Pleotropic effects of hypoxia-inducible factor-prolyl hydroxylase domain inhibitors: are they clinically relevant?

Recent innovations in renal replacement technology and potential applications to transplantation and dialysis patients: a review of current methods

Deep learning predicts the differentiation of kidney organoids derived from human induced pluripotent stem cells

Comparison of dominant and nondominate C3 deposition in primary glomerulonephritis

Hemodialysis facility star rating affects mortality in chronic hemodialysis patients: a longitudinal observational cohort study

The comparative efficacy and safety of basiliximab and antithymocyte globulin in deceased donor kidney transplantation: a multicenter cohort study
Aims and Scope

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

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

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The image on the front cover: Park et al reported the kidney organoids differentiation protocol. Immunofluorescence staining showed the podocytes, proximal tubules, and distal tubules in kidney organoids. Please see the text for more details (pp. 75-85).
Over the past decade, the advent of organoid technology has allowed production of laboratory-grown organ tissues as a model system for human biological studies. The use of human organoids has allowed researchers to learn a great deal about human biology and disease mechanisms [1]. Organoids are unique three-dimensional culture systems that are self-organized and similar to actual human organs. Human organoids represent human physiology rather than human-like animal models or two-dimensional culture systems [2].

Kidney organoids model the structure and function of nephrons, which are subdivided into functionally distinct portions, containing podocytes with proximal and distal tubules [3–5]. Kidney organoids have been shown to recapitulate genetic kidney diseases and acute kidney injury, such as cisplatin-induced toxicity [3,4]. Additionally, organoids generated from patient-derived induced pluripotent stem cells (iPSCs) have potential applications in pharmaceutical drug testing and molecular medicine [6]. Recently, protocols for cost-effective bulk production of organoids have been developed and are well-suited for large-scale assays, such as drug screening and regenerative medicine [2,7].

In spite of the availability of kidney organoids, certain technical limitations are associated with the use of organoids. Extended culture of kidney organoids may lead to expansion of nonrenal cell types, including neuronal cells and myofibroblasts [7]. Additionally, patient-derived or genetically modified iPSCs exhibit a variable nature of differentiation and low maturation efficiency. However, these limitations have been partly overcome using strategies to avoid immaturity or screen out unwanted maturation stages.

A study by Park et al. [8] demonstrated that bright-field optical microscopic images could be used to assess the maturity of kidney organoids. They discriminated organoids according to bright-field morphology and examined renal markers of differentiation using quantitative polymerase chain reaction (PCR). These results revealed that bright-field morphology has a high correlation with actual differentiation of organoids. The distinctive feature of this method is that analysis is performed in a living model. The existing methods such as immunofluorescence analysis, reverse transcription-PCR, and single-cell RNA sequencing analysis require destruction of cells in the organoids. Moreover, analysis of bright-field images can be performed in a relatively short time and can be performed at low cost in the laboratory because it is time-efficient and does not
use expensive equipment.

Because the analysis of bright-field images is subjective, the outcomes may vary by the observer. To overcome this challenge, the authors adopted deep learning algorithms to objectively analyze bright-field images (Fig. 1). Advances in machine learning, especially deep learning, have allowed exploration, classification, and interpretation of patterns in biological images [9]. One of their typical tasks includes an unsupervised comparison of the features of collections of images by identifying changes in cellular morphology in imaging-based screening [10].

Park et al. [8] trained convolutional neural networks (CNNs) using bright-field images of kidney organoids on day 18 after differentiation and compared the best-performing CNNs with a human-based classifier. They concluded that DenseNet121 is most suitable for predicting the differentiation of kidney organoids. This model classified organoid images into useful (positive) and nonuseful (negative) groups. The results revealed that the CNN algorithm had higher accuracy and speed in classifying organoids than human experts.

Park et al. [8] highlighted that a deep learning model could accurately validate kidney organoid maturity based on analysis of bright-field morphology. The use of

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**Figure 1. Conventional and deep learning-based methods to evaluate the maturity of kidney organoids.** The deep learning-based method has advantages over conventional methods in the quality control of organoids in that it can reduce subjectivity and variability depending on observers. In addition, bright-field images can be obtained without destruction of organoids. RT-PCR, reverse transcription polymerase chain reaction; scRNA-seq, single-cell RNA sequencing.
bright-field images is a cost-effective and fast strategy for distinguishing suitable organoids. This noninvasive and nondestructive prediction method could also be applied to standardize the quality control protocol of organoids and to high-throughput imaging analysis for drug screening. However, the current model has only been trained on 150 images of “good” and “bad” kidney organoids, which is a relatively small scale. Therefore, its practical use remains challenging. Breakthrough improvements can be achieved by training with many annotated images, which would enable a more detailed classification than just positive or negative groups. Furthermore, using additional learning models and including images for quality control in publicly available datasets would be helpful for improving this tool. We can also expect that this deep learning algorithm is applicable to kidney organoids induced by other differentiation protocols.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Writing—original draft: SY
Writing—review & editing: HYG
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References


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Cinnamon: an aromatic condiment applicable to chronic kidney disease

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Cinnamon, a member of the Lauraceae family, has been widely used as a spice and traditional herbal medicine for centuries and has shown beneficial effects in cardiovascular disease, obesity, and diabetes. However, its effectiveness as a therapeutic intervention for chronic kidney disease (CKD) remains unproven. The bioactive compounds within cinnamon, such as cinnamaldehyde, cinnamic acid, and cinnamate, can mitigate oxidative stress, inflammation, hyperglycemia, gut dysbiosis, and dyslipidemia, which are common complications in patients with CKD. In this narrative review, we assess the mechanisms by which cinnamon may alleviate complications observed in CKD and the possible role of this spice as an additional nutritional strategy for this patient group.

Keywords: Chronic renal insufficiency, Cinnamomum zeylanicum, Inflammation, Oxidative stress, Spices

Introduction

Cinnamon is a spice used for centuries as a culinary flavoring agent with organoleptic properties in different cultures worldwide. It has been used traditionally as a remedy for respiratory and gastrointestinal complications and has been widely studied because of its potential health-promoting properties [1]. These include antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and antilipemic properties [2–4].

The anti-inflammatory properties of cinnamon have been suggested to be derived via inhibition of nuclear factor kappa B (NF-κB) expression and consequently reduced production of proinflammatory cytokines, such as tumor necrosis factor (TNF), C-reactive protein (CRP), and interleukin (IL) 6 [5–7]. Cinnamon also promotes the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which upregulates a host of cytoprotective defenses and increas-
Cinnamon is an indigenous spice obtained from the inner bark of trees belonging to the genera *Cinnamomum* from the Lauraceae family. It has been used since as early as 3,000 BC in Egypt. The name is of Greek origin (κινάμομον), which translates as ‘sweet wood’ [20]. Today, it is used daily in various cuisines worldwide. Despite there being several varieties of cinnamon, only two, Ceylon cinnamon (also known as true cinnamon, which originates mainly from Sri Lanka) and cassia cinnamon (which originates from China, Vietnam, and Indonesia), are available in American and European food markets (Table 1) [2,21].

Cinnamon contains carbohydrates (52%), fibers (33%), protein (3.5%), and fat (4%). This spice is also a source of potassium (134.7 mg/g), magnesium (85.5 mg/g), calcium (83.8 mg/g), phosphorus (42.4 mg/g), manganese (20.1 mg/g), and iron (7.0 mg/g) [22]. The key components of cinnamon are essential oils of trans-cinnamaldehyde, cinnamyl acetate, and eugenol; a range of bioactive resinous compounds including cinnamaldehyde, cinnamic acid, and cinnamate; water-soluble polyphenols such as catechin, epicatechin, procyanidin, quercetin, and kaempferol; and polyphenolic polymers [23,24]. Eugenol is the main compound in the leaves, whereas cinnamaldehyde is predominant in the bark and camphor in the root [2,23,25]. The spicy flavor and fragrance characteristics of cinnamon are due to cinnamaldehyde (known as cinnamic aldehyde). In addition, the aging of cinnamon leads to color darkening due to higher levels of resinous compounds [25].

The daily intake of cinnamon can be considered safe if it does not exceed the tolerable daily intake of coumarin (0.1 mg/kg of body weight) [2], which is a phytochemical with anticoagulant, carcinogenic, and hepatotoxic properties [2,26]. However, coumarin concentration depends on the type of cinnamon, e.g., cassia cinnamon contains significant amounts of coumarin, whereas Ceylon cinnamon contains only trace quantities [2].

Different species of cinnamon may present an array of other oils with diverse characteristics, and their effects have been widely debated. Various studies have used different species and forms of cinnamon supplementation, leading

<table>
<thead>
<tr>
<th>Cinnamon variety</th>
<th>Scientific names</th>
<th>Common names</th>
<th>Country of origin</th>
<th>Color</th>
<th>Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceylon cinnamon</td>
<td><em>Cinnamomum zeylanicum</em> or <em>Cinnamomum verum</em></td>
<td>Ceylon cinnamon, true cinnamon, Mexican cinnamon</td>
<td>Sri Lanka, southern India</td>
<td>Light to medium reddish brown</td>
<td>Slightly sweetness</td>
</tr>
<tr>
<td>Cassia cinnamon</td>
<td><em>Cinnamomum burmanni</em></td>
<td>Indonesian cassia, Indonesian cinnamon, Korintje cinnamon, Padang cassia</td>
<td>Indonesia, Philippines</td>
<td>Dark reddish brown</td>
<td>Strong spicy</td>
</tr>
<tr>
<td></td>
<td><em>Cinnamomum loureiro</em></td>
<td>Saigon cinnamon, Vietnamese cassia, Vietnamese cinnamon</td>
<td>Vietnam</td>
<td>Dark reddish brown</td>
<td>Spicy and sweet</td>
</tr>
<tr>
<td></td>
<td><em>Cinnamomum aromaticum</em></td>
<td>Chinese cinnamon, Chinese cassia, cassia cinnamon</td>
<td>China, Burma</td>
<td>Dark reddish brown</td>
<td>Mild and slightly sweet</td>
</tr>
</tbody>
</table>
to equivocal findings [1,27,28].

**Cinnamon: antioxidant and anti-inflammatory actions**

High production of reactive oxygen species (ROS) and reactive nitrogen species and reduced antioxidant capacity lead to oxidative stress, which promotes the pathogenesis of several chronic diseases, including diabetes, CKD, and CVD [29,30]. Therefore, modulating antioxidant enzyme production can reduce ROS formation and oxidative stress, slowing chronic disease progression [31]. Various cinnamon extracts, such as *Cinnamomum zeylanicum* Blume essential oil, ethanol extracts of cinnamon bark, cinnamon bark aqueous extract, and methanolic crude extract of *Cinnamomum verum*, display antioxidant activity, which indicates the potential for cinnamon to manage oxidative stress-related disorders [32]. Most cinnamon studies in *vitro* and *in vivo* (Table 2) [9,33-47] demonstrate significant antioxidant activity through multiple mechanisms, including reduction of malondialdehyde level (lipid peroxidation marker), activation of transcription factor Nrf2, and synthesis of antioxidant enzymes such as HO-1, superoxide dismutase, CAT, and GPx [48,49]. Twenty-two chemical ingredients have been isolated from cinnamon in addition to cinnamaldehyde analogues; of these, lignan pinosylvin (PRO) and the flavonol (-)-2R,3R)-5,7-dimethoxy-3',4'-methylenedioxy-flavan-3-ol (MFO) display antioxidant capacity [50].

The primary mechanism by which cinnamon (principally the cinnamaldehyde component) acts as an anti-inflammatory is via the downregulation of NF-κB [33,51] and diminution of inflammatory cytokine expression (e.g., TNF, CRP, and IL-6). Cinnamon also appears to reduce the levels of IL-1β and IL-18 by inhibiting the expression of NLR family pyrin domain containing 3 inflammasome and caspase-1 [34].

Additionally, cinnamaldehyde suppresses the expression of cyclooxygenase 2, nitric oxide synthase and prostaglandin E2 (PGE2) [52,53]. It has been implicated in the decreased phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinases (p38 MAPKs) pathways [35]. The role of cinnamon as an antioxidant and anti-inflammatory agent is illustrated in Fig. 1.

A limited number of studies have described the anti-inflammatory effects of cinnamon in humans, but the results remain inconclusive. Supplementation of 1.5 g/day of *Cinnamomum burmannii* powder in women with rheumatoid arthritis for 8 weeks promoted a reduction in both visual and pain scales, reduced tender and swollen joint counts, and reduced serum CRP and TNF levels [5]. Similarly, cinnamon (1.8 g/day for 2 months) in patients with migraines reduced serum IL-6 and nitric oxide (NO) levels [54]. The frequency, severity, and duration of migraine attacks decreased, suggesting a reduction in the inflammatory process [54]. In contrast, Davari et al. [51] used 3 g/day of cinnamon for 8 weeks in patients with type 2 diabetes (T2D). They found no beneficial effects on NF-κB, sirtuin 1 (SIRT1), or other systemic inflammation markers, including IL-6 and high-sensitivity CRP. The reasons for this outcome disparity remain unclear and may be multifactorial, including differing cinnamon sources, purity, and experimental methodologies.

**Diabetes and cinnamon**

Diabetes is one of the leading causes of CKD, manifesting as diabetic kidney disease. Several studies (Table 3) [51,55-78] have proposed that cinnamon therapy can improve insulin action and glucose metabolism, with procyanidin type-A polymers and cinnamaldehyde being the primary components associated with the antidiabetic effects [79].

Procyanidin type-A polymers in cinnamon can mimic insulin action as they increase insulin receptor autophosphorylation of β-subunit tyrosine residues and reduce oxidative stress in pancreatic β-cells [80,81]. Moreover, cinnamon extract (*C. zeylanicum*) ameliorated glucose transporter 4 translocation via the adiponectin and intracellular 5' adenosine monophosphate-activated protein kinase (AMPK) signaling pathway [82,83] and through stimulation of liver kinase B1 mediated AMPK phosphorylation [84]. Additionally, inhibition of α-glucosidase and pancreatic α-amylase, which promote postprandial glycemic amelioration, has been attributed to the action of the cinnamon extract [85].

Cinnamon also induces the expression of the peroxisome proliferator-activated receptors (PPAR) alpha and gamma (PPAR-α and PPAR-γ) *in vitro* and *in vivo*. This is notable as these regulate adipogenesis and insulin resistance by
**Table 2. Studies involving cinnamon and antioxidant and anti-inflammatory actions**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study/samples</th>
<th>Intervention</th>
<th>Results</th>
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<td><em>In vitro study</em></td>
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<tr>
<td>Uchi et al. (2017)</td>
<td>Human keratinocyte cell line benz[a]pyrene-stimulated</td>
<td>Cinnamaldehyde (25 μM) or Cinnamomum cassia extract (100 mg/mL)</td>
<td>↓ Nrf2 translocation and HO-1 expression ↓ activation of AHR</td>
</tr>
<tr>
<td>Kim et al. (2018)</td>
<td>Raw 264.7 murine macrophage cells</td>
<td>Trans-cinnamaldehyde (25, 50, or 100 μM)</td>
<td>↓ TNF-α, IL-1β, and IL-6 and NO synthesis</td>
</tr>
<tr>
<td>Schink et al. (2018)</td>
<td>THP-1 monocyte-macrophage cell line TIB-202, LPS-stimulated</td>
<td>Cinnamon compounds (25 μg/mL)</td>
<td>Trans-cinnamaldehyde and p-cymene ↓ IL-8 secretion</td>
</tr>
<tr>
<td>Qu et al. (2019)</td>
<td>LPS-stimulated RAW264.7 cells</td>
<td>Cinnamaldehyde (5, 10, or 20 μM) pretreatment</td>
<td>↓ NLRP3 inflammasome, miR-21 and miR-155 ↓ ROS, the phosphorylation of AKT, mTOR, and COX-2 protein level</td>
</tr>
<tr>
<td>Cheng et al. (2020)</td>
<td>Human rheumatoid fibroblast-like synoviocyte line MH7A cells IL-1β-induced</td>
<td>Cinnamaldehyde (40, 60, and 80 nM) pretreatment</td>
<td>40, 60, and 80 nM: ↓ TNF-α, IL-6</td>
</tr>
<tr>
<td>Chen et al. (2020)</td>
<td>Human osteoarthritis chondrocytes</td>
<td>Cinnamaldehyde pretreatment (10, 20, or 50-μM)</td>
<td>All doses: ↓ IL-6, IL-1β, TNF-α ↓ MMP-13 and ADAMTS-5</td>
</tr>
<tr>
<td>Ben Lagha et al. (2021)</td>
<td>The monoblastic leukemia cell line U937 LPS-stimulated</td>
<td>Cinnamon bark aqueous extract (32.5 to 500 μg/mL) pretreatment</td>
<td>250 μg/mL: ↓ IL-6, IL-8, and TNF-α</td>
</tr>
<tr>
<td>Vallion et al. (2022)</td>
<td>Human keratinocytes cells</td>
<td>100 μM of cinnamaldehyde</td>
<td>↑ Nrf2 accumulation</td>
</tr>
<tr>
<td>Chen et al. (2022)</td>
<td>LPS-induced human osteoarthritis synovial fibroblasts</td>
<td>Pretreatment with cinnamic aldehyde (20 and 50 μmol/L)</td>
<td>↓ IL-1β, IL-6, and TNF-α ↓ TLR-4 and MyD88 expression</td>
</tr>
<tr>
<td><em>Experimental study</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tuzcu et al. (2017)</td>
<td>HFD rats</td>
<td>Cinnamon polyphenol (100 mg/kg body weight) for 12 weeks</td>
<td>↓ NF-κB p65 expressions ↓ PPAR-α, IRS-1, Nrf2, and HO-1 expressions in the HFD rat livers ↓ IL-1β levels, MDA, and caspase-3 levels in the hippocampus Activate Nrf2</td>
</tr>
<tr>
<td>Abou El-Ezz et al. (2018)</td>
<td>LPS-induced neuroinflammation mouse model</td>
<td>Trans-cinnamaldehyde (50 mg/kg) intraperitoneally for 1 week</td>
<td>↓ NLRP3 inflammasome, miR-21 and miR-155 ↓ ROS, the phosphorylation of AKT, mTOR, and COX-2 protein level</td>
</tr>
<tr>
<td>Liu et al. (2020)</td>
<td>In vitro: macrophages (Raw246.7)</td>
<td>In vivo: cinnamaldehyde (6.25, 12.5, or 25 μM)</td>
<td>↓ TNF-α and NO (6.25, 12.5, and 25 μM) In vivo: ↓ IL-1β in blood ↓NLRP3 in synovium</td>
</tr>
<tr>
<td>Wang et al. (2020)</td>
<td>Leptin receptor-deficient (db/db) mice</td>
<td>Diet containing 0.02% cinnamaldehyde for 12 weeks</td>
<td>↓ ROS generation, preserved NO production ↓ p-eNOS ↑ Nrf2, HO-1 and NQO-1</td>
</tr>
<tr>
<td>Ryu et al. (2020)</td>
<td>Mice with cognitive dysfunction induced by d-galactose and aluminum chloride</td>
<td>Trans-cinnamaldehyde (30 mg/kg/day) injected intraperitoneally + treadmill exercise for 5 weeks</td>
<td>↑ Nrf2, NQO-1, HO-1, and SOD-1</td>
</tr>
<tr>
<td>Abdel-kawi et al. (2022)</td>
<td>Wistar rats, gastric ulcers ethanol-induced model</td>
<td>2.5 mL/kg of cinnamon oil and omeprazole (20 mg/kg) for 1 week before ulcer induction</td>
<td>↑ CAT, SOD, GPx, and GSH in the stomach ↓ MDA and TNF-α levels</td>
</tr>
<tr>
<td>Zou et al. (2022)</td>
<td>Sepsis-induced C57BL/6 J mice</td>
<td>2 g/kg of cinnamyl alcohol by gavage</td>
<td>↓ IL-1β and IL-18 Expression of NLRP3, caspase-1, and apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain in the liver, heart, lungs, and kidneys</td>
</tr>
</tbody>
</table>

ADAMTS-5, metalloproteinase with thrombospondin motif 5; AHR, aryl hydrocarbon receptor; AKT, protein kinase B; CAT, catalase; COX-2, cyclooxygenase type 2; GPx, glutathione peroxidase; GSH, glutathione; HFD, high-fat diet; HO-1, heme oxygenase 1; IL, interleukin; IRS-1, insulin receptor substrate 1; LPS, lipopolysaccharide; MDA, malondialdehyde; MMP-13, matrix metalloproteinase-13; mTOR, mammalian target of rapamycin; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; NO, nitric oxide; NQO-1, NAD(P)H dehydrogenase [quinone] 1; Nrf2, nuclear factor erythroid 2-related factor 2; p-eNOS, phosphorylated endothelial nitric oxide synthase; PPAR-α, peroxisome proliferator-activated receptors (PPAR) alpha; ROS, reactive oxygen species; SOD-1, superoxide dismutase 1; TLR-4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha.

