 (2.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1 (2.2)</td>
<td>3 (3.6)</td>
<td>1 (1.0)</td>
<td>15 (1.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Pretransplant dialysis vintage (mo)</td>
<td>37.0 ± 28.7</td>
<td>55.0 ± 58.3</td>
<td>45.7 ± 61.5</td>
<td>49.6 ± 55.3</td>
<td>0.40</td>
</tr>
<tr>
<td>DXM positive</td>
<td>3 (6.7)</td>
<td>4 (4.8)</td>
<td>7 (7.1)</td>
<td>44 (5.5)</td>
<td>0.90</td>
</tr>
<tr>
<td>Desensitization</td>
<td>10 (21.7)</td>
<td>9 (10.8)</td>
<td>10 (10.2)</td>
<td>106 (13.3)</td>
<td>0.25</td>
</tr>
<tr>
<td>Rituximab</td>
<td>8 (17.4)</td>
<td>8 (17.4)</td>
<td>10 (10.2)</td>
<td>106 (13.3)</td>
<td>0.50</td>
</tr>
<tr>
<td>No. of HLA mismatches</td>
<td>4.0 ± 1.4</td>
<td>2.8 ± 1.7</td>
<td>2.8 ± 1.7</td>
<td>3.1 ± 1.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Retransplantation</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>66.7 ± 4.9</td>
<td>43.7 ± 12.0</td>
<td>64.2 ± 3.7</td>
<td>41.9 ± 12.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Donor sex, male</td>
<td>22 (47.8)</td>
<td>41 (49.4)</td>
<td>55 (56.1)</td>
<td>409 (51.4)</td>
<td>0.74</td>
</tr>
<tr>
<td>Donor BMI (kg/m²)</td>
<td>23.5 ± 0.5</td>
<td>22.8 ± 2.7</td>
<td>23.9 ± 3.1</td>
<td>23.4 ± 3.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Donor serum creatinine (mg/dL)</td>
<td>0.96 ± 0.28</td>
<td>0.86 ± 0.28</td>
<td>0.97 ± 0.26</td>
<td>0.95 ± 0.21</td>
<td>0.08</td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>Living</td>
<td>26 (56.5)</td>
<td>47 (56.6)</td>
<td>60 (61.2)</td>
<td>500 (62.8)</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>20 (43.5)</td>
<td>36 (43.4)</td>
<td>38 (38.8)</td>
<td>296 (37.2)</td>
<td></td>
</tr>
<tr>
<td>Induction immunosuppression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>42 (91.3)</td>
<td>69 (83.1)</td>
<td>86 (87.8)</td>
<td>651 (81.8)</td>
<td></td>
</tr>
<tr>
<td>Anti-thymocyte globulin</td>
<td>4 (8.7)</td>
<td>14 (16.9)</td>
<td>12 (12.2)</td>
<td>145 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>2 (4.3)</td>
<td>5 (6.0)</td>
<td>3 (3.1)</td>
<td>116 (14.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>43 (93.5)</td>
<td>74 (89.2)</td>
<td>90 (91.8)</td>
<td>651 (81.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>Steroid</td>
<td>45 (97.8)</td>
<td>78 (94.0)</td>
<td>95 (96.9)</td>
<td>770 (96.7)</td>
<td>0.57</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>45 (97.8)</td>
<td>79 (95.2)</td>
<td>93 (94.9)</td>
<td>734 (92.2)</td>
<td>0.42</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>0 (0)</td>
<td>2 (2.4)</td>
<td>1 (1.0)</td>
<td>12 (1.5)</td>
<td>0.72</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1 (2.2)</td>
<td>1 (1.2)</td>
<td>0 (0)</td>
<td>23 (2.9)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%). BMI, body mass index; CVA, cerebrovascular accident; DXM, direct cross-match; HLA, human leukocyte antigen.

elderly cutoff age of 60 years. Both all-cause mortality and infection-related mortality were higher in the elderly recipient groups. When patients were divided into two groups based on the recipient or donor age of 70 years (age of ≥70 and <70 years), neither extremely elderly recipient nor donor KT groups showed higher incidence of graft failure and death of patients than the young recipient or donor KT groups as follows. 1) Extremely elderly recipients (n = 7) vs. young recipients (n = 1,016): graft failure, 0 vs. 111 (10.9%) (p > 0.99); death, 1 (14.3%) vs. 72 (7.1%) (p = 0.41). 2) Extremely elderly donors (n = 21) vs. young donors (n = 1,002): graft failure, 1 (4.8%) vs. 110 (11.0%) (p = 0.37); death, 1 (4.8%) vs. 72 (7.2%) (p = 0.67).