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Figure 1. Antioxidant and anti-inflammatory actions of cinnamon in cells. Bioactive compounds from cinnamon may activate the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), leading to the synthesis of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), heme oxygenase 1 (HO-1), glutathione peroxidase 1 (GPx-1), and NAD(P)H dehydrogenase [quinone] 1 (NQO-1). Also, these compounds can inhibit nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), and NLR family pyrin domain containing 3 (NLRP3), reducing inflammatory cytokine production. TLR-4, toll-like receptor 4; ERK, extracellular signal-regulated kinase; AKT, protein kinase B; Keap1, Kelch-like ECH-associated protein 1; TNF-α, tumor necrosis factor alpha; CRP, C-reactive protein; IL, interleukin.

regulating the expression of genes encoding proteins involved in adipokine synthesis, adipocyte differentiation, and lipid and carbohydrate metabolism [86]. Additionally, cinnamaldehyde may stimulate the expression of PPAR-γ and PPAR delta (PPAR-δ) in differentiated adipocytes, promoting insulin sensitivity and fatty acid β-oxidation in adipose tissue and skeletal muscle [87]. Another component of cinnamon extract, the B-type procyanidin C1, has been demonstrated to stimulate preadipocyte differentiation as well as act as a potential insulin sensitizer through the protein kinase B (AKT)/endothelial NO synthase (eNOS): AKT/eNOS pathway in mature adipocytes [88]. The phosphoinositide 3-kinase (PI3K)/AKT pathway participates in glucose uptake by skeletal muscles, adipose tissues, and liver. Cinnamaldehyde treatment (10 mg/kg) has been reported to increase the expression of insulin receptor substrate 1 (IRS-1), PI3K, and AKT2 in diabetic rats, promoting enhanced insulin signaling by the IRS1/PI3K/AKT pathway and reducing insulin resistance and promoting an antidiabetic effect [55].

Despite the salutogenic effects of cinnamon treatment in diabetes, other human-based studies have yielded equivocal results. In one systematic review, no significant benefits were found for cinnamon in reducing glucose and glycated hemoglobin (HbA1c) levels in patients with type 1 diabetes [89]. Conversely, a meta-analysis has reported that intake of whole cinnamon or cinnamon extract lowered fasting blood glucose (FBG) in T2D and prediabetes [90]. In a meta-analysis of 435 patients, Akilen et al. [91] reported that cinnamon doses ranging from 1 to 6 g/day ingested for
<table>
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<tr>
<td>Hafizur et al. (2015) [58]</td>
<td>STZ-induced diabetic rats</td>
<td>5 and 10 mg/kg of cinnamic acid or cinnamaldehyde</td>
<td>Cinnamic acid: ↓ blood glucose, improved glucose tolerance ↑ Glucose-stimulated insulin secretion in isolated islets. Cinnamaldehyde: ↔ glucose-stimulated insulin secretion</td>
</tr>
<tr>
<td>Qusti et al. (2016) [59]</td>
<td>STZ-induced diabetic in male albino rats</td>
<td>20% (w/w) cinnamon methanol extract for 28 days</td>
<td>↓ Blood glucose ↓ IL-6 and MDA ↑ CAT and SOD ↓ Urea, Cr, and uric acid ↓ Blood glucose ↓ TNF-α and IL-6</td>
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<tr>
<td>Jawale et al. (2016) [60]</td>
<td>STZ-induced diabetic in rats</td>
<td>10, 20, or 40 mg/kg of cinnamaldehyde for 3 weeks</td>
<td>↓ Hyperphagia and glucose intolerance ↓ Fructosamine, TC, TG, leptin ↓ TNF-α, MDA, NO ↑ HDL-C, adiponectin, liver glycogen ↑ PPAR-γ gene expression</td>
</tr>
<tr>
<td>Hosni et al. (2017) [61]</td>
<td>STZ-induced diabetic in female albino rats with gestational diabetes</td>
<td>20 mg/kg oral dose of cinnamaldehyde with or without fatty-sucrose diet, or normal diet for 8 weeks</td>
<td>↓ Hyperphagia and glucose intolerance ↓ Fructosamine, TC, TG, leptin ↓ TNF-α, MDA, NO ↑ HDL-C, adiponectin, liver glycogen ↑ PPAR-γ gene expression</td>
</tr>
<tr>
<td>Taheri et al. (2018) [62]</td>
<td>STZ-induced diabetic in adult male Wistar rats</td>
<td>300 mg/kg cinnamon bark powder for 14 days</td>
<td>↓ OGTT, ITT, FBG ↓ Insulin and HOMA-IR ↑ HOMA-β ↓ MDA ↑ Aortic GSH, SOD, IRS-1, PI3K-p85, AKT2</td>
</tr>
<tr>
<td>Kommula et al. (2020) [63]</td>
<td>Neonatal STZ rat model</td>
<td>3% Cinnamon for 8 months</td>
<td>↓ Fasting and postprandial glucose levels prevented retinal functional abnormalities</td>
</tr>
<tr>
<td>Mohammed et al. (2020) [64]</td>
<td>STZ-induced diabetic rats</td>
<td>200 and 400 mg/kg of cinnamon oil emulsion in whey protein concentrate for 1 month</td>
<td>↓ Blood glucose, amylase, ↓ TC, LDL-C, TG ↑ Insulin, HDL-C ↑ Hepatic SOD, GSH ↓ Hepatic MDA</td>
</tr>
<tr>
<td>Niazmand et al. (2021) [65]</td>
<td>STZ-induced diabetic rats</td>
<td>Cinnamon extract (100, 200, 400 mg/kg) and metformin (300 mg/kg) orally for 42 days</td>
<td>↓ MDA level, SOD and CAT activities in the liver and kidney</td>
</tr>
<tr>
<td>Sampath et al. (2021) [66]</td>
<td>Gastric emptying in obesity-induced diabetic female mice</td>
<td>Cinnamaldehyde 50 mg per body mass per day for 6 weeks</td>
<td>↓ Body weight gain ↓ FBG ↓ HOMA-IR ↑ Reduced/oxidized glutathione ratio ↑ Activities of mitochondrial enzymes ↓ Levels of hepatic marker enzymes (AST, ALT, and ALP) ↓ Urea, Cr, and uric acid</td>
</tr>
<tr>
<td>Vijayakumar et al. (2022) [57]</td>
<td>STZ-induced diabetic rats</td>
<td>Ethanolic bark extracts of <em>Cinnamomum cassia</em> with different concentrations (300, 400, and 500 mg/kg BW) and glibenclamide (3 mg/kg BW)</td>
<td>↓ Body weight gain ↓ FBG ↓ Urea, Cr, and uric acid</td>
</tr>
<tr>
<td>Çelik et al. (2022) [67]</td>
<td>STZ-induced diabetic rats</td>
<td>20 mg/kg of BW of cinnamaldehyde by gavage daily for 1 month</td>
<td>↓ Body weight gain ↓ FBG ↓ TG, TC, VLDL, LDL-C, and urea levels</td>
</tr>
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Table 3. Continued

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<thead>
<tr>
<th>Reference</th>
<th>Study/sample</th>
<th>Intervention</th>
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<tr>
<td>Human study</td>
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<tr>
<td>Bernardo et al. (2015) [68]</td>
<td>Nondiabetic adults</td>
<td>100 mL of cinnamon tea (Cinnamomum burmannii bark) obtained from 60 g sticks of cinnamon soaked into 1,000 mL of water, after OGTT</td>
<td>Slightly ↓ PBG level after OGTT</td>
</tr>
<tr>
<td>Sengsuk et al. (2015) [69]</td>
<td>T2D patients</td>
<td>1,500 mg of cinnamon (divided into 3 times a day capsules) or placebo for 2 months</td>
<td>↓ Median glucose, TG, TG/HDL-C ratio, and BP</td>
</tr>
<tr>
<td>Anderson et al. (2015) [71]</td>
<td>Hyperglycemic adults</td>
<td>1 g (divided into 2 capsules) a day water extract of cinnamon (CinSulin), or placebo for 2 months</td>
<td>↑ HDL-C and eGFR, ↓ Fructoseamine, fasting insulin, ↓ TC, LDL-C, HDL-C</td>
</tr>
<tr>
<td>Azimi et al. (2016) [56]</td>
<td>T2D patients</td>
<td>3 g/day of cinnamon with black tea for 2 months</td>
<td>↓ Median glucose, TG, TG/HDL-C ratio, and BP</td>
</tr>
<tr>
<td>Gutierrez et al. (2016) [70]</td>
<td>Young, sedentary, obese women</td>
<td>5 g of encapsulated cassia cinnamon bark for 3 separate days (30-, 60-, 90-, and 120-minute following glucose ingestion)</td>
<td>↔ BP and endothelial function, ↔ Insulin resistance and sensitivity</td>
</tr>
<tr>
<td>Gupta Jain et al. (2017) [72]</td>
<td>Individuals with metabolic syndrome</td>
<td>3 g (divided into 6 capsules) of cinnamon or placebo, for 4 months</td>
<td>↓ Peak blood glucose at 30-time point, ↓ FBG, ↓ HbA1c, ↓ WC, ↓ BMI, improved lipid profile, waist-hip ratio, and BP</td>
</tr>
<tr>
<td>Talaie et al. (2017) [73]</td>
<td>T2D patients</td>
<td>3 g of cinnamon (divided into 3 capsules-day), for 2 months</td>
<td>↔ FBG, insulin, HbA1c, HOMA-IR, total antioxidant capacity, and MDA</td>
</tr>
<tr>
<td>Zare et al. (2019) [74]</td>
<td>T2D patients</td>
<td>1 g of cinnamon bark powder (divided into 2 capsules daily) or placebo for 3 months</td>
<td>↓ BMI, body fat, visceral fat, ↓ FBG, HbA1c, fasting insulin, insulin resistance</td>
</tr>
<tr>
<td>Kizilaslan and Erdem (2019) [75]</td>
<td>Healthy adult individuals</td>
<td>1 g or 3 g or 6 g/day cinnamon peel (C. cassia), for 40 days</td>
<td>↓ TC, LDL-C, and HDL-C, ↔ BMI, HbA1c, Difference in pre-prandial blood glucose (6 g/day)</td>
</tr>
<tr>
<td>Davari et al. (2020) [51]</td>
<td>T2D patients</td>
<td>3 g of cinnamon for 2 months</td>
<td>↔ NF-κB, SIRT1, hs-CRP, IL-6, and TNF-α plasma levels</td>
</tr>
<tr>
<td>Romeo et al. (2020) [76]</td>
<td>Adults with prediabetes</td>
<td>500 mg cinnamon thrice daily for 3 months</td>
<td>Fasting plasma glucose remained stable only in the cinnamon group</td>
</tr>
<tr>
<td>Lira Neto et al. (2022) [77]</td>
<td>T2D patients</td>
<td>3 g of cinnamon (capsules daily) for 3 months</td>
<td>↓ OGTT</td>
</tr>
<tr>
<td>Rachid et al. (2022) [78]</td>
<td>T2D patients</td>
<td>6 g/100 mL of aqueous cinnamon extract (C. burmannii) after 30, 60, 90, and 120 minutes</td>
<td>↔ Area under the curve, glucose conc., variation, and maximum glucose conc.</td>
</tr>
</tbody>
</table>

AKT, protein kinase B; AKT2, AKT serine/threonine kinase 2; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BW, body weight; CAT, catalase; Cr, creatinine; CYP2D, cytochrome P450; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; GSH, glutathione; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IRS-1, insulin receptor substrate 1; ITT, insulin tolerance test; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; NO, nitric oxide; OGTT, oral glucose tolerance test; PBG, postprandial glucose level; P3K, phosphoinositide 3-kinase; PPAR-γ, peroxisome proliferated activated receptor gamma; SIRT1, silent mating-type information regulation 2 homolog 1; SOD, superoxide dismutase; STZ, streptozotocin; T2D, type 2 diabetes; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor alpha; VLDL, very-low-density lipoprotein; WC, waist circumference.
between 40 days and 4 months reduced HbA1c and fasting glycemia levels. In 2013, a further meta-analysis including 543 patients reported that cinnamon supplementation (powdered cinnamon and aqueous extract) ranging from 120 mg to 6 g ingested for between 4 and 18 weeks reduced blood glucose, total cholesterol, and triglycerides but did not affect HbA1c level [92]. Costello et al. [80] have shown that cinnamon dietary supplements (doses ranging from 120 to 6,000 mg/day ingested for between 4 and 16 weeks) have clinically meaningful effects on glycemic control (FBG or HbA1c) in patients with T2D.

Additionally, a meta-analysis showed no effect of powdered cassia cinnamon intake (1–2 g) on fasting glucose, HbA1c, triglycerides, low-density lipoprotein (LDL), and total cholesterol levels in patients with T2D. On the other hand, a higher (at least 3 g) rather than a lower dose of cassia bark powder or cassia extract associated with lifestyle and diet protocols was more effective for glucose control in T2D [93].

Analyzing the impact of cinnamon on patients with diabetes is very complex as cinnamon contains several compounds, such as coumarin, cinnamic acid, cinnamaldehyde, cinnamic alcohol, and eugenol, with varied concentrations among species [94]. In addition, results are related to the quality of cinnamon, the type of branches, and manufacturing practices among species and formulations [95]. The effectiveness of cinnamon in glucose control may depend on how well the diabetes was controlled during the study. In addition, previous studies have used different parameters and periods [95]. Therefore, administering cinnamon can be a helpful add-on therapy in integrative medicine for managing T2D. Still, long-term trials are required to establish the efficacy and safety of cinnamon. In addition, the differing contributions of various microbiomes between subjects must be addressed [96].

**Cinnamon: benefits in obesity**

Obesity is a strong predictor of renal dysfunction and CKD [97]. Some physiological responses of the kidneys to obesity include increased glomerular filtration rate, tubular reabsorption of sodium, filtration fraction, and renal plasma flow [98]. Central obesity and abdominal fat are risk factors for metabolic syndrome, which is also associated with the development and progression of CKD and CVD [99].

Cinnamon has been studied as a potential nutritional strategy for managing obesity and its complications [9]. Cinnamon’s antiobesogenic effect may be related to its ability to induce thermogenesis in adipocytes as mediated by uncoupling protein 1 which is expressed in brown and beige tissues and improves metabolism to promote weight loss [100].

Moreover, cinnamaldehyde activates a classic thermogenesis pathway through protein kinase A signaling that phosphorylates p38 MAPK, inducing the transcription of thermogenic genes such as hormone-sensitive lipase and lipid droplet-associated protein perilipin 1 [52]. Additionally, as cinnamaldehyde is the primary natural agonist of the transient receptor potential ankyrin 1 (TRPA1), it may also indirectly influence food intake and weight gain, which can be expressed in gastrointestinal functions such as decreasing ghrelin secretion [101,102]. Other natural compounds present in cinnamon oil, such as cumin aldehyde (cumin), p-anisaldehyde (anise), and triglycaldehyde (onion/garlic), can activate human TRPA1 specifically but with lower affinity compared to cinnamaldehyde. Among these compounds, cumin aldehyde demonstrated glucose-dependent insulin secretagogue activity in diabetic rats by TRPA1 stimulation [102].

The AMPK pathway is also relevant to the study of obesity as it is a mediator of cellular energy production, which can improve insulin sensitivity in insulin-sensitive tissues, such as adipose tissue [103]. Cinnamon seems to exert beneficial effects via AMPK activation and enhanced adiponectin concentrations, as demonstrated by Kopp et al. [104]. They evaluated the Gi/Go-protein-coupled receptor 09A, which stimulates adiponectin secretion after binding trans-cinnamic acid from cinnamon.

Other protective effects ascribed to cinnamon appear to result from a reduction of hepatic expression of the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) and NF-κB, in conjunction with up-regulation of PPAR-α, a cluster of differentiation 36 (CD36), fatty acid synthase, carnitine palmitoyltransferase I, and Nrf-2 [105]. Studies of obese rats with hepatic steatosis caused by a high-fat diet suggest enhancement of hepatic beta-oxidation and inhibition of hepatic lipogenesis, oxidative damage, and inflammation resulting from cinnamon intake.
Aqueous extract of *Cinnamomum cassia* bark has been linked to neurochemical and behavioral effects in rats by decreasing food intake through augmentation of 5-hydroxytryptamine in the brain [106].

Only a few studies have reported a relationship between cinnamon and antiobesogenic effects in humans. Yazdanpanah et al. [107] have conducted a systematic review and meta-analysis to investigate the effects of cinnamon on fat and body mass, body mass index (BMI), waist circumference, and waist-hip ratio. In total, 21 randomized controlled trials (RCTs) with 1,480 participants were included, and it was reported that cinnamon supplementation decreased obesogenic parameters. In agreement with the studies discussed, a systematic review and dose-response meta-analysis suggested that cinnamon supplementation could improve obesity measures, particularly in obese subjects aged <50 years at dosages of ≥2 g/day for at least 12 weeks [108]. More recently, Keramati et al. [109] evaluated the effects of cinnamon on obesity rates in humans through an umbrella meta-analysis, which indicated that cinnamon supplementation reduced BMI. The effects of cinnamon were more pronounced at doses of ≥3 g/day and in patients with polycystic ovary syndrome. Table 4 [52,72,105,110–123] lists these associated experimental and clinical studies on the effects of cinnamon on obesity.

**Cinnamon and cardiovascular disease**

Patients with CKD have a high risk of developing premature CVD due to a combination of traditional risk factors, including diabetes, obesity, dyslipidemia, hypertension, and a toxic uremic milieu [124]. Cinnamon may benefit cardiovascular health; indeed, studies have shown hypotensive effects, control of dyslipidemia, and protection of the endothelium and vascular smooth muscle cells (VSMC). As already discussed, cinnamon has anti-inflammatory and antioxidant properties, which can reduce the progress of atherosclerosis [56]. However, postulated hypotensive effects ascribed to cinnamon remain inconclusive [125]. Ghavami et al. [126] evaluated the effects of cinnamon supplementation on blood pressure through a systematic review and meta-analysis of RCTs. Eight studies, including 582 participants, suggested that cinnamon supplementation had beneficial effects only on diastolic blood pressure.

Components of cinnamon, such as catechin, epicatechin, procyanidin B2, and phenolic polymers, can act as agonists of PPARs, inhibiting the formation of advanced glycation end products to reduce oxidative stress and increasing the bioavailability of vasodilator NO [108,125].

Furthermore, cinnamon improves the lipid profile and reduces lipid oxidation and the risk of vascular blockage, mitigating potential hypertensive conditions [127]. Flavonoids and phenolic acids found in cinnamon inhibit pancreatic lipase, which is necessary for forming chylomicrons [110]. Cinnamon ameliorates lipid profiling by suppressing the expression of transcription factor SREBP-1c and liver X receptor alpha enzymes, such as ATP-citrate lyase and NF-κB p65. Furthermore, it upregulates PPAR-α expression to enable modulation of lipid metabolism [9]. Additionally, cinnamon has been reported to inhibit the secretion of proatherogenic apolipoprotein B 48 CD36, and the class A macrophage scavenger receptor, as well as the uptake of acetylated LDL, again suggesting that cinnamon can act as a preventive medicine [128,129].

Despite these promising results, the evidence remains inconclusive. Krittanawong et al. [130] have systematically reviewed the literature and evaluated cinnamon consumption and cardiovascular risk. A meta-analysis that included 23 studies (1,070 subjects) concluded that there was no association between cinnamon consumption and differences in LDL-cholesterol, high-density lipoprotein cholesterol, and HbA1c levels. Studies on cinnamon in *vivo*, in animals, and in humans are listed in Table 5 [9,45,131–144]. Again, allowance for different exposome features, such as microbiota composition, may be pertinent here [145].