Serial changes in eGFR after transplant are shown in Fig. 3.
Table 2. Comparison of graft and patient outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Old-to-old (n = 46)</th>
<th>Young-to-old (n = 83)</th>
<th>Old-to-young (n = 98)</th>
<th>Young-to-young (n = 796)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed graft function</td>
<td>1 (2.2)</td>
<td>3 (3.6)</td>
<td>5 (5.1)</td>
<td>31 (3.9)</td>
<td>0.85</td>
</tr>
<tr>
<td>SCr &gt; 1.5 mg/dL at 1 yr</td>
<td>13 (28.3)</td>
<td>8 (9.6)</td>
<td>27 (27.6)</td>
<td>101 (12.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Graft failure</td>
<td>3 (6.5)</td>
<td>5 (6.0)</td>
<td>14 (14.3)</td>
<td>89 (11.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>BPAR†</td>
<td>2 (4.3)</td>
<td>4 (4.8)</td>
<td>9 (9.2)</td>
<td>46 (5.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>Early</td>
<td>1 (2.2)</td>
<td>3 (3.6)</td>
<td>6 (6.1)</td>
<td>20 (2.5)</td>
<td>0.20</td>
</tr>
<tr>
<td>Late</td>
<td>1 (2.2)</td>
<td>1 (1.2)</td>
<td>3 (3.1)</td>
<td>26 (3.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>Acute TCMR</td>
<td>2 (4.3)</td>
<td>2 (2.4)</td>
<td>4 (4.1)</td>
<td>33 (4.0)</td>
<td>0.91</td>
</tr>
<tr>
<td>Early</td>
<td>1 (2.2)</td>
<td>2 (2.4)</td>
<td>3 (3.1)</td>
<td>17 (2.1)</td>
<td>0.81</td>
</tr>
<tr>
<td>Late</td>
<td>1 (2.2)</td>
<td>0 (0)</td>
<td>1 (1.0)</td>
<td>15 (1.9)</td>
<td>0.66</td>
</tr>
<tr>
<td>Active ABMR</td>
<td>0 (0)</td>
<td>2 (2.4)</td>
<td>5 (5.1)</td>
<td>14 (1.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>Early</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
<td>3 (3.1)</td>
<td>4 (0.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Late</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
<td>2 (2.0)</td>
<td>10 (1.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Infection-related hospitalization</td>
<td>10 (21.7)</td>
<td>20 (24.1)</td>
<td>16 (16.3)</td>
<td>204 (25.6)</td>
<td>0.23</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>8 (17.4)</td>
<td>15 (18.1)</td>
<td>11 (11.2)</td>
<td>168 (21.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Viral infection</td>
<td>2 (4.3)</td>
<td>5 (6.0)</td>
<td>5 (5.1)</td>
<td>39 (4.9)</td>
<td>0.95</td>
</tr>
<tr>
<td>Death</td>
<td>6 (13.0)</td>
<td>14 (16.9)</td>
<td>5 (5.1)</td>
<td>48 (6.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Infection</td>
<td>3 (6.5)</td>
<td>8 (9.6)</td>
<td>1 (1.0)</td>
<td>6 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>0 (0)</td>
<td>1 (1.2)</td>
<td>0 (0)</td>
<td>3 (0.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>Cancer</td>
<td>0 (0)</td>
<td>3 (3.6)</td>
<td>0 (0)</td>
<td>9 (1.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Others</td>
<td>3 (6.5)</td>
<td>2 (2.4)</td>
<td>4 (4.1)</td>
<td>30 (3.8)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).

ABMR, antibody-mediated rejection; BPAR, biopsy-proven acute rejection; SCr, serum creatinine; TCMR, T-cell mediated rejection.

†Early indicates within 1 year of and late indicates at least 1 year after kidney transplantation.

The old-to-old group had significantly lower eGFR than groups with young donors (young-to-old and young-to-young) at 14 days and 1, 2, 3, and 5 years after transplant (p < 0.05).

Patient survival

In the Kaplan-Meier curve for patient survival, elderly recipients showed significantly shorter survival times com-
pared with young recipient groups (log-rank $p < 0.001$) (Fig. 4). In univariable Cox proportional hazard regression analysis, both elderly recipient groups showed a significantly higher risk of patient death than the young-to-young group (Table 3). In multivariable analysis to adjust for confounding effects of baseline characteristics, elderly recipients were consistently at significantly higher risk of patient death than was the young-to-young group (Model 3; old-to-old: aHR, 2.89; 95% CI, 1.14–7.32; $p = 0.03$; young-to-old: aHR, 3.06; 95% CI, 1.51–6.20; $p = 0.002$).