**Does cinnamon benefit the gut microbiota?**

Microbiota dysbiosis is a disruption to the normative microbial community driven by host-related exposome factors such as diet, resulting in perturbations to its composition and function [145,146]. Dysbiosis is associated with many chronic diseases, such as metabolic syndrome, inflammatory bowel disease, and CKD, which present a typical proinflammatory phenotype. Increased permeability in the gut with age and condition enables the entry of microbial metabolites, pathobionts, or endotoxins such as lipopoly saccharides (LPS) into the circulation [147,148]. It also presents a loss of symbiotic microbes.
Table 4. Studies involving cinnamon on obesity

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<td>Lopes et al. (2015) [111]</td>
<td>Adult male Wistar rat</td>
<td>400 mg/kg BW/day of cinnamon aqueous extract (Cinnamomum zeylanicum), for 25 days</td>
<td>↔ Food intake and serum lipid profile</td>
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<td></td>
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<td></td>
<td>↓ Body mass gain</td>
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<td>↓ Relative mass of WAT</td>
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<td>Leptin mRNA expression in the WAT</td>
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<td>↑ Protein content</td>
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<td>Lee et al. (2016) [112]</td>
<td>3T3-L1 preadipocytes cells</td>
<td>50, 100, 200 µg/mL of cinnamon extract (Cinnamomum cassia)</td>
<td>↑ Lipid storage in white adipocytes,</td>
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<td></td>
<td>↑ Fatty acid oxidation capacity</td>
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<td>↑ PGC-1α, CPT-1α, PPARγ, C/EBP-α, and C/EBP-β genes expressions</td>
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<tr>
<td>Khare et al. (2016) [113]</td>
<td>3T3-L1 preadipocytes cells</td>
<td>10, 20, and 40 µM of cinnamaldehyde: in vitro 5 mL/kg and 10 mL/kg BW of</td>
<td>↑ HPL</td>
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<td>Leptin expression in the WAT</td>
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<td>↓ PPARγ and C/EBP-α prevented the increase in visceral fat pad weight</td>
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<td>regulated leptin/ghrelin ratio</td>
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<td></td>
<td>In vivo: male Swiss albino mice</td>
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<td>↑ Anorectic gene expression in hypothalamus (POMC, BDNF, UCN, CARTPT, and CCK)</td>
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<td>↓ Glycerol and free fatty acid levels</td>
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<td>↑ Expression of lipolysis-promoting genes: HSL, PNPLA2, and MGL</td>
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<td>↓ IL-1β, COX, MCP1, TNF-α, and IL-6</td>
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<td></td>
<td></td>
<td></td>
<td>↑ Anorectic and lipolytic gene expression</td>
</tr>
<tr>
<td>Jiang et al. (2017) [52]</td>
<td>Primary preadipocytes from and</td>
<td>200 and 400 µM of cinnamaldehyde</td>
<td>↑ Thermogenesis: ↑ UCP1, FGF21, PKA, phosphorylation of HSL and PLIN1</td>
</tr>
<tr>
<td></td>
<td>human adipose-derived stem cells</td>
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<td>↑ Lipid metabolism: Pdk4</td>
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<td>Kwan et al. (2017) [114]</td>
<td>3T3-L1 preadipocytes and Ex vivo:</td>
<td>80 µg/mL (in vitro) and 500 mg/kg BW (in vivo) cinnamon extract (C. cassia)</td>
<td>Induced browning in white adipocytes: ↑ UCP1 expression; ↑ Prdm16, Cidea, PPARγ, PGC, Cpt1</td>
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<td>subcutaneous adipose tissue from</td>
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<td>Induced browning in subcutaneous adipocytes in db/db mice: UCP1 protein</td>
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<td></td>
<td>db/db mice and in vivo/ex vivo</td>
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<td>and mRNA Cidea and Prdm16</td>
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<td></td>
<td>DIO mice</td>
<td></td>
<td>DIO mice: ↑ UCP1 expression in the subcutaneous adipose tissue; ↓ BW</td>
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<td>Kang et al. (2019) [115]</td>
<td>3T3-L1 and HIB1B preadipocytes</td>
<td>10–200 µM of trans-cinnamic acid of bark (C. cassia)</td>
<td>Induced browning in white adipocytes activation of β3AR-PKA-AMPK, TRPA1,</td>
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<td>cells</td>
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<td>GPR signaling pathways</td>
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<td>↑ Fat oxidation</td>
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<td>↓ Adipogenesis and lipogenesis</td>
</tr>
<tr>
<td>Neto et al. (2019) [116]</td>
<td>Lactating dams (Wistar rats) were</td>
<td>400 mg/kg BW/day of cinnamon aqueous extract (C. zeylanicum) during lactating period</td>
<td>↑ Visceral obesity</td>
</tr>
<tr>
<td></td>
<td>supplemented, and adult male</td>
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<td>Hepatic metabolic dysfunction and ↑ lipid accumulation</td>
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<tr>
<td></td>
<td>offspring were evaluated at 180</td>
<td></td>
<td>↓ Glycogen content in the liver, hyperleptinemia and hyperinsulinemia</td>
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<tr>
<td></td>
<td>days old</td>
<td></td>
<td>↓ Visceral adipose tissue mass</td>
</tr>
<tr>
<td>Neto et al. (2020) [117]</td>
<td>Adolescent rat model of obesity</td>
<td>Cinnamaldehyde 40 mg/kg of body mass per day for 29 days</td>
<td>↓ Plasma nitrate and nitrate</td>
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<td></td>
<td>programmed by early overnutriton</td>
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<td>↓ Inslet insulin secretion</td>
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<td>↓ iNOS activity</td>
</tr>
<tr>
<td>Ataie et al. (2021) [118]</td>
<td>Adult male Wistar rats with</td>
<td>Cinnamaldehyde 20 mg/kg of body mass per day for 16 weeks</td>
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<tr>
<td></td>
<td>HFD-induced</td>
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</tbody>
</table>

(Continued to the next page)
Beyond typical treatments to mitigate dysbioses, such as pro-, pre-, or symbiotics, some bioactive compounds can be effective in modulating the gut microbiota \[149,150\]. Studies of the benefits of cinnamon in this capacity have been increasing \[150,151\].

Cinnamon compounds, such as polyphenols, reach the colon and serve as substrates for bacterial metabolism \[152\]. Normative gut microbiota is dominated by anaerobic bacteria from the Firmicutes and Bacteroidetes phyla. Dysbiosis is characterized by a loss of microbial diversity and symbionts and an increased representation of pathobionts \[96,153\]. Cinnamon effectively enriches gut microbiota by reducing Proteobacteria and increasing Bacteroidetes \[154\]. The essential oil in cinnamon contributes to the growth of salutogenic bacteria capable of short-chain fatty acid production.

### Table 4. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study/sample</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. (2022) [105]</td>
<td>Adult male Wistar rat obesity HFD-induced</td>
<td>Cinnamon powder 50 or 100 mg/kg BW orally for 12 weeks</td>
<td>↓ Hepatic levels of oxidative and inflammatory biomarkers ↓ Serum levels of glucose, liver enzymes, insulin, and lipid profiles ↓ Hepatic expression of SREBP-1c and NF-kB ↑ PPAR-α, CD36, CPT-1, and Nrf-2 ↓ Adipocyte hypertrophy ↑ Oxidative pathways (PGC1α, FGF21) in WAT ↑ Increased BAT thermogenesis markers (PPARα, FGF21, UCP-1) ↓ WAT adipocyte size ↓ TC, LDL-C, and glucose levels ↓ ALT and AST and fat deposition in the liver</td>
</tr>
<tr>
<td>Neto et al. (2022) [119]</td>
<td>Adolescent rat model of obesity programmed by early overnutrition</td>
<td>Cinnamaldehyde 40 mg per kg of body mass per day for 30 days</td>
<td></td>
</tr>
<tr>
<td>Miah et al. (2022) [120]</td>
<td>Adult Swiss albino mice hyperlipidemia and obesity</td>
<td>10% butter with cinnamon 200 mg, 400 mg, or 600 mg powder per liter drinking water for 10 weeks</td>
<td></td>
</tr>
<tr>
<td>Human study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gupta Jain et al. (2017) [72]</td>
<td>Adults with metabolic syndrome</td>
<td>3 g/day (6 capsules) of cinnamon for 16 weeks</td>
<td>↓ BW, WC, waist-to-hip ratio ↓ % Body fat</td>
</tr>
<tr>
<td>Borzoei et al. (2017) [121]</td>
<td>Polycystic ovary syndrome in overweight or obese women</td>
<td>1.5 g cinnamon extract (3 capsules) for 8 weeks</td>
<td>Improved glucose metabolism and lipid profile, ↓ insulin</td>
</tr>
<tr>
<td>Khedr et al. (2020) [110]</td>
<td>Overweight /obese adults</td>
<td>1.2 g of Ceylon cinnamon capsules and 120 mg of Orlistat for 15 weeks</td>
<td>↓ BMI ↓ Lipase activity ↓ Lipid profile</td>
</tr>
<tr>
<td>Wang et al. (2021) [122]</td>
<td>Normal and overweight/obese individuals</td>
<td>1/2 cup dry instant oatmeal with milk prepared with or without 6 g of cinnamon (Korintje cinnamon, from cassia bark), acute intake (4 hours)</td>
<td>↓ Postprandial insulin response in overweight/obese individuals ↓ Postprandial glucagon levels, glucagon and C-peptide response in normal weight participants</td>
</tr>
<tr>
<td>Huang et al. (2022) [123]</td>
<td>Overweight adults</td>
<td>6 g of cinnamon meal on 4 separate visits at least 3 days apart</td>
<td>↓ Postprandial glyemia</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AMPK, adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BAT, brown adipose tissue; BDNF, brain-derived neurotrophic factor; BMI, body mass index; BW, body weight; C/EBP-α, CCAAT/enhancer-binding protein alpha; C/EBP-β, CCAAT-enhancer-binding protein beta; CARTPT, cocaine amphetamine-related transcript; CCK, cholecystokinin; CD36, cluster of differentiation 36; Cidea, DFFA-like effector A; COX, cyclooxygenase; CPT-1, carnitine palmitoyl transferase 1; CPT-1α, carnitine palmitoyltransferase 1 alpha; DIO, diet-induced obesity; FGF21, fibroblast growth factor 21; GPD, glycerol-3-phosphate dehydrogenase; GPR, G-protein-coupled receptor; HFD, high-fat diet; HSL, hormone-sensitive lipase; IL, interleukin; iNOS, inducible nitric oxide synthase; LDL-C, low-density lipoprotein cholesterol; MGLL, monoglyceride lipase; NF-κB, factor nuclear kappa B; Nrf-2, nuclear factor erythroid 2-related factor 2; Pdk4, pyruvate dehydrogenase kinase 4; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PPAR-α, peroxisome proliferator-activated receptor alpha; PPARy, peroxisome proliferator-activated receptor gamma; Prdm16, PR domain containing 16; PYGAME, peroxisome proliferator-activated receptor gamma; PR domain containing 16; SREBP-1, sterol regulatory element-binding transcription factor 1; TC, total cholesterol; TNF-α, tumor necrosis factor alpha; TRPA1, necrosis factor receptor-associated protein 1; UCN, urocortin; UCP-1, uncoupling protein 1; VAT, white adipose tissue; WC, waist circumference; β3AR, β3 adrenergic receptor.
### Table 5. Studies involving cinnamon on cardiovascular health

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study/sample</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Kwon et al. (2015) [131] | Rat aortic vascular smooth muscle cells | Extract *Cinnamomum cassia* bark – 10, 30, and 50 μM | ↓ PLCy1, Akt, and P38  
↑ Percentage of G0/G1 phase cells  
↓ PCNA expression |
| Panickar et al. (2015) [132] | Mouse brain endothelial cells | Cinnamtannin D1 – 10⁻² and 10⁻³ mg/mL | ↓ OGD-induced swelling  
↓ Cell swelling in presence of MCP-1  
↓ Mitochondrial ROS  
↓ OGD-induced fluorescence |
| Chen et al. (2016) [133] | Mice with ischemia/reperfusion-induced brain injury | 10, 20, and 30 mg/kg trans-cinnamaldehyde, an essential oil in cinnamon powder 60 minutes before ischemia surgery | ↓ Infarction area and neurological deficit score  
↓ iNOS, COX-2, NF-κB, mRNA, TNF-α |
| Kang et al. (2016) [134] | Male rats with metabolic syndrome with cardiac oxidative stress | 20, 40, and 80 mg/kg cinnamaldehyde for 5 weeks | ↓ HW/BW, TGF-β, p-Smad 2/3 and Smad4 |
| Tuzcu et al. (2017) [9] | Rats given high-fat feed | 100 mg/kg cinnamon polyphenol extract for 12 weeks | ↑ GSH/GSSG  
↓ Expression of hepatic SREBP-1c, LXRα, ACS, FAS, MDA, NF-κB  
↑ PPAR-α, IRS, Nrf2, HO-1, SOD, CAT  
↓ TG, TC, LDL-C  
↑ BW, visceral fat |
| Nayak et al. (2017) [135] | Mice with dexamethasone-induced atherosclerosis | 500 mg/kg and 250 mg/kg cinnamon extract for 12 days | ↓ TG, TC, LDL-C  
↑ HDL-C  
↓ Atherosclerotic change of aorta |
| Sedighi et al. (2018) [136] | Rats with ischemia | *Cinnamomum zeylanicum* bark extract – 50, 100, or 200 mg/kg - 2 weeks before ischemia | ↓ Infarct size  
↓ Ventricular tachycardia, ventricular ectopic beats episodes  
↓ R-wave amplitude  
↑ Heart rate during ischemia  
↓ MDA, cardiactroponin I, LDH  
↑ SOD, GPx |
| Pulungan and Pane (2020) [137] | Mice (Mus musculus) given high-fat feed | 2, 4, and 8 mg/kg cinnamon extract for 2 weeks | ↓ TC |
| Alsoodeeri et al. (2020) [138] | Rats given high-fat feed | 2 and 4 g/kg cinnamon powder for 4 weeks | ↓ TG, TC, LDL-C  
↑ HDL-C |
| Wang et al. (2020) [45] | Leptin receptor-deficient mice | Diet containing 0.02% cinnamaldehyde for 12 weeks | ↑ Nitrotyrosine, NO, NFR2, HO-1, NQO-1  
↓ ROS, p-eNOS  
↓ R-spondin-1 and -2 |
| Moreno et al. (2022) [139] | Rings from male Wistar rat thoracic aorta pre | Cinnamon extract (0-380 μg/mL) | Induced concentration-dependent vasodilation  
Inhibited induced cardiac hypertrophy |
| Tian et al. (2022) [140] | Male, cardiac hypertrophy model C57BL/6 | Trans-cinnamaldehyde daily at a dosage of 50 mg/kg or 100 mg/kg via oral gavage for 2 weeks | SBP, DBP |
| **Human study** | | | |
| Ranasinghe et al. (2017) [141] | Healthy adults | 85 mg, 250 mg, and 500 mg of *C. Zeylanicum* (water extract) for a period of 3 months, with dose increased at monthly intervals | ↓ Renal and liver function, fasting blood glucose, HDL-C, VLDL, and TG  
↓ TC and LDL-C |
| Mirmiran et al. (2019) [142] | Type 2 diabetes patients | 3 g cinnamon extract capsules, for 2 months | ↓ ICA-1 and VCAM-1 in both cinnamon and placebo groups, but not between groups |

(Continued to the next page)
production. These can produce butyrate, acetate, and propionate, which not only serve as the substrate for the host cells but also regulate inflammation [154,155]. Cinnamon oil may improve microbiota diversity and downregulate inflammatory processes [154]. Moreover, cinnamon oil can protect against LPS-induced intestinal injury through upregulation of epidermal growth factor, claudin-1, occludin, alkaline phosphatase (ALP), and pregnane X receptor expression, improving gut barrier integrity [156]. The evidence supports cinnamon or cinnamon compounds as nutritional adjuvants for maintaining intestinal integrity [156,157].

An experimental study conducted with early-weaned rats, highly susceptible to intestinal stress and alterations, has shown that treatment with 100 or 200 mg/kg body weight/day cinnamaldehyde for 2 weeks improved the gut barrier and was accompanied by an increase in mucin production, reduced inflammation, and improved microbiome diversity [158]. These authors suggested that the beneficial effects were due to inhibition of NF-κB activation; upregulated expression of mucin 2, trefoil factor 3, and tight junction proteins; and reduced IL-6 and TNF-α expression, potentially mediated by increased in gut microbe diversity [158].

Another recent study has supported this assertion, indicating that the microbiota in ovariecotomized mice displayed improved diversity after treatment with cinnamic acid. This result was accompanied by an elevation in transforming growth factor beta levels in bone marrow cells, which induced osteoblast differentiation and increased the expression of osteogenic markers [159].

Based on these data, cinnamon usage is encouraged not only to manage diseases influenced by microbiota, such as CKD but also for general health. The role of the microbiota in the health of the general population has recently been exemplified by a report linking poor renal function with accelerated aging and an imbalanced diet [160]. These data are pertinent to the treatment and management of CKD, as well as other diseases of aging.

**Table 5. Continued**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study/sample</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shirzad et al. (2021)</td>
<td>Stage 1 hypertension patients</td>
<td>Cinnamon capsules, 1,500 mg/day for 2 months</td>
<td>Moderate clinical decrease in mean ambulatory SBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ HDL-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ LDL-C levels</td>
</tr>
<tr>
<td>Zhang et al. (2022)</td>
<td>Patients with mild stroke or transient ischemic attack</td>
<td>Aspirin-cinnamon group (100 mg/day aspirin + 5 g of cinnamon granules) and aspirin-placebo group (100 mg/day aspirin + placebo granules) for 2 months</td>
<td>Aspirin-cinnamon group:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ TG, LDL-C, fasting plasma glucose, Hba1c, Lp-PLA2, and hs-CRP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑ HDL-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓ Carotid atherosclerosis</td>
</tr>
</tbody>
</table>

ACLY, ATP-citrate lyase; Akt, protein kinase B; BW, body weight; CAT, catalase; COX-2, cyclooxygenase type 2; DBP, diastolic blood pressure; FAS, fatty acid synthase; GPX, glutathione peroxidase; GSH/GSSG, glutathione/oxidized glutathione ratio; Hba1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HO-1, heme oxygenase 1; hs-CRP, high-sensitivity C reactive protein; HW/BW, heart-to-body weight; icAM-1, intercellular adhesion molecule 1; iNOS, inducible nitric oxide synthetase; IRS, insulin receptor; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Lp-PLA2, plasma lipoprotein-related phospholipase A2; LXRα, liver X receptor; MCP-1, monocyte chemotactic protein 1; MDA, malondialdehyde; mRNA, messenger RNA; NF-κB, nuclear factor kappa B; NO, nitric oxide; NQO-1, NAD(P)H dehydrogenase [quinone] 1; NRF2, factor erythroid nuclear factor 2 related to factor 2; OGD, oxygen-glucose deprivation; P38, anti-phospho-p38; PCNA, antiproliferating cell nuclear antigen; p-eNOS, phosphorylated endothelial nitric oxide synthase; Ploy1, anti-phospho-phospholipase C gamma 1; PPAR-α, peroxisome proliferator-activated receptor alpha; p-Smad 2/3, phosphorylated Smad2/3; p-SmaD4, phosphorylated Smad4; ROS, reactive oxygen species; SBP, systolic blood pressure; SOD, superoxide dismutase; SREBP-1c, sterol regulatory element-binding proteins; TC, total cholesterol; TG, triglyceride; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; Vcam-1, vascular cell adhesion molecule-1; VLDL, very-low-density lipoprotein.

**Cinnamon: could it be of benefit in chronic kidney disease?**

Although studies evaluating the effect of cinnamon on the kidneys are scarce, the salutogenic effects suggested by the literature (as shown in Fig. 2) suggest an overall benefit [161]. CKD is a significant cause of mortality globally, and its prevalence is growing in low-middle-income countries, where social deprivation amplifies its effects [145]. The reenvisioning of the Hippocratic concept of ‘food as medicine’ champions the use of natural bioactives as potential therapeutics to tackle the emerging diseases of aging [12].
The use of cinnamon is merited for evaluation to be included in the physician’s and nutritionist’s armamentarium. Common pathways underpin the salutogenic effects of cinnamon in CKD, including the inactivation of the ERK/JNK/p38 MAPK pathway leading to reduced renal interstitial fibroblast proliferation and hypertrophy [162]. Nrf2 pathway stimulation, promoting attenuation of renal damage and preservation of renal function, is also a key element in this mechanism [8, 163–165]. Other reported benefits of cinnamon are the inhibition of peroxynitrite-induced nitrination and lipid peroxidation and its influence on the production of NO and PGE2 [166, 167].

Patients with CKD experience premature and accelerated aging [145], and cinnamon may also benefit in mitigating the effects of cellular aging. In support of this, it has been reported that cinnamaldehyde attenuates cellular senescence in the kidney through PI3K/AKT pathway-mediated autophagy via downregulation of microRNA-155 [168].

Cinnamon is a promising candidate in the dietetic management of CKD, as it can mitigate complications such as dyslipidemia and diabetes. Studies have suggested possible improvements in kidney function through dietetic approaches aimed at upregulating antioxidant and anti-inflammatory defenses [12, 169]. However, despite the known properties of cinnamon, its effect on patients with CKD has not been explored, and most studies are experimental (Table 6) [65, 168, 170–173]. This highlights the need for further investigations.

**Toxicity caused by cinnamon**

Contrary to popular belief, herbal medicines are not entirely safe and may have adverse effects. The available data suggest that cinnamon is safe for use as a spice, and mod-
Table 6. Experimental studies involving cinnamon on kidney diseases

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study/sample</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hussain et al. (2019) [170]</td>
<td>Administration of acetaminophen in BALB/c mice</td>
<td>Pretreatment with 200 mg/kg/day i.g. of cinnamon bark aqueous extract for 2 weeks</td>
<td>Prevention against elevation in serum ALT, AST, Cr, urea Prevention against macroscopic and histological alterations in liver and kidney Improvement of oxidative balance ↓ MDA level, SOD and CAT activities in the liver and kidney ↑ GSH and total thiol contents and NO production</td>
</tr>
<tr>
<td>Niazmand et al. (2021) [65]</td>
<td>STZ-induced diabetic rats</td>
<td>100, 200, or 400 mg/kg of cinnamon extract for 6 weeks</td>
<td>Improvement in serum biochemical markers and oxidative parameters: Protected cellular injury in kidney tissue ↓ IL-1β, IL-6, and caspase 3 and 9 ↑ GSH level and ameliorates antioxidative enzymes (SOD, CAT, GR, and GPx in kidney tissue)</td>
</tr>
<tr>
<td>Alshahrani et al. (2021) [171]</td>
<td>Male Wistar rats with nephrotoxicity induced by acetaminophen</td>
<td>50, 100, and 200 mg/kg of cinnamon oil with 2 g/kg of acetaminophen, for 15 days</td>
<td>Prevention of alterations in body weight, serum total proteins, calcium level, kidneys’ relative weight, Cr, urea, and uric acid ↓ MDA, and TNF-α, IL-1β, and IL-6 and nitrates ↑ GSH, SOD, CAT Prevention of histological alterations</td>
</tr>
<tr>
<td>Atsamo et al. (2021) [172]</td>
<td>Male Wistar rats with gentamicin-induced nephrotoxicity</td>
<td>200 and 400 mg/kg/day of Cinnamomum zeylanicum stem bark aqueous extract for 2 weeks concomitantly with gentamicin administration</td>
<td>Prevention of alterations in body weight, serum total proteins, calcium level, kidneys’ relative weight, Cr, urea, and uric acid ↓ IL-10 and Bcl-2 ↓ Blood urea nitrogen and Cr In the kidneys: the contours of the proximal and distal convoluted tubules were improved, ↓ the number of nuclear pyknosis, ↓ hyperemia ↑ Ratio of p-P13K to P13K and the ratio of p-Akt to Akt</td>
</tr>
<tr>
<td>Elshopakey and Elazab (2021) [173]</td>
<td>Broiler chickens with copper-induced nephrotoxicity</td>
<td>200 mg/kg of C. zeylanicum alone or plus probiotic for 6 weeks</td>
<td>Both supplemetations: ↓ Urea, Cr, and uric acid In renal tissue: ↓ MDA ↑ CAT, and GSH, ↓ Copper ↓ TNF-α, IL-2, Bax, and COX-II in kidneys ↑ IL-10 and Bcl-2</td>
</tr>
<tr>
<td>Xiao (2022) [168]</td>
<td>Sprague-Dawley rats (male) kidney senescence model D-galactose-induced</td>
<td>40 mg/kg/day of cinnamaldehyde for 6 weeks</td>
<td>In the kidneys: the contours of the proximal and distal convoluted tubules were improved, ↓ the number of nuclear pyknosis, ↓ hyperemia ↑ Ratio of p-P13K to P13K and the ratio of p-Akt to Akt</td>
</tr>
</tbody>
</table>

Akt, protein kinase B; ALT, alanine transaminase; AST, aspartate transaminase; Bcl-2, B-cell lymphoma 2; CAT, catalase; COX-II, cyclooxygenase; Cr, creatinine; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; IL, interleukin; MDA, malondialdehyde; NO, nitric oxide; p21, p21/WAF1/Cip1; PARP, poly (ADP-ribose) polymerase; PI3K, phosphoinositide 3-kinase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha.

Erste ingestion has several health benefits, as previously reported. However, its use for medicinal purposes in high doses or over a long duration may lead to adverse effects, such as gastrointestinal disturbances and self-limiting allergic reactions that should be clinically monitored [174]. Yun et al. [175] have reported that cinnamon extract (2 g/kg body weight/day for 13 weeks) might result in nephrotoxicity and hepatotoxicity in rats due to high doses of coumarin. In animals, despite all the extracts tested showing possible antioxidant activity in vitro, they showed acute dose-dependent toxicity (1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg body weight) in vivo, with increased levels of aspartate transaminase, alanine transaminase ALP, urea, and creatinine reported in animals treated with the highest dose [57].

In a systematic review of the adverse effects of cinnamon, the authors report that most studies did not identify the cinnamon species responsible for these effects. Knowing that different cinnamon species contain other components, such as coumarin, studies on herbal medicines should be standardized to include their exact identification, dose, and duration of treatment [174]. Recently, Gu et al. [176] evalu-
ated the safety of cinnamon in humans through a study using relevant meta-analyses and systematic reviews of RCTs and concluded that there are no adverse effects caused by cinnamon.

There is no exact recommendation for the daily intake of cinnamon. Still, studies recommend approximately 1 to 4 g per day, and attention should be paid to the amount of coumarin in different types of cinnamon and symptoms such as diarrhea, nausea, and vomiting [161].

**Conclusion**

Cinnamon compounds have several beneficial effects for consideration for inclusion in a ‘food as medicine’ strategy to treat CKD. These reside in inherent antioxidant, anti-inflammatory, cardioprotective, antiobesogenic, and antidiabetic properties. Additionally, they may reside in the ability of cinnamon to influence the composition of the gut and microbiota. Though most reported studies are preclinical, they indicate that human clinical studies are merited. Therefore, different clinical trials need to be planned regarding the dose and period of supplementation, the types of cinnamon species, and other populations. This review highlights the need for further studies on patients with CKD who suffers from several comorbidities, in which the use of cinnamon supplementation has demonstrated potential advantages.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

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**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: LSGM, ISCB, DCMVR, LT, TRC, ME, LFMFC, PS, DM  
Funding acquisition, Methodology: DM  
Supervision: PS, DM, PGS  
Writing–original draft: LSGM, ISCB, DCMVR, LT, TRC, ME, LFMFC, DM  
Writing–review & editing: LSGM, LFMFC, PS, PGS, DM  
All authors read and approved the final manuscript.

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Introduction

Anemia is a major complication of chronic kidney disease (CKD) and impacts patients overall outcomes and quality of life [1]. Impairment of synthesis of erythropoietin from the pericytes in fibrotic kidney primarily contributes to renal anemia [2]. Therefore, the use of recombinant human erythropoietin related products (erythropoiesis-stimulating agents, ESAs) has been the major therapeutic strategy for over three decades [3]. The prevalence rate of anemia rises up to 90.2% in CKD stage 5 [4]. In a CKD registry with 14,415 patients, anemia occurred in 60% of nondial-

Pleiotropic effects of hypoxia-inducible factor-prolyl hydroxylase domain inhibitors: are they clinically relevant?

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Anemia is common in patients with chronic kidney disease (CKD) and is mainly caused by insufficient production of erythropoietin from fibrotic kidney. Because anemia impairs quality of life and overall prognosis, recombinant human erythropoietin-related products (erythropoiesis-stimulating agents, ESAs) have been developed to increase hemoglobin level for decades. However, many safety concerns have been announced regarding the use of ESAs, including an increased occurrence of cardiovascular events, vascular access thrombosis, cancer progression, and recurrence. Hypoxia-inducible factor (HIF) is crucial to erythropoietin production, as a result, prolyl hydroxylase domain (PHD) enzyme inhibitors have been new therapeutic agents for the treatment of anemia in CKD. They can be administered orally, which is a preferred route for patients not undergoing hemodialysis. In clinical trials, PHD inhibitor could induce noninferior effect on erythropoiesis and improve functional iron deficiency compared with ESAs. Although no serious adverse events were reported, safety is still a concern because HIF stabilization induced by PHD inhibitor has pleotropic effects, such as angiogenesis, metabolic change, and cell survival, which might lead to unwanted deleterious effects, including fibrosis, inflammation, cardiovascular risk, and tumor growth. More molecular mechanisms of PHD inhibition and long-term clinical trials are needed to observe these pleotropic effects for the confirmation of safety and efficacy of PHD inhibitors.

Keywords: Anemia, Chronic renal insufficiency, Erythropoietin, Hypoxia-inducible factor
ysis and 93% of dialysis-dependent patients. More ESAs were administered in dialysis-dependent patients than in nondialysis patients (82% vs. 24%) [5]. However, ESA therapy is associated with cardiovascular adverse effects, including hypertension and thromboembolism in some studies, especially when using ESAs to target a hemoglobin level of greater than 11 g/dL [6–8]. Cancer progression or recurrence is also a significant concern with ESA therapy, and the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for anemia in CKD recommended using ESA therapy with caution in those with current or previous malignancy [9].

Anemia results in tissue hypoxia, and hypoxia-inducible factor (HIF) is a crucial transcription factor by activating the hypoxia-responsive genes, including erythropoietin. HIF is a heterodimer that consists of an α and β unit. This heterodimer translocates to the nucleus and binds to DNA sequences named hypoxia response elements. The β unit is present consistently (HIF-β), while there are three isoforms of a subunit (HIF-1α, HIF-2α, and HIF-3α) whose activity is primarily controlled by their degradation rate. In normoxia, HIF-α subunits are hydroxylated by a family of prolyl hydroxylase domain (PHD) enzymes, of which there are three isoforms: PHD1, PHD2, and PHD3. The hydroxylated HIF-α subunits are degraded by the von Hippel-Lindau tumor suppressor (VHL)-mediated ubiquitination [10]. Under hypoxia, the hydroxylation activity of PHDs is suppressed, which results in HIF-α accumulation and combines with HIF-β, leading to increased transcription of hypoxia-responsive genes [11]. Elucidation of these mechanisms led to the development of PHD inhibitors as promising therapeutic agents to stabilize HIF and correct anemia in CKD. Currently, several PHD inhibitors including roxadustat, vadadustat, daprodustat, molidustat, enarodustat, and desidustat have been developed and undergoing clinical trials.

Nevertheless, in addition to erythropoietin, more than 60 direct target genes of HIFs have been identified [12]. Therefore, in addition to erythropoiesis, HIF also plays key roles in angiogenesis, energy metabolism, immune modulation, cell proliferation, and survival (Fig. 1). As a result, pleotropic effects of HIF stabilization may be concerned during the treatment with PHD inhibitor. In this review, we will discuss these pleotropic effects and clinical relevance. Through investigating the molecular mechanisms and clinical evidence of pleotropic effects of PHD inhibition, we hope to avoid deleterious off-target effects and develop therapeutic agents with more safety and efficacy.

**The pleotropic effects of prolyl hydroxylase domain inhibition**

**Iron metabolism**

PHD inhibitors can increase hemoglobin levels by decreasing hepcidin and ferritin levels and decrease transferrin saturation by increasing total iron-binding capacity. Through inhibition of ferroportin (FPN), hepcidin inhibits iron absorption from intestine and impedes the release of iron from liver and reticuloendothelial macrophage. The hepcidin levels are elevated in CKD patients due to decreased renal clearance and upregulated systemic inflammation, which results in the malabsorption and impaired availability of iron, contributing to renal anemia [13]. In an animal study, HIF stabilization in global Vhl knockout mice could suppress the hepcidin gene (Hamp1) messenger RNA levels in the liver via increasing erythropoietic activity and serum growth differentiation factor 15 level. Reduced iron and ferritin levels were noted as well [14]. Another murine model showed that increase of HIF-2α, not HIF-1α was essential for FPN activation in intestinal cells under conditions of low iron [15]. And experiments of intestinal cell line demonstrated that PHD enzymes were down-stream of hepcidin-FPN pathway in the regulation of HIF-2α, and pharmacological inhibition of PHD could stabilize HIF-2α [16].

Clinical study of PHD inhibitor (roxadustat) in nondialysis CKD or dialysis-dependent patients, hepcidin decreased more significantly in roxadustat-treated group than in placebo or ESA group [17,18]. In study of daprodustat and vadadustat in dialysis-dependent patients, the hepcidin levels were also lower in PHD inhibitor group than in darbepeotin alfa group [19,20]. As a result, PHD inhibitors might decrease the use of iron supplement to avoid the risk of anaphylactic reaction and the adverse effects such as increase of oxidative stress or iron overload.

**Metabolic change**

In obese type 2 diabetic mice, the enarodustat-treated group had lower body weight compared with vehicle group.
without the difference in the amount of food intake between groups. Enarodustat treatment also improved insulin sensitivity and glucose tolerance [21]. A similar improvement was reported in another murine model of Hif-p4h-2–hypomorphic mice (partial loss of Phd2 function), whether fed a normal or high-fat diet. These mice had higher HIF levels because of PHD deficiency and higher levels of glucose transporter 2 (GLUT2) in the liver as well as glycolysis enzymes in the skeletal muscle and white adipose tissue (WAT), contributing to the improved glucose tolerance. In addition, decreased size of adipocytes, a reduced number of adipose tissue macrophages, and increased glucose transporter GLUT4 expression were noticed in the Hif-p4h-2–hypomorphic mice, which seemed likely the mechanisms of increased insulin sensitivity. After administration of another PHD inhibitor, FG-4497, to wild-type mice with metabolic dysfunction, these beneficial effects could be reproduced, indicating that this is a plausible class effect of PHD inhibitors [22]. HIF-1α is crucial for islet β cell function. Deletion of HIF-1α in β cells caused glucose intolerance in mice due to impaired first-phase insulin release [23]. The increase of HIF-1α expression during exercise in skeletal muscle was important for glucose metabolism and insulin action by maintaining the translocation of GLUT4 to the cell surface [24]. Additionally, HIF-2α stabilization increased Irs2 and cyclic AMP-specific phosphodiesterase gene expression, leading to upregulated and downregulated insulin and glucagon signaling, respectively. Human and mice hepatocyte-specific deletion of HIF-2α markedly attenuated these effects of PHD inhibitors, showing the effect of PHD inhibition on glucose metabolism was mainly HIF-2α dependent in the liver [25]. However, this beneficial effect on glucose control of diabetic patients has not yet been determined in clinical trials.

Regarding lipid metabolism, the levels of plasma total cholesterol and the mass of WAT were lower, while adi-
Acute kidney injury

HIF is a master regulator of energy metabolism, inflammation, and cell proliferation, contributing to the protective role of acute kidney injury (AKI). Hill et al. [29] showed that either hif1a\(^{-/-}\) and hif2a\(^{-/-}\) mice had higher injury score and worse renal function in the ischemia-reperfusion injury (IRI) model. They also found that PHD inhibitor L-mimosine administered 6 hours before IRI could protect mouse kidneys from injury. HIF-1\(\alpha\) was detected predominantly at the corticomedullary junction as well as distal tubular cells and was obviously increased, while HIF-2\(\alpha\) increased expression mainly in interstitial cells at the corticomedullary junction compared with control group [29]. On the other hand, in a model of ischemia produced by oxygen-glucose deprivation of cultured renal proximal tubule cells, the cell viability was increased and the levels of reactive oxygen species (ROS) were reduced significantly by enarodustat treatment or small interfering RNA (siRNA) knockdown of PHD2, but not of PHD1 or PHD3. These protective effects were mediated by the increase of glycogen storage and the upregulation of genes of glycogen synthesis by HIF-1\(\alpha\) before injury. Oxidative stress could be attenuated because sufficient glycogen produced reduced nicotinamide adenine dinucleotide phosphate that reduced glutathione, and ROS was then scavenged by reduced glutathione. In an IRI model of rat, pretreatment with the PHD inhibitors could upregulate glycogenesis, prolong glycolysis, and delay adenosine triphosphate consumption, contributing to renal protection [30]. For endothelial cell, specific inactivation of endothelial PHD2 attenuated IRI-AKI. Although PHD2 inhibition was not sufficient to induce detectable HIF activity in the kidney endothelium, the therapeutic effect was dependent on HIF-1\(\alpha\) but not HIF-2\(\alpha\) and was generated by suppressing the expression of proinflammatory genes and recruitment of neutrophils and macrophage [31]. Moreover, hyper-methylation of pericytes in the kidney was noted after AKI and was the major cause of ensuing CKD [32], whether PHD inhibitors influence methylation after AKI should also be investigated in the future. Taken together, PHD inhibitors have the potential to prevent AKI clinically. AKI frequency and severity could be monitored in long-term PHD inhibitor users. Clinical trials of the preventive effects by PHD inhibition for AKI could be performed in patients with operation (e.g., cardiac surgery associated-AKI).

Renal fibrosis and chronic kidney disease progression

PHD inhibition effect on renal fibrosis is still controversial and possibly cell-dependent. Early study demonstrated that mice in a 5/6 nephrectomy model with VHL deletion in tubular epithelial cells could stabilize HIF-1\(\alpha\) but contribute to renal fibrosis. The data showed the increase of fibroblasts number and collagen production in VHL-deleted mice, and also had higher albuminuria and profibrotic gene expression, including transforming growth factor (TGF)-\(\beta\)1, plasminogen activator inhibitor-1, and connective tissue growth factor [33]. In cultured renal medullary interstitial cells, angiotensin II remarkably increased HIF-1\(\alpha\) levels, which induced the accumulation in collagen I/III and tissue inhibitors of metalloproteinases (TIMP)-1 protein levels. HIF-1\(\alpha\) siRNA rather than HIF-2\(\alpha\) siRNA completely blocked the effects of angiotensin II on collagen I/III and TIMP-1 production. HIF-1\(\alpha\) siRNA also attenuated angiotensin II-induced elevation of proliferating cell nuclear antigen and vimentin, a marker of cell proliferation and cell transdifferentiation, respectively. In addition, it was reported that overexpression of PHD2 gene attenuated...
angiotensin II-induced profibrotic action and silencing of PHD2 gene enhanced angiotensin II-induced profibrotic action [34].

Nevertheless, recent studies showed neutral results of PHD inhibition on renal fibrosis. The study deleted PHD1, 2, 3 in renal erythropoietin-producing cells and found no difference of collagen production compared to control group [35]. There was also no significant increase of profibrotic gene and most inflammatory gene expression in mice with PHD deficiency compared to control group in the kidneys on day 7 and day 14 after unilateral ureteral obstruction surgery [35]. Furthermore, the concern for the impact of HIF in pericytes on kidney fibrosis arises from the fact that pericytes are the major precursor cells of myofibroblasts in fibrotic kidney. Previous study reported that Gli1 is one of the markers of pericyte and the ablation of Gli1+-cell attenuated renal fibrosis [36]. Hence, we induced Gli1+-specific HIF stabilization via Vhl or Phd2 knockout showed increased serum erythropoietin and polycythemia without a significant difference in profibrotic gene expression and kidney fibrosis [37]. However, because there are many other markers of fibroblasts/pericytes such as platelet-derived growth factor (PDGF) receptor β, CD73, smooth muscle myosin protein, and tenasin-C which manifest as different lineages, we need further study to clarify the effect of HIF activation in pericytes from other lineages in various models (ischemia-reperfusion, adenine-induced CKD, aristolochic acid nephropathy, and diabetic nephropathy) and clinical trials to observe the effect of PHD inhibitors on renal fibrosis [38].

For CKD progression, a diabetic mice model showed that enarodustat-treated mice also exhibited reduced albuminuria and amelioration of glomerular epithelial and endothelial damage compared with the vehicle group. In vitro experiments demonstrated that reduced C-C motif chemokine ligand 2/monocyte chemoattractant protein-1 (CCL2/MCP-1) expression in mesangial cells was contributed by both local HIF-1α activation and restoration of adiponectin [21]. However, clinical reports of renal outcome in patients using PHD inhibitors were rare, only a 3-year follow-up study of patients with daprodustat treatment showed no impact on CKD progression [39].

**Immunity and inflammation**

For innate immunity, HIF-1α and HIF-2α have been shown to express both in macrophages and neutrophils. In hypoxia, HIF-1α is essential for neutrophil function and survival [40]. Thompson et al. [41] found that the neutrophils from the blood and lungs of mice or zebrafish with acute lung injury showed early expression of HIF-1α. In contrast, HIF-2α was expressed later and maintained during the resolution phase of acute lung injury. Through the study of patient cells and in vivo in a zebrafish model, HIF-2α gain-of-function mutations resulted in a reduction in neutrophil apoptosis and promoted the persistence of neutrophilic during chronic inflammation [41]. Moreover, Sadiku et al. [42] identified PHD2 as the dominant HIF-hydroxylase in neutrophils. Myeloid-specific genetic deletion of Phd2 or pharmacological inhibition of PHD2 activity led to robust neutrophilic inflammation in response to Streptococcus pneumonia and in a lipopolysaccharide (LPS)-induced acute lung injury model. This exaggerated inflammatory response was due to increased glycolytic flux and glycogen stores in neutrophil, which enhanced neutrophil functional capacity, motility, and survival [42]. Unlike PHD2, loss of PHD3 in neutrophils was associated with reduced inflammation because of the unique role for PHD3 in prolonging neutrophil survival during hypoxia [43].