**Graft survival and biopsy-proven acute rejection-free survival**

Death-censored graft survival was significantly different among the four groups in the Kaplan-Meier survival curve (log-rank $p = 0.02$) (Fig. 5A). In univariable and multivariable Cox proportional hazard regression analyses, there was no significant difference in graft failure in elderly recipients compared with the young-to-young group ($p > 0.05$) (Table 4). However, the old-to-young group showed a consistently increased risk of death-censored graft failure compared with the young-to-young group (Model 3: aHR, 2.41; 95% CI, 1.30–4.46; $p = 0.005$).

BPAR-free survival did not differ among the four groups as per the Kaplan-Meier survival curve (log-rank $p = 0.389$) (Fig. 5B). Table 5 shows a risk of BPAR among groups in univariable and multivariable Cox proportional hazard regression analyses. There were no significant differences in BPAR risk in groups other than the young-to-young group ($p > 0.05$).

**Discussion**

We demonstrated that graft survival is not inferior in elderly recipients regardless of donor age compared to young recipients with young donors. However, patient survival was lower in elderly recipients from both young and elderly donors. In particular, as infection-related mortality was higher in elderly recipients, special attention is needed in this area. This is the first Asian cohort study to evaluate patient and graft prognosis considering the combined effects of elderly recipients and donors. Our findings will assist physicians of elderly patients with ESKD in selecting appropriate RRT modality and to help decrease mortality in elderly recipients.

The prognosis of KT in generally better in Asian samples than in those of patients of other ethnicities [8,16,17]. Although the rate of KT in patients with ESKD has been steadily increasing, it is still low in elderly patients with ESKD in Korea [3,6,18]. KT improves the quality of life in patients with ESKD and has a better prognosis than dialysis [19], but elderly ESKD patients are often reluctant to receive KT, especially from elderly donors. Moreover, due to a shortage of donated organs, the waiting time for deceased donor KT is longer, making it more difficult for elderly ESKD patients to receive KT, especially from young donors [20]. However, survival benefit has been demonstrated in elderly KT recipients compared to dialysis patients on the waiting list for transplantation in several studies [7,9,10,21].
**Figure 4. Kaplan-Meier curve for patient survival.** A p-value less than 0.008 indicates significant difference in post hoc Bonferroni correction.

**Table 3. Cox regression analysis of patient death**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>aHR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Young-to-young</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Old-to-young</td>
<td>1.30 (0.52–3.29)</td>
<td>0.58</td>
<td>1.38 (0.54–3.51)</td>
<td>0.498</td>
</tr>
<tr>
<td>Young-to-old</td>
<td>4.00 (2.19–7.29)</td>
<td>&lt;0.001</td>
<td>3.35 (1.76–6.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Old-to-old</td>
<td>4.28 (1.81–10.12)</td>
<td>&lt;0.001</td>
<td>3.56 (1.46–8.70)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.

<sup>a</sup>Adjusted for sex and diabetes.

<sup>b</sup>Adjusted for sex, diabetes, recipient body mass index, donor body mass index, and transplant type.

<sup>c</sup>Adjusted for sex, diabetes, recipient body mass index, donor body mass index, transplant type, tacrolimus use, number of human leukocyte antigen mismatches, anti-thymocyte globulin induction, and maintenance mycophenolate use.
Figure 5. Kaplan-Meier curves for graft outcomes. (A) Death-censored graft survival. (B) Biopsy-proven acute rejection (BPAR)-free survival. A p-value less than 0.008 indicates significant difference in post hoc Bonferroni correction.

Table 4. Cox regression analysis of death-censored graft failure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Model 1\textsuperscript{a}</th>
<th>Model 2\textsuperscript{b}</th>
<th>Model 3\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>aHR (95% CI) p-value</td>
<td>aHR (95% CI) p-value</td>
</tr>
<tr>
<td>Young-to-young</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Old-to-young</td>
<td>2.28 (1.29–4.04)</td>
<td>0.005</td>
<td>2.33 (1.28–4.23) 0.005</td>
<td>2.16 (1.18–3.98) 0.01</td>
</tr>
<tr>
<td>Young-to-old</td>
<td>0.87 (0.35–2.15)</td>
<td>0.77</td>
<td>0.81 (0.32–2.05) 0.66</td>
<td>0.90 (0.35–2.30) 0.82</td>
</tr>
<tr>
<td>Old-to-old</td>
<td>1.43 (0.45–4.54)</td>
<td>0.55</td>
<td>1.33 (0.41–4.31) 0.63</td>
<td>0.96 (0.23–4.00) 0.96</td>
</tr>
</tbody>
</table>

aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.
\textsuperscript{a}Adjusted for sex and diabetes. \textsuperscript{b}Adjusted for sex, diabetes, recipient body mass index, donor body mass index, and transplant type. \textsuperscript{c}Adjusted for sex, diabetes, recipient body mass index, donor body mass index, transplant type, tacrolimus use, number of human leukocyte antigen mismatches, anti-thymocyte globulin induction, and maintenance mycophenolate use.