Regarding macrophage, HIF-a isoforms crucially regulate macrophage function via metabolic change. HIF-1α was induced by M1 signals such as interferon gamma (IFN-γ) and LPS, whereas M2 signals such as interleukin (IL)-4 and IL-13 favored HIF-2α expression. HIF-1α mediated a metabolic switch from oxidative phosphorylation (OxPhos) to glycolysis and was crucial for macrophage phagocytosis and inducible nitric oxide synthase (NOS) generation. Conversely, HIF-2α enhanced the M2 gene arginase 1 expression and polarized an M2 macrophage phenotype [44]. In contrast to the findings in neutrophilic inflammation, loss of PHD3 resulted in increased mortality in a murine model of LPS-induced peritonitis, where inflammation was predominantly induced by macrophage [45]. Enarodustat reduced expression of CCL2/MCP-1 and macrophage infiltration in diabetic mice [21].

For adaptive immunity, HIF-1α activation favored T helper 17 cells (Th17) development over regulatory T cell (Treg) differentiation via enhancing glycolytic activity, upregula-
tion of the transcription factor retinoic acid-related orphan receptor gamma t, and degradation of the Treg transcription factor FOXP3 [46,47]. And mammalian target of rapamycin (mTOR) signaling was essential to induce HIF-1α expression in Th17 cells [46]. On the other hand, a recent study investigating the role of microRNAs in Tregs also identified that Treg cell development could be suppressed by both HIF-1α and HIF-2α, demonstrating similar roles of the HIF protein in Treg differentiation [48]. Furthermore, PHD3 was found to be upregulated in Tregs and required for the development of Tregs. The PHD3 inhibitors di-methyl oxalylglycine or siRNAs, which upregulated HIF-1α, could down-regulate Foxp3 expression, and enhanced the development of Th17 cells [49]. Similar to CD4 T cell, CD8 T cell differentiation and activation required upregulation of HIF-1α and metabolic adaptations, including a switch from OxPhos to glycolysis. HIF-1α enhanced viral and tumor cell clearance, but was also associated with excessive inflammation and mortality in chronic viral infections [50,51]. Finlay et al. [52] also showed that mTOR complex 1 (mTORC1)-mediated expression of HIF-1α was required not only to sustain glycolysis in CD8 T cells but also to generate inflammatory mediators such as cytolytic effector molecules, including perforin, granzymes, and IFN-γ.

Regarding B cell population, it was found that HIF-1α was stabilized following B cell receptor activation, and that B cell-specific deletion of Hif resulted in fewer IL-10-producing B cell population and exacerbated collagen-induced arthritis and experimental autoimmune encephalomyelitis. And HIF-1α-dependent glycolysis facilitated CD11bhi-CD5 B cells expansion [53]. However, sustained hypoxia or HIF-1α stabilization by B cell-specific Vhl deletion inhibited mTORC1 activity in germinall center B cell, reduced antigen-specific germinal center B cells, undermined class-switching, and reduced generation of early memory B cells [54].

Overall, these data on immune cells highlight that, even within a single cell population, targeting HIFs or PHDs can have highly complex and variable outcomes of infection, inflammation, and resolution. The final outcome of HIF activation in an inflammatory response depends on various factors, including which cell and tissue type are affected, which HIF or PHD isoform is targeted, and other environmental factors such as the cytokine profile, metabolite availability, tissue-specific factors, and duration and severity of hypoxia [55].

In clinical trials, upper respiratory infection, pneumonia, and urinary tract infection were more common in roxadustat than in control group [18,56], which might be due to immune modulation by PHD inhibition. Herein, we should continue monitoring the incidence of infection rate under the use of PHD inhibitors, and discuss the discontinuation of inhibitors if patients are undergoing any infection.

**Cancer development and progression**

Because most solid cancers are in hypoxia environment and HIF expression is high, cancer development and progression are the major concern in PHD inhibitor users. HIF-1α acts as a versatile regulator that is involved in crucial aspects of cancer biology, including angiogenesis, glucose metabolism, cell survival, resistance to cell death, and tumor metastasis. In most cases, HIF-1α overexpression is associated with treatment failure and increased mortality, and inhibitors of HIF-1α have been studied as anticancer therapeutics [12].

HIF-1α induced metabolic reprogramming in tumors with shifting OxPhos to aerobic glycolysis (also called the Warburg effect) for their energy production [57,58]. Additionally, HIF-1α could inhibit acetyl-CoA dehydrogenases and fatty acid β-oxidation, which was beneficial for cancer proliferation via enhanced Glut2 expression and glycolysis, reduced ROS production, and activation of pro-survival cancer signaling pathway [59]. These metabolic reprogramming effects provide sufficient energy for tumor growth and metastasis even in hypoxia microenvironment. Of note, HIF-1α also upregulates proangiogenic genes encoding NOS, vascular endothelial cell growth factor (VEGF), and PDGF-B to augment vascular density and decreases O2 diffusion distances [60]. In addition, HIF-1α facilitated cancer cell proliferation by activating numerous growth factors and pathways such as insulin-like growth factor-2 [61], TGF-α [62], phosphoinositide 3-kinases, and mitogen-activated protein kinase pathways [63]. HIF-1α deterred apoptosis by activating the expression of the inhibitor of apoptosis protein 2 and inducing G1/S arrest through the upregulation of protein phosphatase 2A [64,65]. Nevertheless, HIF-1α also mediated hypoxia-related apoptosis by increasing the expression of Bcl-2 binding proteins 3 thereby inhibiting the antiapoptotic effect of Bcl-2 [66], or...
by stabilizing wild-type p53 [67,68]. The function of HIF-1α in the regulation of apoptosis by hypoxia is complex and should be further investigated. Furthermore, a recent study showed that long noncoding RNA (lncRNA) DANCR with the NF90/NF45 complex could stabilize HIF-1α, leading to increased cancer proliferation and metastasis in nasopharyngeal carcinoma [69]. Another lncRNA, MTA2 transcriptional regulator RNA, functioned as a promoter and enhanced the proliferation and metastasis of pancreatic cancer cells by enhancing the stability of HIF-1α protein via metastasis-associated protein 2-induced deacetylation [70]. On the other hand, clear cell renal cell carcinoma (ccRCC) has been reported to develop in approximately 70% of patients with VHL disease [71]. HIF-2α has been implicated tumorigenesis in VHL-reconstituted ccRCC [72], and HIF-2α inhibitor has been tested in phase 2 clinical trials for ccRCC in VHL disease [73].

Current PHD inhibitors have different impact on PHD isoforms, contributing to variable effect on HIF stabilization. These inhibitors have been tested in various cancer models, resulting in positive and negative outcomes. For example, roxadustat inhibited all PHD isoforms and reduced tumor growth of macrophage-abundant tumors via normalization of tumor vessels [74,75]. Sensitivity to chemotherapy was repaired by roxadustat as well in a murine tumor model [76]. Vadadustat had a major impact on PHD3 and PHD2, resulting in higher upregulation of HIF-2α than HIF-1α without increase of plasma VEGF in patients [77]. Daprodustat influenced PHD1 more than other isoforms. High dose of daprodustat did not pose carcinogenic risk in vivo [78]. The results from the ASCEND-ND trial demonstrated that daprodustat for the treatment of anemia in patients with CKD not receiving dialysis had higher risk of cancer-related death or tumor progression or recurrence compared to the group of darbepoetin alfa [39]. However, another post-hoc modified intention-to-treat analysis of pooled data from the ASCEND-D and ASCEND-ND trials showed that daprodustat was not associated with an increased risk of cancer, or cancer mortality, relative to ESA [79]. Molidustat inhibited PHD2 more than other isoforms and inhibited breast cancer cell survival, self-renewal capacity, and enhanced the efficacy of chemotherapeutic gemcitabine [80]. Overall, HIF-1α stabilization is required for tumor growth and metastasis. Nevertheless, aforementioned findings indicate that under certain circumstances, HIF-1α stabilization may exert protective effects and even decrease tumor cell aggressiveness. Different cancer showed distinct PHD isoform expression, and the impact of the PHDs on tumor progression is diverse and cell-dependent. These studies highlight that longer follow-up data period and more patients with dialysis or nondialysis are desirable to evaluate the impact of PHD inhibitors on cancer development and progression compared to that of ESAs. Furthermore, head-to-head comparison of different PHD inhibitors should be performed in the future not only for the investigation of efficiency of erythropoiesis but also for the impact on cancers.

Cardiovascular risk and atherosclerosis

According to previous studies, HIF-1α stabilization could reduce lipogenesis [22], which might ameliorate atherosclerosis. However, the pathogenesis of atherosclerosis includes the dysfunction of inflammatory signaling pathway as well [81]. Some studies displayed that HIF-1α activation contributed to atherosclerosis. One finding was that the upregulation of nuclear factor kappa B, VEGF, and ROS by HIF-1α led to endothelial cell dysfunction contributing to the progression of atherosclerosis [82]. Another mechanism was that HIF-1α regulated macrophages and promoted inflammation and migration of macrophages. HIF-1α also promoted the formation of foam cells by reducing the ABCA1 cholesterol efflux protein [82]. This along with smooth muscle migration and proliferation contributed to plaque growth and atherosclerosis [83]. Furthermore, pulmonary arterial hypertension (PAH) may contribute to cardiovascular risk as well. It had been found that endothelial HIF-2α was required for the increase of the expression of vasoconstrictor molecule endothelin-1 and a concomitant decrease in vasodilatory apelin receptor signaling, contributing to the development of increased pulmonary artery pressures [84]. PHD2 plays a crucial role in the mechanisms of severe PAH. PHD2-deficient endothelial cells were demonstrated to promote smooth muscle cell proliferation in part through HIF-2α-induced C-X-C motif chemokine 12 expression [85].

Regarding cardiovascular risk in clinical trials, roxadustat demonstrated a similar cardiovascular safety profile and overall mortality compared with ESAs in a pooled analysis of cardiovascular safety of dialysis-dependent CKD [86].
In subgroup analysis, patients who received roxadustat compared with ESA in the incident dialysis subgroup had a lower risk of all-cause mortality as well as time to the first cardiovascular event than those in the stable dialysis subgroup [86]. In another roxadustat study in nondialysis CKD patients, there was no difference in cardiovascular risk between roxadustat and placebo group [56]. A recent study of daprodustat in nondialysis patients also showed no difference in the major adverse cardiovascular event (MACE), thromboembolic event, hospitalization for heart failure, death from any cause between daprodustat and darbepoetin alfa group [39]. Moreover, a meta-analysis from nine randomized controlled trials in a total of 8,022 enrolled patients revealed that there was no increase in cardiovascular risk of daprodustat treatment in patients undergoing dialysis or not [87]. There was also no difference in MACEs between molidustat and darbepoetin treatment groups [88]. More importantly, although there was no increase in cardiovascular risk of vadadustat treatment in patients with incident or maintenance dialysis of the INNOVATE trial [89], vadadustat treatment in patients with non-dialysis-dependent CKD of the PROTECT trial resulted in significant higher hazard ratio of MACE, which failed to prove cardiovascular safety of vadadustat [90]. In addition to cardiovascular adverse effect, thromboembolism is also a concern of PHD inhibitors. Pilli et al. [91] showed that stabilization of HIF-1α in the liver was associated with reduced protein S (PS) expression and a lower plasma PS level, suggesting increased likelihood of thrombosis. Animal model study showed that hypoxia contributed to intimal hyperplasia formation in arterial prosthetic grafts [92,93]. Another animal model study also demonstrated that HIF-1α expression was associated with the degree of intimal hyperplasia in the venous grafts possibly by inducing the switch of adventitial fibroblasts to myofibroblasts and migration into the neointima [94]. In PYRENEES study of roxadustat in dialysis patients, the percentage of arteriovenous fistula thrombosis event in roxadustat group was higher than ESA group (12.1% vs. 7.4%) [95]. Hence, the U.S. Food and Drug Administration expressed the concern about these potential adverse effects of PHD inhibitors in 2021 [96].

### Summary and perspective

The studies described above highlight various pleotropic effects of HIF stabilization by PHD inhibitors. Different PHD inhibitors might have variable impact on each PHD isoform (PHD1, 2, and 3), resulting in different levels of HIF-1α or HIF-2α and subsequent pleotropic effects. Some pleotropic effects are beneficial but other unwanted effects should be avoided. Current evidence is summarized in Table 1. More efforts should be performed to elucidate what are the alternative hydroxylation targets of PHD inhibitors and whether isoform-specificity of PHD inhibitors can be improved for specific targeting rather than pan-suppression of PHD activity. The timing and dosing of PHD inhibition should also be tested to improve the specificity and side-effect profiles.

Clinically, we should evaluate the trade-off between erythropoietic effects and avoidance of adverse effects. Therefore, larger number of patients and longer observation period are required to evaluate pleotropic effects. In nondialysis patients, the effect of PHD inhibitors on CKD progression could be evaluated. If no significant MACEs are noticed in the future study, higher target hemoglobin

**Table 1. Current evidence of pleotropic effects by PHD inhibition**

<table>
<thead>
<tr>
<th>Pleotropic effect</th>
<th>Current evidence of PHD inhibition effect</th>
<th>Study model</th>
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<tbody>
<tr>
<td>Iron metabolism</td>
<td>Improve</td>
<td>In vitro, in vivo, clinical trial</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td>Improve</td>
<td>In vitro, in vivo</td>
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<tr>
<td>Lipid metabolism</td>
<td>Improve</td>
<td>In vitro, in vivo, clinical trial</td>
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<tr>
<td>Acute kidney injury</td>
<td>Protective</td>
<td>In vitro, in vivo</td>
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<td>Renal fibrosis &amp; CKD progression</td>
<td>Controversial</td>
<td>In vitro, in vivo</td>
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<tr>
<td>Inflammation</td>
<td>Induce</td>
<td>In vitro, in vivo</td>
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<tr>
<td>Cancer development and progression</td>
<td>Controversial</td>
<td>In vitro, in vivo, clinical trial</td>
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<tr>
<td>Cardiovascular risk and atherosclerosis</td>
<td>Controversial</td>
<td>In vitro, in vivo, clinical trial</td>
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CKD, chronic kidney disease; PHD, prolyl hydroxylase domain.
level could be tested (near normal, >12 g/dL) for potential better quality of life and outcomes.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: YHC, SLL
Investigation: YHC
Supervision: SLL
Validation: SYP
Writing—original draft: YHC
Writing—review & editing: all authors
All authors read and approved the final manuscript.

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Uremic pruritus: pathophysiology, clinical presentation, and treatments

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Uremic pruritus is one of the most common and bothersome symptoms in patients with end-stage renal disease. Most patients with uremic pruritus experience a prolonged and relapsing course and significant impairments of quality of life. The pathophysiology of uremic pruritus is not completely understood. A complex interplay among cutaneous biology and the nervous and immune systems has been implicated, with the involvement of various inflammatory mediators, neurotransmitters, and opioids. Uremic pruritus treatment outcomes are often unsatisfactory. Clinical trials have mostly been small in scale and have reported inconsistent results. Recent evidence shows that gabapentinoids, nalfurafine, and difelikefalin are effective for relieving uremic pruritus in hemodialysis patients. This review provides an overview of the epidemiology and proposed mechanisms of uremic pruritus, then highlights the manifestations of and clinical approach to uremic pruritus. Current evidence regarding treatment options, including topical treatments, treatment of underlying disease, phototherapy, and systemic treatments, is also outlined. With a better understanding of uremic pruritus, more therapeutic options can be expected in the near future.

Keywords: Gabapentin, Chronic kidney failure, Pregabalin, Pruritus, Chronic renal insufficiency

Introduction

Uremic pruritus is one of the most common and distressing comorbid diseases in patients with end-stage renal disease (ESRD) and also occurs in patients with chronic kidney disease (CKD). Uremic pruritus significantly affects multiple aspects of quality of life, including mood, sleep, and social relationships, and is often refractory to treatment [1,2]. Moreover, in ESRD patients, a higher intensity of pruritus is associated with worse patient survival and more technique failures of peritoneal dialysis (PD) [3–5].

In this review, we summarized the current knowledge...
regarding the epidemiology, pathophysiology, clinical presentation, clinical approach, and treatment of uremic pruritus. Due to the various definitions of uremic pruritus used in the literature, we defined uremic pruritus as symptoms of chronic itch secondary to declining renal function. Articles reporting studies on pruritus secondary to ESRD or CKD were reviewed. For the pathophysiology and treatment of other pruritic diseases, we refer readers to other review articles [6–8].

**Epidemiology**

The prevalence of uremic pruritus varies by country, dialysis modality, dialysis unit, and study population. Uremic pruritus affects 25% to 62% of patients receiving PD [9,10] and 38% to 84% of patients receiving hemodialysis (HD) [1,11,12]. In an international survey conducted from 1996 to 2015, the prevalence of bothersome uremic pruritus in HD patients gradually declined from 28% to 18% [11]. However, comparisons between HD patients and PD patients with regard to the prevalence and severity of uremic pruritus remain inconsistent [13,14]. In a multinational cross-sectional study of stage 3–5 CKD patients, up to 24% of participants experienced moderate to extreme pruritus [15]. Severe uremic pruritus is rare among pediatric dialysis patients, but the reason for this remains unclear. A study of 199 children on dialysis reported that only 9.1% had pruritus, and the intensity of pruritus was also mild [16].

**Pathophysiology**

The pathophysiology of uremic pruritus has not been fully elucidated. Along the itch-sensory pathway, the proposed origins of itch have been classified as follows: 1) pruritoceptive: itch induced by pruritogens in the skin, e.g., allergic contact dermatitis; 2) neuropathic: itch resulting from pathology in the afferent conduction pathway of the peripheral and central nervous system, e.g., itch related to multiple sclerosis; 3) neurogenic: itch originating in the nervous system without neural damage, e.g., opioid-induced pruritus; 4) psychogenic: itch owing to psychiatric and psychosomatic causes without organic problems, e.g., parasitophobia [17,18]. The mechanism of uremic pruritus may involve complex interactions of more than one proposed origin (Fig. 1).

Skin moisture is lower in dialysis patients, and dry skin is very common in patients with uremic pruritus [19,20]. Dialysis patients with uremic pruritus showed lower levels of stratum corneum hydration than nonpruritic patients [20], while some studies did not find an association between pruritus and skin hydration or transepidermal water loss [19,21]. Whether there are more skin mast cells in patients

![Figure 1. The pathophysiology of uremic pruritus.](https://www.krcp-ksn.org)
with uremic pruritus remains unclear. Some studies have reported that the number of dermal mast cells in HD patients is significantly higher than that in healthy controls [22,23], while another report showed no relationship between the extent of pruritus, the number of skin mast cells, or the level of plasma histamine in dialysis patients [24]. Divalent ions, calcium-phosphate products, hyperparathyroidism, and uremic neuropathy have also been implicated in uremic pruritus [5,13,20,25,26]. The results of our previous study and those of others identified dialysis adequacy as an independent predictor of pruritus intensity in HD patients, which suggested that the clearance of pruritogenic substances could influence the severity of pruritus [27–29].

Immune dysregulation plays a critical role in the pathophysiology of uremic pruritus. Compared with nonpruritic patients, those with uremic pruritus show higher levels of C-reactive protein [4,30] and various inflammatory mediators, including histamine, interleukin (IL)-2, and IL-6 [30,31]. Animal studies reported that IL-31 induced severe pruritus and dermatitis in transgenic mice [32], and serum levels of IL-31 were positively associated with the intensity of uremic pruritus in HD patients [33]. In addition, patients with uremic pruritus were found to have an increased proportion of T-helper 1 cells [30] and altered monocyte subsets [34]. The relationship between the immune system and the itch-sensory pathway is thus an interesting field for further study.

Morphine has been reported to trigger itching, which suggests that the opioid system is involved in the mechanism of uremic pruritus [35]. There are three major types of opioid receptors: μ, κ, and δ. Itch is observed after the activation of μ-opioid receptors following systemic or neuraxial opioid administration [36], while κ-opioid receptor agonists exert antipruritic effects [37]. Although the effects of opioid receptor agonists/antagonists are mainly activated through the central nervous system [35], opioid receptors are also present on peripheral nerve fibers and various skin cells, such as keratinocytes, melanocytes, and hair follicles [38]. Expression of κ-opioid receptor was lower in the skin of patients with uremic pruritus [39], indicating a significant role of the peripheral opioid system in uremic pruritus. In addition, a peripherally restricted, selective κ-opioid receptor agonist showed a significant antipruritic effect in a recent trial on HD patients [40].

### Clinical presentation

Patients suffering from uremic pruritus often experience itch daily or nearly daily [1]. Pruritus can involve all areas of the body, affecting more than 25% of the body surface area in more than half of patients with uremic pruritus [2,34]. The course is fluctuating and prolonged, usually lasting for more than one year [1,41]. Patients with uremic pruritus often have pruritus in the absence of a primary cutaneous eruption. However, the vicious cycle of itch and scratching behaviors may lead to secondary skin changes, including excoriations, prurigo nodularis, lichen simplex, or nonspecific eczema [16].