Table 5. Cox regression analysis of biopsy-proven acute rejection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th>Model 1\textsuperscript{a}</th>
<th>Model 2\textsuperscript{b}</th>
<th>Model 3\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>aHR (95% CI) p-value</td>
<td>aHR (95% CI) p-value</td>
</tr>
<tr>
<td>Young-to-young</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Old-to-young</td>
<td>1.84 (0.90–3.76)</td>
<td>0.10</td>
<td>1.85 (0.90–3.80) 0.09</td>
<td>1.61 (0.77–3.35) 0.21</td>
</tr>
<tr>
<td>Young-to-old</td>
<td>0.94 (0.34–2.60)</td>
<td>0.90</td>
<td>0.73 (0.26–2.08) 0.56</td>
<td>0.69 (0.24–1.97) 0.49</td>
</tr>
<tr>
<td>Old-to-old</td>
<td>0.92 (0.22–3.80)</td>
<td>0.92</td>
<td>0.71 (0.17–3.01) 0.65</td>
<td>0.83 (0.20–3.51) 0.80</td>
</tr>
</tbody>
</table>

aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio.
\textsuperscript{a}Adjusted for sex and diabetes. \textsuperscript{b}Adjusted for sex, diabetes, recipient body mass index, donor body mass index, and transplant type. \textsuperscript{c}Adjusted for sex, diabetes, recipient body mass index, donor body mass index, transplant type, tacrolimus use, number of human leukocyte antigen mismatches, anti-thymocyte globulin induction, and maintenance mycophenolate use.
In addition, we showed that graft prognosis was not inferior in elderly recipients receiving kidneys from elderly donors compared to that of young recipients from young donors. Therefore, elderly patients with ESKD should be positively considered for KT from elderly donors.

In this study, recipient age had a significant impact on patient survival regardless of donor age. Even after adjusting for various confounding factors, elderly recipients consistently showed increased risk of death. Patients with ESKD on dialysis have the highest mortality from cardiovascular disease [6], but elderly recipients have increased mortality primarily due to infection. Although the overall incidence of infection was not higher than that in young recipients, many severe/critical infection cases lead to death of elderly patients. This suggests vulnerability to immunosuppressive therapy and the need for tailored immunosuppression in elderly recipients. The aging of the immune system, or immunosenescence, causes immune system alterations in that macrophages, neutrophil granulocytes, and dendritic cells have reduced capability to react to mediators and cytokines [22,23]. Therefore, theoretically, the intensity of immunosuppressive therapy in elderly recipients can be lowered during the maintenance period [22,24]. Our results showed that the mean tacrolimus trough level kept low during the first year after transplant for the old-to-old group, but the level was not low compared with the other groups thereafter. Therefore, it is recommended that clinicians maintain steady low immunosuppression after transplant, considering the risk of severe infection in elderly recipients.

The risk of graft failure was not increased in elderly recipients compared with young recipients receiving kidneys from young donors. This agrees with previous studies that examined the impact of donor age on graft survival in young recipients rather than elderly recipients [18,25,26]. Although elderly recipients have higher mortality than young recipients receiving kidneys from young donors, death-censored graft survival was not inferior in these elderly recipients regardless of donor age. Therefore, elderly recipients do not need to hesitate to receive a transplant from elderly donors because donor age has a minor effect on long-term function of the graft. In contrast, young recipients from elderly donors did not show increased mortality. However, the risk of death-censored graft failure was increased compared with that of young recipients receiving kidneys from young donors, so long-term graft survival will be the major issue when determining the prognosis of such young recipients.

Neither early nor late BPAR incidence was increased in elderly recipients compared with young recipients, and all groups showed a low incidence of BPAR in the range of 3% to 5%. BPAR episodes are a risk factor for patient death and graft failure in KT recipients [18]. BPAR has a greater association with mortality than does underlying diseases in elderly recipients [24,27,28]. Some previous studies have shown similar incidence of BPAR in elderly recipients to that of young recipients [29], whereas others showed a lower risk of BPAR [18,30,31]. We found that donor or recipient age had a neutral impact on BPAR, which might be due to the low overall incidence of BPAR in our study patients. Thus, it would be reasonable to maintain a low intensity of immunosuppression, especially for elderly recipients with high risk of infection-related death.

The strength of this study is that it demonstrates high infection-related mortality in elderly KT recipients, but not low immunosuppression intensity compared to young KT recipients. However, there are some limitations to this study. First, this is a retrospective observational study, so factors that affect patient or graft outcomes cannot be fully controlled, limiting the interpretation of the results. However, we analyzed the impact of donor or recipient age using multiple multivariable analysis models to minimize possible confounding variables. Second, the sample of elderly recipients was small, and results were based on a single transplant center, so the results of this study might not represent overall national results for Korea. However, this study observed prognosis over long periods for the entire cohort of a single transplant center and minimized variation based on differences between transplant centers or clinicians. Third, postoperative complications after transplant are higher in elderly recipients [32], but postoperative complications could not be examined based on the available data in this study. Therefore, prospective studies using a large-scale national cohort with various clinical information are warranted to overcome these limitations.

In conclusion, elderly recipients had increased risk of mortality compared with young recipients. Notably, infection-related mortality was higher in elderly recipients. However, graft survival and BPAR-free survival of elderly recipients were comparable with those of young recipients, and donor
age did not significantly impact long-term graft function in elderly recipients. Thus, low immunosuppression intensity might help decrease mortality in elderly KT recipients.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This study was supported by the Young Investigator Research Grant from the Korean Society of Nephrology (2021) and supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, South Korea (grant number: HI15C0001).

**Authors’ contributions**

Conceptualization: JHL, GYL, CDK

Formal analysis: JHL, GYL, YJ

Funding acquisition: JHL, JHC

Investigation: JHL, GYL, HYJ, JYC, JHC, YLK, HKK, SH, ESY, DIW, CDK

Supervision: CDK

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**References**


Coronavirus disease 2019 (COVID-19), the rapidly ongoing pandemic caused by spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), led to a significant impact on transplant systems [1]. Because of intensified induction therapy as well as maintenance of immune suppressant, there is significant concern about transplantation after COVID-19 infection. Meanwhile, the immune response to SARS-CoV-2 is crucial in detecting primary infection and in confirming postinfection recovery or reinfection [2]. In this case, we serially monitored humoral and cellular immunity to SARS-CoV-2 in patients who were diagnosed with COVID-19 during preparation for an ABO-incompatible kidney transplantation (ABO icKT) and who successfully received kidney transplantation (KT) without reinfection or complication before or after.

A 47-year-old male patient with chronic kidney disease (stage 5) secondary to diabetic nephropathy for which he was on hemodialysis was planned for a living donor ABO icKT. The potential donor was his spouse, and the isoagglutinin titer was 1:16. According to our center’s protocol, we infused rituximab 4 weeks before KT and planned three plasmapheresis procedures. However, 3 weeks prior to KT, the patient was diagnosed as COVID-19 positive by reverse transcriptase-polymerase chain reaction (SARS-CoV-2 test). Fortunately, the disease course was not severe, and he only required quarantine and symptomatic treatment.

Both humoral and cellular immunity against SARS-CoV-2 had been assessed approximately 2 months prior to KT.
Humoral immunity of COVID-19 was detected by measuring anti-SARS-CoV-2 antibodies (Elecsys Anti-SARS-CoV-2 chemiluminescent immunoassay, cutoff of 0.8; Roche Diagnostics, Rotkreuz, Switzerland). That antibody level in serum samples was 0.77 U/mL. The low pre-KT humoral immunity level could be due to the immunosuppressive effect of rituximab for ABO incompatibility. Cellular immunity against SARS-CoV-2 was detected by enzyme-linked immune-spot (ELISPOT, the T-SPOT Discovery SARS-Cov-2 assay kit; Oxford Immunotec, Abingdon-on-Thames, London, UK) and flow cytometry of T cell, regulatory T cell (Treg), and natural killer (NK) subset cytokine expression. Our case was characterized by predominant CD4+ and CD8+ T cell responses, which suggests that he had developed SARS-CoV-2–specific T cell responses [3] (Fig. 1). Expression of Treg and NK subset cytokine also was detected. In Fig. 2A, adaptive immunity to SARS-CoV-2 virus was confirmed by measurement of SARS-CoV-2–specific T cell immunity (spike protein, N protein, and M protein) by the ELISPOT method.