### Clinical approach

The first step to managing itch in patients with reduced kidney function is accurate diagnosis. In addition to uremic pruritus, various pruritic skin diseases, such as scabies, atopic dermatitis, and drug allergies, can occur in dialysis and CKD patients. A detailed medical history and skin examination are crucial to correct diagnosis [18]. Other causes in addition to uremic pruritus should be considered if an itchy skin condition occurred before the onset of kidney disease. If pruritus is confined to localized areas or is exacerbated in a short period, exposures or aggravating factors should be evaluated. A careful review of the patient’s medication history may exclude drug-related itch or drug-related hypersensitivity reactions. If skin examination reveals primary skin eruptions, such as wheals, morbilliform eruptions, or bullae, other dermatological diseases should be included in differential diagnosis. A skin biopsy is usually not necessary for diagnosis of uremic pruritus. Laboratory and imaging studies can be considered for patients with manifestations suggesting other causes of itchy skin like hyperthyroidism or cutaneous T cell lymphoma.

### Treatments

Uremic pruritus is frequently refractory to multiple treatments. However, many studies on the treatment of uremic pruritus in recent years have shed light on this intractable disease (Table 1, 2 [40, 42–75]).
Moisturizer
A high percentage of patients with uremic pruritus have dry skin [20]. Maintaining adequate skin hydration is the cornerstone of antipruritic treatment. In a noncontrolled study, 16 of 21 dialysis patients with uremic pruritus reported a reduction in the severity of pruritus after 1 week of regular emollient use [20].

Steroids
Approximately 10% of physicians prescribe topical steroids as a first-line treatment for uremic pruritus in HD patients [11], but no trials have assessed their efficacy. As microinflammation plays an important role in the pathogenesis of uremic pruritus, topical steroids may provide antipruritic effects against uremic pruritus, especially for skin areas with secondary scratch-induced eczema or obvious inflammation. However, as uremic pruritus usually involves a large percentage of the body surface area, the use of potent topical steroids on large skin areas may cause systemic absorption and adverse cutaneous effects, including skin atrophy and folliculitis. Topical steroids should be prescribed with caution, and patients should be educated on how to use them properly.

Capsaicin
Capsaicin, the active compound in chili peppers, depletes neuropeptide substance P from sensory nerve terminals in the skin and blocks the conduction of pain and pruritus [76]. Topical capsaicin has been used to relieve itch, especially neuropathic itch conditions, such as postherpetic itch, brachioradial pruritus, and notalgia paraesthetica [76]. Two double-blind, crossover randomized controlled trials (RCTs) of HD patients showed that capsaicin 0.025% cream was significantly more effective for alleviating uremic pruritus than placebo [42, 43]. Local burning, stinging, and erythema at the site of application are common side effects.

Calcineurin inhibitors
Topical calcineurin inhibitors, including tacrolimus and pimecrolimus, selectively inhibit calcineurin and thus prevent the transcription of IL-2 and other cytokines in T lymphocytes [77]. Topical calcineurin inhibitors have been used in inflammatory skin disorders [78]. In a noncontrolled study of 25 dialysis patients, Kuypers et al. [77] showed that tacrolimus ointment significantly reduced the severity of uremic pruritus after 6 weeks. However, in a 4-week double-blind RCT of 22 HD patients, Duque et al. [44] demonstrated that 0.1% tacrolimus ointment was not more effective than placebo for relieving uremic pruritus. In another 8-week double-blind RCT of 60 dialysis patients, Ghorbani et al. [45] showed no significant antipruritic benefit of topical pimecrolimus 1% compared with placebo.

Pramoxine
Pramoxine is a topical local anesthetic with a potential antipruritic effect that interferes with the transmission of impulses along sensory nerve fibers [79]. In a double-blind
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<td>Tarng et al., 1996 [42]</td>
<td>Crossover</td>
<td>Taiwan</td>
<td>HD</td>
<td>19</td>
<td>4</td>
<td>Capsaicin 0.025% cream 4-Point scale vs. placebo</td>
<td>82.4% of participants experienced relief of pruritus after receiving capsaicin cream; capsaicin cream was more effective in improving itching score than placebo (p &lt; 0.001).</td>
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<td>Cho et al., 1997 [43]</td>
<td>Crossover</td>
<td>Taiwan</td>
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<td>Capsaicin 0.025% cream 4-Point scale vs. placebo</td>
<td>86.4% of participants experienced relief of pruritus after receiving capsaicin cream; 22.7% experienced relief of pruritus after placebo treatment (p &lt; 0.001).</td>
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<td>Calcineurin inhibitors</td>
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<td>Duque et al., 2005 [44]</td>
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<td>0.1% Tacrolimus ointment vs. placebo VAS</td>
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<td>Young et al., 2009 [46]</td>
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<td>4</td>
<td>1% Pramoxine lotion vs. placebo VAS</td>
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<td>2.2% Gamma-linolenic acid cream vs. placebo VAS, QPS</td>
<td>More reduction of VAS in the gamma-linolenic acid group (–4.5) than the placebo group (–0.5) (p &lt; 0.01).</td>
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<td><strong>Treatment of underlying disease</strong></td>
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<td>High-flux HD vs. low-flux HD VAS</td>
<td>More reduction of VAS in the high-flux group (–3.99) than the low-flux group (–0.71).</td>
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<td>Lim et al., 2020 [50]</td>
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<td>MCO group had lower scores for morning pruritus distribution and frequency of scratching during sleep at week 12. Between-group differences for VAS were not significant.</td>
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<td>Phototherapy</td>
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<td>Broadband UVB vs. UVA 4-Point scale</td>
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<td>Narrowband UVB vs. UVA VAS</td>
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<td>Crossover</td>
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<td>25</td>
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<td>Gabapentin vs. placebo VAS</td>
<td>More reduction of VAS in the gabapentin group (–6.7) than the placebo group (–0.8) (p &lt; 0.001).</td>
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<td>Gabapentin vs. placebo VAS</td>
<td>More reduction of VAS in the gabapentin group (–6.7) than the placebo group (–1.5) (p &lt; 0.001).</td>
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<td>Gabapentin vs. pregabalin</td>
<td>VAS</td>
<td>Change in VAS was not different between the gabapentin (–4.41) and the pregabalin groups (–4.43) (p = 0.844).</td>
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<td>Yue et al., 2015 [57]</td>
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<td>Pregabalin vs. ondansetron vs. placebo</td>
<td>VAS, QPS</td>
<td>More reduction of VAS in the pregabalin group (–6.6) than the ondansetron (–2.5) or placebo (–2.0) groups (p &lt; 0.05).</td>
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<td>Gabapentin vs. placebo</td>
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<td>More reduction of VAS in the gabapentin group (–5.82) than the placebo group (–0.1) (p &lt; 0.001).</td>
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<td>Foroutan et al., 2017 [59]</td>
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<td>Pregabalin vs. doxepin</td>
<td>VAS</td>
<td>More reduction of VAS in the pregabalin group (–5.4) than the doxepin group (–2.9) (p &lt; 0.001).</td>
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<td>Compared with the placebo group (–1.1%), there was a higher percentage of VAS reduction in both the gabapentin (–4) and the deschlorpheniramine groups (–4) (p &lt; 0.7).</td>
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<td>Rossi et al., 2019 [61]</td>
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<td>Gabapentin vs. doxepin</td>
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<td>Change in VAS was not different between the gabapentin (–4.41) and the pregabalin groups (–4.43) (p = 0.844).</td>
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<td>Naltrexone vs. placebo</td>
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<td>More reduction of VAS in the naltrexone group (–8.3) than the placebo group (–1.1).</td>
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<td>Pauli-Magnus et al., 2000 [64]</td>
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<td>Naltrexone vs. placebo</td>
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<td>Percentage of reduction in VAS was not different between the naltrexone (29.2%) and placebo groups (16.9%) (p = 0.095).</td>
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<td>Nalfurafine vs. placebo</td>
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<td>Change in VAS was not different between the nalfurafine (–2.5) and placebo groups (–1.27) (p = 0.0649).</td>
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<td>A greater reduction of VAS in the cromolyn sodium group (–7.78) than the placebo group (–2.90) (p &lt; 0.001).</td>
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<td>Mahmudpour et al., 2017 [70]</td>
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<td>Montelukast vs. placebo</td>
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<td>A greater reduction of VAS in the montelukast group (–2.73) than the placebo group (–5.47) (p &lt; 0.001).</td>
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RCT of 28 HD patients, Young et al. [46] reported that a lotion containing 1% pramoxine was more effective than the control lotion for reducing the intensity of uremic pruritus.

**Gamma-linolenic acid**

Gamma-linolenic acid is an essential fatty acid found in some plant seed oils that provides possible relief of pruritus through local anti-inflammatory or immunoregulatory effects [47]. In a double-blind, crossover RCT of 17 dialysis patients, Chen et al. [47] showed that cream containing 2.2% gamma-linolenic acid was more effective than control cream for alleviating uremic pruritus.

**Cannabinoids**

Cannabinoids are chemical compounds derived from cannabis and have therapeutic potential in several diseases, including chronic pruritus [80]. In a noncontrolled study of 23 HD patients, a topical cream containing endocannabinoids (N-acetylcarnosamine and N-palmitoylethanolamine) completely eliminated pruritus in 38.1% of patients and significantly reduced xerosis after 3 weeks of treatment [81].

**Treatment of underlying disease**

**Optimization of dialysis dosage and modality**

Optimizing dialysis dosage and increasing the clearance of middle molecules could remove more pruritogenic substances and decrease the severity of pruritus; however, there is no standard dialysis target or dialysis modality for pruritus symptoms. In an interventional study of 22 HD patients with uremic pruritus, Hiroshige et al. [82] reported that 78% of patients had a significant reduction in the severity of pruritus after increasing Kt/V (the assessment of the dialysis dose) from 1.08 to 1.19, while only 8% of patients who remained on the same dialysis dose had reduced pruritus severity. In our 5-year cohort study of 111 HD patients, we found that a target of Kt/V ≥ 1.5, which was slightly above the standard of ≥1.4, reduced the intensity of uremic pruritus [27]. In another 2-year cohort study of 85 PD patients, we found that a weekly total Kt/V of ≥1.88, which was higher than the standard of ≥1.7, was associated with a lower intensity of uremic pruritus [3].

In a double-blind RCT of 116 HD patients with a similar Kt/V, patients who used a high-flux dialyzer showed more
reduction of pruritus intensity than those who used a low-flux dialyzer [48]. In another 12-week RCT of 51 ESRD patients with chronic pruritus, high-flux HD showed better efficacy in the treatment of pruritus than hemodialfiltration [49]. Additionally, a 12-week RCT of 50 HD patients, those who used a medium cut-off dialyzer showed a greater reduction in morning pruritus distribution and sleep disturbance than those who used a high-flux dialyzer, but differences in pruritus intensity assessed by visual analog scale scores were not significant between groups [50].

Control of hyperparathyroidism
In a case series of 37 dialysis patients with uremic pruritus and hyperparathyroidism, Chou et al. [83] found significantly reduced pruritus intensity 1 week after parathyroidectomy. In a 36-week open-label RCT of 82 HD patients with hyperparathyroidism, El-Shafey et al. [51] reported better alleviation of pruritus intensity in patients who received cinacalcet, a calcimimetic-targeting calcium-sensing receptor on parathyroid cells, compared with those who received conventional therapy with vitamin D and phosphate binders. Currently, parathyroidectomy or cinacalcet should only be considered based on the severity of hyperparathyroidism rather than as a standard treatment for uremic pruritus.

Kidney transplantation
Successful kidney transplantation should be able to cure uremic pruritus, as a functioning graft kidney alleviates uremic status [84]. However, a considerable number of kidney transplant recipients with good graft function still experience chronic pruritus [84]. In a cohort study of 74 kidney transplant recipients with a functional graft, the prevalence of chronic itch after transplantation (12%) was lower than that before transplantation (35%) [85]. The etiology of chronic pruritus in patients after kidney transplantation remains uncertain, and proposed mechanisms include drug-related skin manifestations, new-onset itchy dermatoses, persistent hyperparathyroidism, or decreased graft function [84,85].

Phototherapy
Ultraviolet (UV) phototherapy is effective for various skin diseases and is more tolerated than many systemic treatments. In a 4-week RCT of HD patients with intractable pruritus, broadband UVB phototherapy showed better antipruritic effects than UVA phototherapy [52]. In a single-blind RCT of patients with refractory uremic pruritus, narrowband UVB phototherapy showed a marginal effect at reducing pruritus intensity [53]. UVB phototherapy may cause xerosis, erythema, changes in pigmentation, and skin aging [86]. Despite concerns about photocarcinogenesis, UVB phototherapy has not been reported to increase the risk of nonmelanoma skin cancer and cutaneous melanoma in patients with uremic pruritus [87].

Systemic treatments

Gabapentinoids
Gabapentinoids, including gabapentin and pregabalin, bind to voltage-dependent calcium channels to decrease neurotransmitter release and are used for the treatment of postherpetic neuralgia, neuropathic pain, and fibromyalgia [88]. In a meta-analysis of five RCTs with 297 HD patients, there was a significant benefit in favor of gabapentinoids compared with placebo for reducing the degree of uremic pruritus [89]. In addition, a meta-analysis of five RCTs with 220 HD patients showed a better reduction in pruritus intensity in gabapentinoid users than in antihistamine users [89]. In a single-blind RCT of 90 HD patients, pregabalin was found to be more effective for reducing the severity of uremic pruritus than doxepin [59]. In a crossover RCT of 50 HD patients, gabapentin and pregabalin showed similar antipruritic effects [56]. Somnolence and dizziness are common adverse effects of gabapentinoids, and dosage adjustment in patients with impaired renal function is necessary [89].

Opioid antagonists and agonists
Central μ-opioid receptors participate in the processing of itching sensation, and the activation of central κ-opioid receptors antagonizes the μ-opioid receptor-mediated process of itch development [35]. Thus, μ-opioid receptor antagonists and κ-opioid receptor agonists have been used in the treatment of pruritic skin diseases, such as prurigo nodularis, cholestatic pruritus, and uremic pruritus [90,91].

Double-blind RCTs on the antipruritic effect of naltrexone, a μ-opioid receptor antagonist, showed conflicting results in dialysis patients [63,64]. In a crossover RCT of 15
HD patientsconstipated, dizzy, and vomiting are common side effects of using oral activated charcoal [94]. An early double-blind RCT of 10 HD patients, Silverberg et al. [72] demonstrated that nalfurafine improved considerably in four of five patients using 5-g cholestyramine twice daily compared with improvement in only one of five patients in the placebo group.

**Cholestyramine**

Cholestyramine is a nonabsorbable resin used for the treatment of hyperlipidemia and pruritus in patients with chronic liver disease and biliary obstruction [72]. In an early double-blind RCT of 10 HD patients, Silverberg et al. [72] demonstrated that nalfurafine improved considerably in four of five patients using 5-g cholestyramine twice daily compared with improvement in only one of five patients in the placebo group.

**Biologics**

Serum IL-31 is positively associated with itching and may play a critical role in uremic pruritus [33]. Nemolizumab, an anti-IL-31 receptor A antibody, has been shown to reduce pruritus intensity in patients with atopic dermatitis [73]. However, a phase II double-blind RCT comparing nemolizumab with placebo did not show a significant difference in pruritus intensity among HD patients with uremic pruritus [73]. Dupilumab, a human monoclonal antibody that blocks IL-4 and IL-13, has been approved for the treatment of moderate-to-severe atopic dermatitis [98,99]. In a case report and a case series, dupilumab significantly reduced uremic pruritus in CKD and dialysis patients [99,100].

**Thalidomide**

Thalidomide has been shown to have sedative, immunomodulatory, and antiangiogenic properties [101]. In a dou-
ble-blind crossover RCT of 29 HD patients with refractory uremic pruritus, Silva et al. [74] showed the antipruritic efficacy of thalidomide, as 55.6% of thalidomide users had reduced pruritus intensity compared with 13.3% of placebo users. However, the benefits and risks should be carefully assessed before initiating thalidomide therapy due to its potential side effects, including teratogenicity, peripheral neuropathy, constipation, and sedation [101].

**Sertraline**

Sertraline, a selective serotonin reuptake inhibitor, is used for the treatment of major depressive disorder, panic disorder, obsessive-compulsive disorder, and posttraumatic stress disorder [102]. In a retrospective cohort study of 17 patients with pruritus related to later stages of CKD, patients had reduced pruritus severity after using sertraline for a mean duration of 5.1 weeks [103]. In a noncontrolled study of 19 HD patients with uremic pruritus, the prevalence of severe pruritus decreased from 52.6% to 10.5% after treatment with 50-mg oral sertraline daily for 4 months [104]. In a double-blind RCT comparing sertraline with placebo in HD patients with uremic pruritus, both groups showed a reduction in pruritus intensity [75]. Common adverse reactions of sertraline include nausea, tremor, and somnolence [102].

**Conclusions**

Correct assessment and diagnosis, optimization of metabolic profiles and dialysis regimens, proper skincare and protection, selection of appropriate topical and oral medications, and monitoring of the side effects of drugs are all important in the management of uremic pruritus. Recent evidence shows that gabapentinoids, nalfurafine, and difelikefalin are effective for relieving uremic pruritus in HD patients. Topical steroids, topical capsaicin, phototherapy, antihistamines, mast cell stabilizers, leukotriene receptor antagonists, activated charcoal, and optimization of dialysis dose and modality may also be therapeutic options, although further trial results are necessary. With a better understanding of the pathophysiology of pruritus and updated clinical trials, more treatment options for uremic pruritus can be expected.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: All authors
Writing—original draft: All authors
Writing—review & editing: All authors
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Introduction

Currently, over two million people around the world suffer from end-stage renal disease (ESRD). The best treatment for current patients with ESRD is a kidney transplant or dialysis when a donor organ is not available. The growing gap between patients who require a kidney transplant and the availability of donor organs as well as the negative effects of long-term dialysis, such as infection, limited mobility, and risk of cancer development, drive the impetus to develop alternative renal replacement technology. The goal of this review is to assess the potential of two of the most recent innovations in kidney transplant technology—the implantable bioartificial kidney (BAK) and kidney regeneration technology—in addressing the aforementioned problems related to kidney replacement for patients with ESRD. Both innovations are fully implantable, autologous, personalized with patient cells, and can replace all aspects of kidney function. Not only do these new innovations have the potential to improve the possibility of transplantation for more patients, they also have potential to improve the outcome of transplantation or dialysis-related renal cancer diagnosis. A major limitation of the current technology is that both implantable BAK and kidney regeneration technology are still in preclinical stages, and thus their potential effects cannot be comprehensively generalized to human patients.

Keywords: Chronic kidney failure, Dialysis, Kidney regeneration, Kidney technology, Kidney transplant

Recent innovations in renal replacement technology and potential applications to transplantation and dialysis patients: a review of current methods

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The current standard of care for patients with end-stage renal disease (ERSD) is a kidney transplant or dialysis when a donor organ is not available. The growing gap between patients who require a kidney transplant and the availability of donor organs as well as the negative effects of long-term dialysis, such as infection, limited mobility, and risk of cancer development, drive the impetus to develop alternative renal replacement technology. The goal of this review is to assess the potential of two of the most recent innovations in kidney transplant technology—the implantable bioartificial kidney (BAK) and kidney regeneration technology—in addressing the aforementioned problems related to kidney replacement for patients with ERSD. Both innovations are fully implantable, autologous, personalized with patient cells, and can replace all aspects of kidney function. Not only do these new innovations have the potential to improve the possibility of transplantation for more patients, they also have potential to improve the outcome of transplantation or dialysis-related renal cancer diagnosis. A major limitation of the current technology is that both implantable BAK and kidney regeneration technology are still in preclinical stages, and thus their potential effects cannot be comprehensively generalized to human patients.
to the immunosuppressant drugs required after transplant surgery [3].

The field of kidney replacement technology has evolved greatly over the last two decades, with improvements in nanotechnology, cell growth techniques, and bioreactors. Two of the most recent technological advancements in this field are the implantable bioartificial kidney (BAK) and kidney regeneration technology. Both techniques are in preclinical stages and aim to fully replace normal kidney functionality. Both technologies address donor organ shortages as well as complications from dialysis and immunosuppressants. The purpose of this review is to analyze recent progress in kidney replacement technology and assess its potential impact on reducing risks associated with dialysis and donor organ kidney transplants, specifically donor shortages, renal failure, and risk of cancer.

**History of renal replacement technology**

Historically, kidney failure following ESRD is best treated by a full kidney transplant with a donor organ. Given the extremely limited availability of donor organs, most patients with a failing kidney end up on dialysis, either HD or PD. The problem with dialysis is that it is suboptimal in terms of morbidity and mortality. While dialysis accounts for kidney filtration function of small solute clearance, it does not make up for the loss of metabolic, endocrine, and reclamation functions of the kidney, resulting in poor outcomes. Innovations in renal replacement technology have been a growing focus over the last two decades, aiming to create a product that will replace full kidney functionality, not just the filtration aspect. Two less recent models of renal replacement technology are the automated wearable artificial kidney (AWAK) and the wearable artificial kidney (WAK) (Fig. 1-3) [4].

The AWAK is a tidal PD-based artificial kidney that uses dialysate regeneration to reduce fluid requirements. It consists of tubing, a disposable storage module, and a system controller compacted into a device the size of an average handbag. Dialysate (the reserve volume) is instilled into the peritoneal cavity and absorbs toxins, waste products, and fluid through the peritoneal membrane. The reserve volume of the regenerated dialysate is returned to the peritoneal cavity, and the remaining fluid (ultrafiltrate) is drained into an ultrafiltration bag and can be discarded. The procedure can be repeated after replacing the used cartridge with a new one. This device has been approved for trials in humans [4].

The WAK is a portable blood-based renal replacement device that is battery operated and can be worn like a belt or vest. The blood flowing through this system is anticoagulated with heparin using a syringe pump and then moves on to a two-channel pump that alternately propels blood and dialysate to a small dialyzer. Next, blood exits the dialyzer and travels through a bubble detector before being returned to the patient. The dialysate is regenerated for further use. An ultrafiltration pump controls fluid removal by portioning off a part of the regenerated dialysate to a waste bag for removal. This device is U.S. Food and Drug Administration (FDA)-approved for clinical trials [4].

The major limitation in both current devices is that they still do achieve full kidney functionality; they are focused on ultrafiltration and remain lifestyle limiting, albeit less so than traditional dialysis. They require external machinery and systems, which limit mobility for the patient using it. Additionally, the lack of endocrine and metabolic functionality causes poorer outcomes for dialysis patients over time compared with patients who received a full transplant.

The two newest iterations in renal replacement technology, the implantable BAK and kidney regeneration technology, address the limitations of the AWAK and WAK devices...
in that both aim to provide full kidney functionality as well as improved patient mobility and autonomy.

### Implantable bioartificial kidney

#### State of technology

The implantable BAK will provide another alternative for ESRD patients. This device not only reduces time on dialysis, but also replaces total kidney functionality. The filtration component of kidney function occurs at the glomerulus. Ultrafiltration of the blood is performed to remove toxic waste from circulation and retain important materials within systemic circulation, such as albumin. The regulatory component of the kidney occurs at tubular segments attached to the glomerulus. Ultrafiltrate from the glomerulus...
moves along the kidney tubule, which reabsorbs fluid and solutes to finely regulate the excretion of various amounts of solutes and water in urine. Both of these functionalities are necessary in a fully functional kidney unit. The implantable BAK combines a high efficiency filter connected in series with a bioreactor of cultured renal tubule epithelial cells to achieve classification as a fully functioning kidney unit (Fig. 4, 5) [5,6].

The implantable BAK achieves solute transfer with convective transport, which is independent of concentration gradient and instead depends on a hydraulic pressure gradient across a membrane. This method of transport for toxin removal is advantageous because it mimics the natural glomerular process of toxin clearance of solutes with a higher molecular weight and solutes of the same diffusion rate. Convective transport in an implantable device can be achieved with polysulfone hollow fibers, which can be lined with renal endothelial cells and placed into the arteriovenous circuit using the common iliac artery and vein [6]. This arteriovenous connection allows the device to operate on blood pressure rather than an externally- or battery-powered pump.

Designing the technology

The first step toward a fully implantable BAK is to perfect the combination of hemofilter and bioreactor devices in an extracorporeal setting. In 2002, a working prototype of the BAK was created with the development of an extracorporeal device that consisted of a hemofiltration cartridge containing over 109 renal tubule cells grown as monolayers along the inner surface of the fibers. These hollow fibers act as scaffolds for the renal tubule cells because they are non-biodegradable and have an optimal pore size for an immunoprotective barrier. In vitro renal tubule assist device (RAD) studies showed that the cells retained differentiated active transport properties, differentiated metabolic activities, and important endocrine processes. The studies also showed that the device replaces endocrine, filtration, transport, and metabolic kidney functions when connected in series with a blood filtration device [7].

In 2004, Phase I and Phase II clinical trials were approved by the FDA for assessing the response of 10 patients with acute renal failure and multiple organ failure to a BAK device. A synthetic hemofilter was connected in series with a bioreactor cartridge that contained around 109 human proximal tubule cells, acting as a RAD, within an extracorporeal perfusion circuit utilizing standard hemofiltration pump systems. The results showed 6 of 10 patients surviving past 30 days [8].

The kidney was the first solid organ whose partial func-
Limitations and challenges

A major limitation in creating the implantable BAK is the miniaturization aspect. An avenue that has been explored to create the device prototype is microelectromechanical systems (MEMS) (Fig. 6). MEMS is an industrial toolkit that applies mature manufacturing techniques from the semiconductor industry to miniature electromechanical devices, such as pumps, valves, and sensors. This technology can be used to produce silicon membranes containing ‘slit-shaped’ pores that are necessary for producing an implantable BAK [10]. Another engineering challenge for the implantable BAK is to design it such that the membrane maximizes water permeability while minimizing leakage of albumin and other important macromolecules. This challenge is overcome using silicon nanotechnology slit pores. A challenge in using silicon is the oxide coating that can form when exposed to oxygen. The coating can be prevented by modifying the silicone surface with a highly hydrated polymer by grafting an organic polymer to the silicon nanopore surface [10,11]. A long-term challenge for the implantable device is combating coagulation; a sustainable anticoagulation solution will be essential for a fully implantable BAK device. Further limitations of the implantable device are the size and pump requirements of modern dialyzers, and the water volume required for lytic therapy [11].

Novel renal replacement technological components: BioCartridge and HemoCartridge

A newly pioneered collaboration among multiple academic institutions is working on creating the first fully functioning, FDA-approved implantable BAK. The device contains both a “HemoCartridge” and “BioCartridge,” where the HemoCartridge is a high efficiency filter that utilizes the silicon slit-pore nanotechnology and the BioCartridge is a bioreactor containing cultured renal tubule epithelial cells. Ultrafiltrate is generated in the HemoCartridge component and then flows to the BioCartridge component where it is processed to return salt, water, and glucose to the blood and filter toxins into a small volume of fluid comparable to urine. The implantable BAK is connected directly to the patient’s vasculature and does not require an electrical pump due to connection to the circulatory system.

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pressure pumps the blood through the device starting at the HemoCartridge via the membranes that imitate the slit-shaped pores of podocytes. Then, the blood flows through the BioCartridge that contains living tubular cells to imitate the glomerulus functionality of the kidney [5].

The implantable BAK device removes the need for dialysate since the reabsorption of salt and water in the BioCartridge maintains approximately neutral fluid balance with removal of concentrated waste. The silicon membranes in this device are coated with hydrated biocompatible polymers that protect blood from stagnation and excess shear, allowing anticoagulant-free clinical implantation [5]. After achieving proof of concept with the HemoCartridge and BioCartridge device, this project is currently in the preclinical testing stage. A pilot study is being conducted to test the two components together once they are miniaturized. The next phase of development is clinical trials with human subjects.

Successful implementation of this device into clinical practice could drastically reduce the length of time a potential kidney transplant patient spends on dialysis due to the ability of the implantable BAK to supplement the small number of donor organs available each year. Additionally, since the BioCartridge component of the implantable BAK contains the patient’s own cell line, it is more biologically compatible. Ideally, this device would lessen the need for the long-term immunosuppression that typically follows transplantation procedures.

**Kidney regeneration technology**

Another recent development in bioengineering kidney technology is cellular regeneration. Progress in stem cell and developmental biology has realized the vision of creating a transplantable kidney graft composed of a patient’s own cells. Directed differentiation allows control of stem cell development through key milestones to create the building blocks for autologous kidney regeneration. Natural kidney development and regeneration need to be continuously studied to further understand the tissue regeneration process [12].

The main method currently used in kidney generation involves scaffolding [12,13]. An ECM scaffold is used for three-dimensional structural support for vasculature and
specific cell types of the organ. Creating scaffolds similar in complexity, structure, and size to human organs is a persistent challenge. Detergent-based perfusion decellularization of mammalian organs, which utilizes a carefully formulated solution to distill an organ down to the ECM scaffold, has been successfully applied to multiple organ systems, including the kidney \[12,13\]. However, kidney scaffolds of human origin require more thorough examination of composition, growth factor levels, and mechanical properties of native developing and adult kidneys. Further research will provide a better understanding of the components preserved versus lost during the decellularization process, and the results will provide a roadmap for building kidney scaffolds with optimal accommodation factors for the cell types necessary for organ functionality (Fig. 7) \[13\].

Success in decellularizing rat kidneys was achieved with detergent perfusion to create whole organ scaffolds with perfusable vascular, glomerular, and tubular compartments that can facilitate whole organ functionality. Rat kidneys decellularized using the perfusion method were shown to preserve structure and composition of the ECM, which is essential for filtration, secretion, and reabsorption \[14\]. The decellularized scaffolds were repopulated with endothelial and epithelial cells grown from the rat’s baseline stem cells to create functional kidney grafts. Then, the bioengineered kidneys were transplanted in orthotopic position, and urine production was found to be normal \[13,14\].

Kidney regeneration technology is currently in the preclinical trial stage. Overall, cadaveric kidneys can be decellularized to structural scaffolds, recellularized with endothelial and epithelial cells, matured in a bioreactor to a functional kidney, and transplanted in orthotopic position to provide normal kidney functions \textit{in vivo}. Progression of the technology will necessitate scaling of the cell seeding processes to larger human organ scaffolds. Perfusion decellularization techniques also have potential benefits in the three-dimensional (3D) bioprinting of kidney cells. In order to artificially 3D bioprint the cells necessary for a functional organ, a specific “bioink” is needed to create a particular setting that reinforces cellular growth and proliferation. A recent study demonstrated the viability of these decellularized ECM scaffolds from porcine kidneys to generate a hydrogel for the bioink of 3D-printed kidneys. This ECM-derived bioink improved cell growth and proliferation and even achieved organizational features of innate renal tissue \[15\]. Successful implementation of this technology in clinical practice will likely eliminate the need for immunosuppressant prescriptions for kidney transplant patients since the kidneys are grown from the patient’s own tissue. This technology will also likely decrease the amount of time ESRD patients remain on dialysis by supplementing the small pool of available donor organs each year.

**Potential application to kidney cancer**

As mentioned before, both implantable BAK and kidney regeneration technology have the potential to address donor organ shortages, the need for immunosuppression, and the shortcomings of previously designed renal replacement devices, such as mobility and full kidney functionality. Another issue they can address is the risk of cancer presentation, specifically renal cell carcinoma (RCC), in long-term dialysis patients and/or kidney transplant recipients.

In a population-based study of the United States’ kidney transplants, over 100,000 kidney transplant recipients were observed. The risk of RCC in these kidney transplant recipients was 5.7-fold higher than that of the general population. The risk of papillary RCC was much higher than for clear cell RCC. The overall RCC risk was highest in recipients who had dealt with long-term dialysis before their transplant procedure. The vast majority (89%) of RCC occurrences in these cases occurred in the patient’s native

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**Figure 7. Simplified process of kidney regeneration using decellularization, recellularization, and bioreactor maturation.**

3D, three-dimensional.
kidney [9]. Additionally, a bimodal distribution of RCC onset after transplantation was observed, as a high risk for cancer was recorded in the time period immediately following transplant and another high-risk spike approximately 30 months after procedure. Following the second spike, the risk for RCC remains gradually increasing over time [9].

It is hypothesized that some RCC tumors appear in the native kidney as a resulting complication of ACKD. The complication of ACKD that is associated with RCC development in the native kidney is renal cysts that transform into malignant cancers [9]. According to the National Institute of Diabetes and Digestive and Kidney Diseases, 20% of patients who begin dialysis already have ACKD, between 60% and 80% of patients on dialysis for at least 4 years develop ACKD, and 90% of patients on dialysis for at least 8 years develop ACKD [9]. Since most kidney transplant patients are on dialysis for extended periods of time before their transplant procedures due to the lack of donor organs, these patients subsequently have a higher probability of RCC associated with the renal cyst complication from ACKD.

Additionally, post-procedure transplant patients are put on a regimen of immunosuppressants, which are thought to be linked to higher susceptibility to cancer because these drugs decrease the ability of a patient’s immune system to detect cancer cells or fight against infections that may cause cancer [3,9,16]. Moreover, a study of 7,217 kidney transplant patients confirmed the hypothesis that kidney transplant recipients have higher risk of de novo cancers, with non-Hodgkin lymphoma, lung, kidney, and prostate cancers as the most common types. That study associated the increased risk of cancer post-transplant with immunosuppressive drugs prescribed after the transplant procedure [17].

Both previously mentioned kidney technologies have the potential to address the higher frequency of kidney cancer in transplant patients. The implantable BAK and kidney regeneration technique are theorized to reduce the need for immunosuppressants since these devices would be seeded from a patient’s own tissue, lessening the overall immune response to the devices [4,18,19]. Additionally, both technologies would inherently increase the amount of donor organs available, decreasing the time patients would spend on dialysis. An increase in transplantation accessibility in a timelier manner could potentially reduce the occurrence of ACKD in these patients since ACKD is associated with dialysis duration. A reduction in ACKD instances would subsequently reduce the instances of known ACKD-associated complications, one of which is kidney cancer (Table 1).

Advancements in implantable BAK device and kidney regeneration technology could drastically improve the lives of hundreds of thousands of people on dialysis each year and save the lives of thousands who die each year waiting for a kidney. These technologies also have potential applications to the risk factor of kidney cancer that is associated with patients who receive a transplant.

### Machine learning/artificial intelligence

The application of artificial intelligence (AI) and machine learning (ML) to medicine has gained traction in recent years. FDA has approved the use of AI in monitoring for atrial fibrillation, coronary calcium scoring, and diagnosis of CT brain bleeds [20]. While renal regeneration and BAK are promising novel technologies, the advent of AI and ML has shown beneficial results with HD and kidney transplantation [20]. Concepts such as artificial neural networks (ANN), internet of things, and “deep” learning technology use extensive data that can predict and execute “personalized” medical decisions for each patient. These algorithms have been shown to better predict changes in HD, such as

<table>
<thead>
<tr>
<th>Variable</th>
<th>AKAK</th>
<th>WAK</th>
<th>IAK</th>
<th>Kidney regeneration</th>
</tr>
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<td>Increases no. of alternatives to transplant</td>
<td>V</td>
<td>V</td>
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<td>Patient mobility</td>
<td>V</td>
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<tr>
<td>Replacement of total kidney functionality</td>
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<td>X</td>
<td>V</td>
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<td>Impact on renal cancer</td>
<td>X</td>
<td>X</td>
<td>Potential to reduce risk</td>
<td>Potential to reduce risk</td>
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<td>Stage of testing of the technology</td>
<td>Clinical trials</td>
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<td>Preclinical testing</td>
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AWAK, automated wearable artificial kidney; WAK, wearable artificial kidney; IAK, implantable artificial kidney.
hypotension and heart rate variability/volumes, than nephrologists [20]. These technologies can be programmed to interactively adapt and react to such complications that occur in HD in real time. This data-driven technology would be beneficial for patients to reduce additional unnecessary medications and the cost of any corrective interventions. Regarding kidney transplantation, ANN were able to better predict the probability of chronic renal allograft rejection, which can enable more precise allotment of organ transplants [20].

The implementation of AI/ML in medicine is still years away as there is a paucity of studies proving its benefit in real-world patients. Additionally, issues with patient privacy and data security persist and must be dealt with before the use of such algorithms [20]. As BAK and cellular kidney regeneration are at the forefront in kidney replacement technology, application of data-driven AI and ML concepts to former technologies such as dialysis or kidney transplantation may provide an alternative route for patients who are comfortable with remaining on their dialysis routine or are steadfast in waiting for a kidney transplant.

**Conclusion**

The two newest innovations in renal replacement technology—implantable BAK and cellular kidney regeneration—create a fully functioning alternative to long-term dialysis or a donor organ. They improve on previous iterations of renal replacement technology by accomplishing all aspects of normal kidney functionality, while also being fully implantable and autologous to allow patients maximum mobility. Additionally, these technologies may have the potential to address many associated risks of dialysis and kidney transplants, such as potential infections, effects of immunosuppression, and the risk of cancer - specifically renal cancer. As these technologies move out of preclinical testing stages into clinical testing and eventual clinical practice, they must be further studied to analyze their impact on instances of renal cancer in kidney transplant patients.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: CLD, EBS, KEO
Data curation: CLD, EBS
Formal analysis: CLD
Investigation: KM
Methodology: CLD, KEO, TGK, KM
Project administration: KEO, TGK, KB
Resources, Supervision: KB
Writing–original draft: CLD, EBS, KEO, TGK
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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**References**

Background: The spectrum of biopsy-confirmed kidney disease varies with regions and periods. We describe the distribution of pathological types and epidemiological characteristics of kidney diseases in Northwest China due to regional differences in geographical environment, social economy, and dietary habits.

Methods: Kidney biopsy cases from 2005 to 2020 in Xijing Hospital were retrospectively analyzed. Pathological characteristics of patients in different periods were analyzed using the t test or chi-square test. Joinpoint regression was used to analyze trends in pathological types and disease spectrum.

Results: A total of 10,528 eligible patients were included. Primary glomerular disease (PGD) accounted for the majority of the cases and exhibited an obvious downward trend, whereas secondary glomerular disease (SGD) showed an obvious upward trend. Among PGD, immunoglobulin A nephropathy (IgAN) remained the most common pathological type, and the detection rate of membranous nephropathy (MN) was significantly increased. Among SGD, Henoch-Schönlein purpura nephritis (HSPN) was the most common pathological type and may present a significant characteristic of Northwest China. Diabetic nephropathy (DN) exhibited the most obvious upward trend in the whole process, whereas the fastest growth since 2012 was in hypertensive nephropathy.

Conclusion: The proportion of SGD increased whereas PGD declined. IgAN remained the most common PGD, and HSPN was the most common SGD. MN and DN showed the most obvious upward trend among PGD and SGD, respectively. Changes in the spectrum of kidney disease, especially the constituent ratio of SGD, pose a great challenge to public health.

Keywords: China, Biopsy, Epidemiology, Kidney disease, Pathology
Introduction

In 2020, the Global Burden of Disease organization indicated that the global burden of kidney disease was increasing year by year [1]. At present, there are over 100 million people with chronic kidney disease (CKD) in China, and the percentage of hospitalized patients with the disease has increased from 3.58% (2010) to 4.95% (2017) [2]. To delay the progression to end-stage kidney disease (ESKD), the underlying etiology and pathology need to be clarified at the initial diagnosis.

The epidemiological study of nephropathy is helpful for early diagnosis and treatment in addition to understanding the epidemic trend of the disease. However, the differences in geographical environment, social economy, and dietary habits in different regions or countries contribute to the variation in the spectrum of kidney disease [3]. Intriguingly, the kidney disease spectrum varied in the same region even during a short period [4]. For example, in countries in East Asia, the detection rate of immunoglobulin A nephropathy (IgAN) and mesangial proliferative glomerulonephritis (MsPGN) can exceed 50% and the prevalence of IgAN and MsPGN has decreased in recent decades [5–7]. In China, the spectrum of kidney disease has changed due to the increasing incidence of hypertension, obesity, diabetes, and the increasing average age of the population [8]. In the United States, the frequency of diabetic kidney disease has increased dramatically over the past 30 years, whereas focal segmental glomerulosclerosis (FSGS) has declined over the past decade [9]. Therefore, it is of great significance to understand the updated epidemiological characteristics of kidney disease in a certain area and a fixed period to guide policy decisions on prevention in public health and therapeutic strategies in clinical practice [2].

Northwest China, located in the interior of China, is characterized by a vast area, drought and water shortages, extensive deserts, sandstorms, and a fragile ecology [10]. Due to the change in people’s economic conditions and lifestyles in recent years, we hypothesize that the population of Northwest China has a distinct spectrum of kidney diseases. However, contemporary large-scale epidemiological studies of kidney disease from Northwest China are lacking. Thus, we retrospectively analyzed a kidney biopsy cohort from the largest clinical center in Northwest China to explore the specific epidemiological characteristics of the kidney disease spectrum.

Methods

Study objective

Pathological and clinical data of 10,879 inpatients who underwent a kidney biopsy in Xijing Hospital (Air Force Military Medical University, Xi’an, China) from December 2005 to December 2020 were screened. Inclusion criteria were as follows: (1) patients who underwent a percutaneous kidney biopsy with ultrasound localization; (2) light microscopy, immunofluorescence, and electron microscopy were performed; (3) undiagnosed renal amyloidosis that required an abdominal fat pad biopsy and Congo red staining; and (4) in patients who had multiple biopsies, only the first result was included. Exclusion criteria were: (1) absence of original pathology report; (2) kidney transplant donors and patients; and (3) unsatisfactory biopsy specimens (the number of glomeruli in biopsy specimens was less than eight).