Therefore, we decided to proceed with the ABO icKT. The patient had received rituximab before the COVID-19 diagnosis, and CD19 and CD20 cell counts in peripheral blood were <0.1%. Hence, the patient underwent only plasmapheresis three times just before KT. Antibody measurement and ELISPOT assay were performed at post-KT intervals of 1 and 3 months. One month after KT, the antibody level was less than 0.4 U/mL, and ELISPOT assay and flow cytometry results also showed decreased cellular immune response (Fig. 2B). Three months after KT, CD8+ absolute cell count abruptly rebounded, but NK and Treg subset expression remained low. The patient remained stable with an estimated glomerular filtration rate of 104 mL/min/1.73 m² up to 10 months posttransplant.

There are approximately four previous case reports of successful transplantation in patients with a mild disease.

**Figure 1.** Expression of T cell, NK cell, and Treg subset cytokine as detected by flow cytometry from COVID-19 infection had recovered at 6 weeks after COVID-19 diagnosis. (A) T cells (CD8+ and CD4+) producing cytokines were detectable in flow cytometry. (B) NK (CD16− CD56+ and CD16+ CD56−) subsets producing cytokines were detectable in flow cytometry. (C) Treg (CD25+ CD127low) subsets producing cytokines were detectable by flow cytometry. Flow cytometry results confirm adaptive immunity toward COVID-19 (SARS-CoV-2) in the case patient.

COVID-19, coronavirus disease-19; NK, natural killer; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; Treg, regulatory T cell.
course of COVID-19 and two case reports of such patients with a severe disease course [4–8]. No previous case report or cohort study showed adaptive immunity in both precise humoral and cellular patterns. T cell-mediated immune response is required for protection against SARS-CoV-2 and is a surrogate of acquired immune protection from reinfection [2]. Though detected humoral immunity value was below the cutoff value, we verified adaptive immunity by detection of more precise cellular immunity with ELISPOT.

In conclusion, our data are reassuring that functional SARS-CoV-2–specific T cell responses are acquired as soon as 6 weeks after initial COVID-19 diagnosis and retained for 6 months following infection. This suggests that adaptive T cell immunity is sustained, and the ideal wait time for transplant could be as soon as only 6 weeks after initial diagnosis of COVID-19. Most KT recipients slowly recover their immune status as immunosuppressant dosages are tapered over time. Our patient recovered his immune status, and CD8+ expression level was normal at 3 months after KT. This case is promising for patients who have suffered from COVID-19 before KT and suggests that recovery from COVID-19 and the presence of T cell immunity against SARS-CoV-2 could be a surrogate marker for safe and successful KT.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP; NRF-2020R1A2B5B01001859) and was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (grant No. HI20C0317).
Authors’ contributions

Conceptualization: SCP, SYH
Data curation: HB, SCP, EJO, SYH
Formal analysis: HB, EJO, SYH
Methodology: HB
Funding acquisition: BHC
Supervision: CWY, EJO, BHC
Visualization: HB, SCP, CWY, EJO
Writing–original draft: SYH
Writing–review & editing: BHC

All authors read and approved the final manuscript.

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References

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**Important information**

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

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

1. A cover letter. It must include your name, address, telephone and fax numbers, e-mail address, and state that all authors have contributed to the paper and have never submitted the manuscript, in whole or in part, to other journals.
2. A conflict of interest disclosure statement (see relevant section 4.2 below).
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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9. Articles should be written in English (using American English spelling) and meet the following basic criteria: the material is original; the information is important; the writing is clear, concise and grammatically correct; the study methods are appropriate; the data are valid; and the conclusions are reasonable and supported by the data. The articles should be readable to native English users, and we recommend using professional language editing service (e.g., American Journal Experts) prior to submission to avoid delays with the review processes.
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2. Types of Articles

2.1. Original Articles

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

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

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

2.4. Correspondence
Correspondence generally takes one of the following forms: (1) Reader’s comment on an article previously published in KRCP and/or a reply from the authors; (2) An article that may not fit to the format of original or review article but suggest creative perspectives for medical issues; (3) A brief report of any kind that presents important research findings adequate for the journal’s scope and of particular interest to the readers. The submitted manuscript includes title page, main text, conflict of interest, acknowledgments (if applicable) and references. No abstract is included, and the text should be limited to 800 words (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://locatorplus.gov/cgi-bin/Pwebrecon.cgi?DB=local&v1=1&ri=1,1&Search_Arg=1013184411&Search_Code=0359&CNT=1&SID=1). The authors may format the citations and references using the KRCP EndNote style file, but we generally recommend the authors to type the citation numbers and references manually.

Journal articles:

Online publication but not yet in print:

Entire Book:

Book chapter:

Website:

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 (\(^a\), \(^b\), ...) should be used for special remarks.

3.7. Figures

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

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

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

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

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

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include a title, methods, outcome and a concluding sentence. Please fill in the template as it’s laid out and do not alter the basic components of the template.

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OPTIMIZE TROUGH LEVEL
START LIFE-LONG JOURNEY
INDICATIONS
1. Renal anaemia
2. Chemotherapy-induced anaemia in solid cancer patients

DOSEAGE AND ADMINISTRATION
<Adolescents> (patients ≥16 years)

- Initial dose
  The usual dose of NES® in adult patients is 20 µg, to be administered as a single intravenous injection once weekly.

- Initial dose at the switching from erythropoiesis preparation: See Precautions related to Doseage and Administration

- Maintenance dose
  When correction of anaemia is achieved, the usual dose of NES® in adult patients is 30-100 µg as darbepoetin alfa (erythropoietin recombinant), to be administered as a single injection once every two weeks (subcutaneously or intravenously). If deviation of anaemia 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 two-fold of the dose in the once every two weeks injection. In the case, the usual dose in adult patients is 100-200 µg administered as a single injection once every four weeks or subcutaneously or intravenously. In all cases, the dose should be adjusted in case of the degree of anaemic symptoms and the patient’s age, and should not exceed 300 µg as a single injection. The target of anaemia correction is around 11 g/l of haemoglobin level.

- Precautions related to Doseage and Administration
  1. Initial dose at the switching from an erythropoiesis preparation.
  2. When NES® is started in substitution for an erythropoiesis preparation, the dose and the frequency of administration should be determined on the basis of the dose of the erythropoiesis preparation that was used. See the Table (package insert).
  3. Patients who have already been treated with the erythropoiesis preparation twice weekly or three times weekly. Calculate the total dose of the erythropoiesis preparation administered during the week before the switching, and divide the initial dose of NES® given according to the table below. The treatment should be started on once weekly basis.
  4. Patients who have been treated with the erythropoiesis preparation once weekly or once every two weeks. Calculate the total dose of the erythropoiesis preparation administered during the two weeks before the switching, and then determine the initial dose of NES® given according to the Table below. The treatment should be started on once every two weeks basis. See the Table insert.

2. Dose adjustment
1. Dose adjustment is required, for example, when the appropriate increase in the haemoglobin concentration over the maintenance phase can not be achieved in correction phase, or when the haemoglobin concentration on the maintenance dose 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 step by step, in principle.

- Precautions
  See the package insert.

- STORAGE
  Store in a lightproof container at 2-8 °C and avoid freezing.

- PACKAGING
  1 vial, 10 vials
  for NES® 100 µg, 30 µg, 50 µg, 60 µg, 120 µg, respectively

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원단 효능 

1) 발작성 야간 혈색소뇨증 (PNH : Paroxysmal Nocturnal Hemoglobinuria)용혈을 감소시키기 위한 발작성 야간 혈색소뇨증 (PNH : Paroxysmal Nocturnal Hemoglobinuria) 환자의 치료. 수혈 이력과 관계없이, 높은 질병 활성을 의미하는 임상 증상이 있는 환자의 용혈에 임상적 이익이 확립되었다.

2) 비정형 용혈성 요독 증후군 (aHUS : atypical Hemolytic Uremic Syndrome) 보체 매개성 혈전성 미세혈관병증을 억제하기 위한 비정형 용혈성 요독 증후군 (aHUS : atypical Hemolytic Uremic Syndrome) 환자의 치료

사용제한 : 
시가(Shiga) 톡신 성 대장균에 의한 용혈성 요독 증후군 (STEC-HUS) 환자 대상의 적용을 권장하지 않는다.

3) 전신 중증 근무력증 (Generalized Myasthenia Gravis) 
항아세틸콜린 수용체 항체 양성인 환자의 불응성 전신 중증 근무력증 (Refractory gMG: Refractory Generalized Myasthenia Gravis)의 치료

4) 시신경 척수염 범주 질환 (Neuromyelitis optica spectrum disorder) 
항아쿠아포린-4 (AQP-4) 항체 양성인 환자의 시신경 척수염 범주질환 (NMOSD: Neuromyelitis optica spectrum disorder)의 치료

용법·용량

심각한 감염에 대한 위험을 줄이기 위해서 환자들은 최신의 백신 접종 지침 (Advisory Committee on Immunization Practices (ACIP) recommendations)에 따라 백신 접종을 해야 한다. (사용상의 주의사항 1. 경고 항 참고)

이 약은 정맥투여되어야 하며 급속정맥투여 (IV push) 또는 일시정맥투여 (IV bolus)로 투여해서는 안된다.

성인
1) 발작성 야간 혈색소뇨증 (PNH) : 첫 4주간은 매 7일마다 600 mg, 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 900 mg을 투여하고, 그 후부터는 매 14일마다 900 mg을 투여한다. 이 약은 권장 투여량과 일정에 맞게 투여, 혹은 예정된 일정의 2일 전/후로 투여 되어야 한다.

2) 비정형 용혈성 요독 증후군 (aHUS) 및 불응성 전신 중증 근무력증 (Refractory gMG) 및 시신경 척수염 범주질환 (NMOSD) : 첫 4주간은 매 7일마다 900 mg, 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 1200 mg을 투여하고, 그 후부터는 매 14일마다 1200 mg을 투여한다.

소아
1) 비정형 용혈성 요독증후군 (aHUS) 만 18세 미만의 aHUS 환자일 경우, 체중에 따라 권장 일정으로 투여한다. (제품정보 원문 용법·용량 [표 1] 만 18세 미만 환자에서의 권장투여법 참고) 이 약은 권장 투여량과 일정에 맞게 투여, 혹은 예정된 일정의 2일 전/후로 투여되어야 한다.

2) 실험실적 검사 결과 모니터링: PNH 환자는 LDH 수치를 확인하여 혈관 내 용혈을 관찰, aHUS 환자는 혈소판 수, 혈청 LDH, 혈청 크레아티닌을 측정하여 미세혈관병증 여부를 관찰하여야 하며, 유지기간 동안 권장 투여일정 (14±2일)내에서 용법·용량 조정이 필요할 수 있다 (매 12일까지).

약물이상반응

상급 후 보고 및 완료된 임상시험에서 보고된 약물이상반응 (발생률 1% 이상 발췌): 매우 흔하게 (≥1/10) - 두통, 흔하게 (≥1/100 ~ <1/10) - 폐렴, 상기도감염, 비인두염, 기관지염, 요로 감염, 구강 헤르페스, 백혈구감소증, 빈혈, 불면, 현기증, 미각이상, 고혈압, 기침, 입인두통, 설사, 구토, 구역, 복부통증, 발진, 탈모, 소양증, 관절통, 근육통, 열, 피로감, 인플루엔자 유사질환 모든 임상시험에서, 가장 중대한 이상반응은 수막구균 패혈증이었고, 이는 이 약으로 치료받은 환자에서 수막구균 감염증이 흔한 증상이었다. 수막구균 패혈증의 징후와 증상에 대해 환자에게 알리고 즉시 의료 조치를 받아야 한다. Neisseria gonorrhoeae, Neisseria sicca/subflava, Neisseria spp unspecified로 인한 패혈증이 보고되었다.
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Powder/Granule/Suspension

REFERENCES
1. 식품의약품안전처, 온라인약국도서관: 약학정보-카리메트
2. 2019 IQVIA DATA 기준(국내 고갈혈증 치료제 판매량)

카리메트 산과필
[효능·의도] 고갈혈증 예방 및 치료。
[용도·용량] 일반 성인 1회 15~20mg을 2회로 나누어, 식전에 200~300ml의 물에 펄프한 후 복용한다.

The most prescribed treatment agent of Hyperkalemia in Korea

The 1st launched medicine of Calcium polystyrene sulfonate in Korea

Various formulations for medication convenience (Powder/Granule/Suspension)

Treatment agent of Hyperkalemia

KALIMATE
Powder/Granule/Suspension

REFERENCES
1. 식품의약품안전처, 온라인약국도서관: 약학정보-카리메트
2. 2019 IQVIA DATA 기준(국내 고갈혈증 치료제 판매량)
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- Intuitive Triage Dashboard
- Patient Snapshot
- Treatment Summary
**For your patients of Hypertension**

**ACERTIL® ARGinine**

**4mg, 8mg**

**ACERTIL® ARGinine**

**5mg, 10mg**

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**INDICATIONS/DOSAGE AND ADMINISTRATION**

Please refer to the most current prescribing information for the detailed information.

**References.**


COUNT ON FABRAZYME

Treat your Fabry disease patients with Fabrazyme

1 mg/kg once every 2 weeks


FABRAZYME agalsidase beta 1 mg/kg once every 2 weeks

Sanofi Genzyme

SANOFI GENZYME