Data collection

Clinical and pathological data were collected by two researchers (Qin and Zhao). Pathological diagnosis was determined by consultation between the pathologist and clinician. Demographic data, diagnostic information, and laboratory data at the time of kidney biopsy were retrieved from electronic medical record information systems. This retrospective study was approved by the ethics committee of Xijing Hospital (No. KY20213027-1) and performed in accordance with the Declaration of Helsinki. Because of the retrospective design of the study, the need to obtain informed consent from eligible patients was waived by the ethics committee. We strictly protected the privacy of subject information during and after data collection.

Classification of clinicopathological diagnosis

According to the 1982 World Health Organization (WHO) histological classification of glomerular diseases [11] and the 1995 revision for glomerular diseases, renal histopathological diagnosis was classified as follows: (1) primary glomerular disease (PGD): IgAN, FSGS, MsPGN, mem-
branoproliferative glomerulonephritis (MPGN), membranous nephropathy (MN), minimal change disease (MCD), crescentic glomerulonephritis (CreGN), endocapillary proliferative glomerulonephritis (EnPGN), IgM nephropathy (IgMN), sclerosing glomerulonephritis (SCGN), and unclassified; (2) secondary glomerular disease (SGD): Henoch-Schönlein purpura nephritis (HSPN), lupus nephritis (LN), diabetic nephropathy (DN), hypertensive nephropathy (HTN), hepatitis B associated nephropathy (HBVN), systemic vasculitis-associated renal damage (SVARD), proliferative glomerulonephritis with monoclonal immunoglobulin deposition (PGNMID), obesity-associated glomerulopathy (OAG), etc.; (3) hereditary nephropathy (HN): thin basement membrane nephropathy, Alport syndrome, Fabry disease etc.; (4) tubulointerstitial nephritis (TIN); and (5) other nephropathies were defined as unqualified morphological changes for WHO classification. Patients with two or more types of pathological diagnoses were reanalyzed by clinical nephrologists and pathologists in our center to determine the most important pathologic lesions.

The clinical diagnoses for kidney biopsy were categorized into six groups: (1) asymptomatic urinary abnormalities (AUA); (2) chronic nephritic syndrome (CNS); (3) nephrotic syndrome (NS); (4) acute kidney injury (AKI); (5) rapidly progressive glomerulonephritis (RPGN); and (6) others. These biopsy indicators have remained roughly the same over the past two decades. However, with the progression of technology, the relative contraindications of biopsy age and blood pressure requirements for elderly patients have been relaxed.

Statistical analysis

All patients enrolled were grouped according to a 5-year interval (period 1: 2005–2010, period 2: 2011–2015, and period 3: 2016–2020) and divided into five age groups for stratified analysis: ≤14, 15–30, 31–45, 46–60, and >60 years of age. The proportion of each pathological type in the total number was converted into a constituent ratio: (number of observed units of a certain component/total number of observed units of each component of the same object) × 100%. The Joinpoint Regression Program (JPR) statistical software version 4.9.0.0 (Statistical Research and Applications Branch, National Cancer Institute, USA) established the time component ratio sequence data using multiple joinpoint models (https://surveillance.cancer.gov/help/joinpoint) and was used for the regression analysis of the trend of the constituent ratio. The annual percentage change (APC) and average APC (AAPC) within the complete time interval of each rate were estimated using the optimal joinpoint model. IBM SPSS version 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as means ± standard deviation, and intergroup comparisons were performed by the chi-square test. A statistically significant difference was set at p < 0.05.

Results

A total of 10,528 patients with clinicopathological data were included (Table 1). In summary, the proportions of various pathological subgroups were as follows: PGD (71.1%), SGD (26.1%), TIN (2.1%), HN (0.6%), and others (0.1%). The leading cause of PGD was IgAN (30.1%), followed by MN (20.1%), MsPGN (8.0%), FSGS (4.6%), MCD (3.7%), IgMN (1.6%), and SCGN (1.1%). The leading cause of SGD was HSPN (7.8%), followed by LN (6.4%), DN (3.8%), HTN (2.3%), HBVN (1.6%), renal amyloidosis (1.5%), and SVARD (1.2%) (Supplementary Table 1, available online).

### Table 1. Baseline characteristics of kidney biopsy patients (n = 10,528)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>6,004 (57.0)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>38.73 ± 16.53</td>
</tr>
<tr>
<td>No. of glomeruli</td>
<td>22.19 ± 10.34</td>
</tr>
<tr>
<td>Asymptomatic urinary abnormalities</td>
<td>1,486 (14.1)</td>
</tr>
<tr>
<td>Chronic nephritic syndrome</td>
<td>5,429 (51.6)</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>3,157 (30.0)</td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>405 (3.8)</td>
</tr>
<tr>
<td>Rapidly progressive glomerulonephritis</td>
<td>29 (0.3)</td>
</tr>
<tr>
<td>Isolated microscopic hematuria</td>
<td>879 (8.3)</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>1,850 (17.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3,147 (29.9)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>3,963 (37.6)</td>
</tr>
<tr>
<td>Renal anemia</td>
<td>1,066 (10.1)</td>
</tr>
</tbody>
</table>

All data are presented as values at the time of kidney biopsy. Data are expressed as number (%) and mean ± standard deviation.

*Estimated glomerular filtration rate, <60 mL/min/1.73 m².
Demographic characteristics

The geographical distribution of enrolled cases was mainly from Northwest China, including Shaanxi (60.2%), Gansu (18.5%), Shanxi (9.7%), Ningxia (4.4%), Qinghai (1.7%), and Xinjiang (0.3%), with a male/female ratio of 1.37:1. Their ages range from 6 to 88 years, and the average age was 38.73 ± 16.53 years. Regarding common pathologic types, the youngest participant had EnPGN (23.69 ± 18.49), whereas the oldest had renal amyloidosis (58.88 ± 9.01) (Table 2). During different periods, there was a significant increase in the average age (period 1: 34.34 ± 16.19, period 2: 37.75 ± 16.52, period 3: 41.93 ± 16.07; p < 0.001) (Table 3). Patients aged 46–60 years increased from 17.4% to 28.0%, and those aged >60 years increased from 7.9% to 14.7% (p < 0.001). IgAN and HSPN had age distribution peaks in the 15–30-year age group (41.1% and 39.2%), whereas MN had age distribution peaks in the 45–60-year age group (34.3%) (Fig. 1A). The largest proportion of male appears in the HTN group, whereas the largest proportion of female appears in the LN group (Fig. 1B).

Table 2. Comparative analysis of common pathological types and age stratification

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Total (n = 10,528)</th>
<th>≤14</th>
<th>15–30</th>
<th>31–45</th>
<th>46–60</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN</td>
<td>34.53 ± 13.63</td>
<td>129 (4.1)</td>
<td>1,301 (41.1)</td>
<td>1,078 (34.1)</td>
<td>504 (15.9)</td>
<td>153 (4.8)</td>
</tr>
<tr>
<td>MN</td>
<td>46.16 ± 16.11</td>
<td>17 (0.8)</td>
<td>399 (18.9)</td>
<td>491 (23.3)</td>
<td>725 (34.3)</td>
<td>479 (22.7)</td>
</tr>
<tr>
<td>MsPGN</td>
<td>36.34 ± 15.53</td>
<td>48 (5.7)</td>
<td>267 (31.9)</td>
<td>260 (31.0)</td>
<td>190 (22.7)</td>
<td>73 (8.7)</td>
</tr>
<tr>
<td>FSGS</td>
<td>28.49 ± 14.96</td>
<td>18 (3.7)</td>
<td>126 (25.9)</td>
<td>181 (37.3)</td>
<td>117 (24.1)</td>
<td>44 (9.1)</td>
</tr>
<tr>
<td>MCD</td>
<td>35.14 ± 17.84</td>
<td>35 (9.1)</td>
<td>147 (38.1)</td>
<td>87 (22.5)</td>
<td>67 (17.4)</td>
<td>50 (13.0)</td>
</tr>
<tr>
<td>IgMN</td>
<td>31.39 ± 14.03</td>
<td>14 (8.3)</td>
<td>71 (42.3)</td>
<td>46 (27.4)</td>
<td>31 (18.5)</td>
<td>6 (3.6)</td>
</tr>
<tr>
<td>SCGN</td>
<td>38.92 ± 15.34</td>
<td>5 (0.8)</td>
<td>30 (5.0)</td>
<td>44 (36.7)</td>
<td>27 (22.5)</td>
<td>13 (10.8)</td>
</tr>
<tr>
<td>CreGN</td>
<td>45.41 ± 16.69</td>
<td>0 (0)</td>
<td>17 (22.4)</td>
<td>18 (23.7)</td>
<td>21 (26.6)</td>
<td>20 (26.3)</td>
</tr>
<tr>
<td>EnPGN</td>
<td>23.69 ± 18.49</td>
<td>37 (52.9)</td>
<td>13 (18.6)</td>
<td>8 (11.4)</td>
<td>7 (10.0)</td>
<td>7 (10.0)</td>
</tr>
<tr>
<td>MPGN</td>
<td>49.56 ± 15.89</td>
<td>2 (3.0)</td>
<td>10 (15.2)</td>
<td>9 (13.6)</td>
<td>25 (37.9)</td>
<td>20 (30.3)</td>
</tr>
<tr>
<td>HSPN</td>
<td>24.91 ± 15.15</td>
<td>215 (26.3)</td>
<td>371 (45.3)</td>
<td>130 (15.9)</td>
<td>62 (7.6)</td>
<td>41 (5.0)</td>
</tr>
<tr>
<td>LN</td>
<td>35.14 ± 13.65</td>
<td>23 (3.4)</td>
<td>243 (36.0)</td>
<td>231 (41.2)</td>
<td>146 (21.6)</td>
<td>32 (4.7)</td>
</tr>
<tr>
<td>DN</td>
<td>54.03 ± 9.78</td>
<td>0 (0)</td>
<td>7 (1.8)</td>
<td>51 (12.7)</td>
<td>214 (53.4)</td>
<td>129 (32.2)</td>
</tr>
<tr>
<td>HTN</td>
<td>45.34 ± 13.15</td>
<td>0 (0)</td>
<td>28 (11.8)</td>
<td>93 (39.1)</td>
<td>75 (31.5)</td>
<td>42 (17.7)</td>
</tr>
<tr>
<td>HBVN</td>
<td>42.31 ± 13.65</td>
<td>2 (1.2)</td>
<td>31 (18.8)</td>
<td>59 (35.8)</td>
<td>56 (33.9)</td>
<td>17 (10.3)</td>
</tr>
<tr>
<td>Renal amyloidosis</td>
<td>58.88 ± 9.01</td>
<td>158 (1.5)</td>
<td>0 (0)</td>
<td>9 (5.7)</td>
<td>73 (46.6)</td>
<td>76 (48.1)</td>
</tr>
<tr>
<td>SVARD</td>
<td>58.45 ± 13.38</td>
<td>123 (1.2)</td>
<td>0 (0)</td>
<td>5 (4.1)</td>
<td>14 (11.4)</td>
<td>32 (26.0)</td>
</tr>
<tr>
<td>TIN</td>
<td>45.96 ± 14.85</td>
<td>216 (2.1)</td>
<td>35 (16.2)</td>
<td>59 (27.3)</td>
<td>73 (33.8)</td>
<td>47 (21.8)</td>
</tr>
</tbody>
</table>

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

Table 3. Characteristics of age distribution in different periods

<table>
<thead>
<tr>
<th>Period</th>
<th>Total</th>
<th>Average age (yr)</th>
<th>≤14</th>
<th>15–30</th>
<th>31–45</th>
<th>46–60</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,258 (21.5)</td>
<td>34.34 ± 16.19</td>
<td>190 (8.4)</td>
<td>852 (37.7)</td>
<td>645 (28.6)</td>
<td>392 (17.4)</td>
<td>179 (7.9)</td>
</tr>
<tr>
<td>2</td>
<td>3,926 (37.3)</td>
<td>37.75 ± 16.52</td>
<td>245 (6.2)</td>
<td>1,314 (33.5)</td>
<td>1,072 (27.3)</td>
<td>877 (22.3)</td>
<td>418 (10.8)</td>
</tr>
<tr>
<td>3</td>
<td>4,344 (41.3)</td>
<td>41.93 ± 16.07</td>
<td>128 (3.0)</td>
<td>1,120 (25.8)</td>
<td>1,240 (28.6)</td>
<td>1,217 (28.0)</td>
<td>639 (14.7)</td>
</tr>
<tr>
<td>Total</td>
<td>10,528</td>
<td>38.73 ± 16.53</td>
<td>563 (5.4)</td>
<td>3,286 (31.2)</td>
<td>2,957 (28.1)</td>
<td>2,486 (23.6)</td>
<td>1,236 (11.7)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%) and mean ± standard deviation.
Figure 1. The distribution of kidney biopsy based on different age groups and genders. (A) The age distribution according to pathological findings. (B) The gender distribution according to pathological findings. (C) Changes in the constituent ratio of kidney biopsy disease spectrum in different periods.

CreGN, crescentic glomerulonephritis; DN, diabetic nephropathy; EnPGN, endocapillary proliferative glomerulonephritis; FSGS, focal segmental glomerulosclerosis; HBVN, hepatitis B associated nephropathy; HN, hereditary nephropathy; HSPN, Henoch-Schönlein purpura nephritis; HTN, hypertensive nephropathy; IgAN, immunoglobulin A nephropathy; IgMN, immunoglobulin M nephropathy; LN, systemic lupus erythematosus nephritis; MCD, minimal change disease; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; MsPGN, mesangial proliferative glomerulonephritis; OAG, obesity-associated glomerulopathy; PGD, primary glomerular disease; SCGN, sclerosing glomerulonephritis; SGD, secondary glomerular disease; SVARD, systemic vasculitis-associated renal damage; TIN, tubulointerstitial nephritis.

*p < 0.01.
Changes in the constituent ratio of the kidney biopsy disease spectrum

According to the constituent ratio analysis of all cases grouped by period (Fig. 1C), the proportion of SGD increased significantly from 23.2% (period 1) to 29.1% (period 3), whereas that of PGD decreased relatively from 74.2% (period 1) to 67.7% (period 3). In the multiple joinpoint models, a key joinpoint came in 2012 (Fig. 2). In summary, the proportions of PGD and SGD fluctuated steadily from 2005 to 2012, with APC values of 0.5% (95% confidence interval [CI], –1.1 to 2.1; p = 0.51) and –1.5% (95% CI, –5.8 to 3.1; p = 0.496), respectively. Nevertheless, from 2012 to 2020, the APC of PGD changed to –2.0% (95% CI, –3.2 to –0.7; p = 0.01), whereas that of SGD was 4.4% (95% CI, 0.6–8.3; p = 0.03) (Supplementary Table 2, available online), indicating that the proportion of PGD showed an obvious downward trend after 2012, while SGD showed an obvious upward trend.

Trend analysis of the constituent ratio of the common type of primary glomerular disease

IgAN was the most common type of PGD during the study period, whereas the proportion showed a decreasing trend (Fig. 3) (APC, –0.9%; 95% CI, –2.9 to 1.1; p = 0.35) (Supplementary Table 4, available online). A downward trend was also shown for MsPGN (APC, –14.2%; 95% CI, –17.8 to –10.4; p < 0.001) and FSGS (APC, –1.7%; 95% CI, –6.2 to 2.9; p = 0.43). MN and MCD presented an increasing trend, of which MN was divided into two segments (2005–2011: APC, 25.8%; 95% CI, 6.3–48.7; p = 0.01; 2011–2020: APC, 3.7%; 95% CI, –5.3 to 13.5; p = 0.40). Despite the growth rate of MN slowing down after 2011, MN had the most significant growth trend both in terms of constituent ratio and the number of patients, and MCD showed a continuous increase (2005–2020; APC, 20.0%; 95% CI, 15.3–25.0; p < 0.001).
Trend analysis of the constituent ratio of secondary glomerular disease

HSPN was the most common type of SGD with the highest proportion (29.9%). Nevertheless, the proportion of HSPN showed an obvious downward trend (Fig. 4) (2005–2020; APC, –8.8%; 95% CI, –10.6 to −7.0; p < 0.001) (Supplementary Table 6, available online). LN also showed a significant downward trend (2005–2020; APC, –3.1%; 95% CI, –5.0 to –1.1; p < 0.001). Meanwhile, DN and HTN showed a huge growth trend over time, with APC achieving 20.0% (2005–2020; 95% CI, 16.4–23.7; p < 0.001) and 46.6% (2012–2020; 95% CI, 18.3–81.7; p = 0.002), respectively. Since 2018, DN has become the most common type of SGD. Another notable change in trend appeared for HBVN, with a clear joinpoint in 2013. From 2005 to 2013, there was a significant upward trend (APC, 13.5%; 95% CI, 2.2–26; p = 0.02), whereas a continuous declining trend manifested thereafter (APC, −29.9%; 95% CI, −38.4 to −20.4; p < 0.001).

The relationship between pathological diagnosis and clinical diagnosis

The indications for kidney biopsy mainly included AUA (14.1%), CNS (51.6%), NS (30.0%), AKI (3.9%), RPG (0.3%), and others (0.2%). MN (47.3%) was the dominant cause of NS, followed by MsPGN (10.1%) (Supplementary Table 8, available online). From the age-stratified comparative analysis of indications for kidney biopsy (Supplementary Table 9, available online), AUA had an obvious distribution peak in the 15–30-year age group (48.7%). On the other hand, most clinical manifestations of MCD (73.8%), MN (70.8%), and renal amyloidosis (67.7%) were NS. The major complications in our group were hypertension (29.9%), hyperlipidemia (37.6%), and renal anemia (10.1%). In addition to HTN, hypertension was the main complication of
DN (80.1%), SCGN (68.3%), and MPGN (51.5%). Hyperlipidemia was the main complication of MN (76.2%), MCD (75.1%), and renal amyloidosis (69.0%). Renal anemia was the main complication of SVARD (52.0%), SCGN (40.8%), and DN (37.9%).

**Discussion**

This report selected the largest kidney disease center in Northwest China for epidemiological statistical data from 10,528 kidney biopsy patients. The descriptive results of kidney biopsies indicated that kidney disease in Northwest China is more common among the young and middle-aged populations, and the age of onset is increasing. During the study period, the composition ratio of various pathological types changed significantly.

PGD was the most common kidney disease in this study, which is consistent with the data from other centers including East [12-14], Central [15], and South [16] China (ranging from 65.1% to 71.1%). It is also the main kidney disease in East Asia in countries such as South Korea and Japan [5,17]. Conversely, in Western countries such as the United States and the United Kingdom, there was a higher proportion of SGD [2,18]. Interestingly, we indicated an obvious upward trend in SGD, which may reflect the evolution of the renal disease spectrum from developing countries to developed countries under the context of rapid economic growth and urbanization. In the specific analysis of PGD, we identified that IgAN still accounted for the highest proportion. This is consistent with the report from East Asia in countries [17,19] such as Japan and South Korea, as well as some other international data (Spain, Czech Republic, Denmark, Italy, Scotland, Kuwait, and Turkey) [6,20-22]. FSGS showed the highest proportion in Europe [22] and MPGN showed the highest proportion in South Africa [15]. In addition to the findings in our research, a decreasing trend in IgAN would be expected to be observed.
was also observed in Northeast [12], South [16], and Central [15] China, which may be due to the sudden increase in MN morbidity and the gradual rise in SGD rates.

MN was the disease with the second greatest incidence in our study, and the majority of patients were middle-aged and elderly (>45 years, 57.0%). The rapid growth of MN not only appears in Northwest China, but also in other regions of the country [14,15,23], and has attracted the attention of researchers. In addition to ethnic or genetic factors, a lot of attention has been focused on environmental effects. Air pollution increases the circulating levels of inflammatory mediators, such as tumor necrosis factor-α, interleukin-6, and plasminogen activator inhibitor-1, and genetic polymorphisms in these cytokines are associated with the development of MN [24]. In a large kidney biopsy series in China [23] and India [25], researchers found that the increased frequency of MN was associated with long-term exposure to high levels of particulate matter less than 2.5 μm (PM2.5). In contrast, in developed countries with lower PM2.5 exposure levels, such as Japan [5], Korea [19], France [26], and the United States [9], the MN prevalence has remained stable or even reversed [24]. Regarding current trends, MN has already become the most common type of PGD in some parts of China with high PM2.5 levels [14,15,23]. Judging from the trend chart, coupled with the aggravation of air pollution, the frequent occurrence of haze, and the increase in PM2.5 pollution in Northwest China [27], the prevalence of MN may further increase. This is probably the most notable change in the spectrum of kidney disease in Northwest China.

In regards to the pathological types of SGD, HSPN ranked first in Northwest China and was significantly higher than in other regions of China [7,15,16]. The pathological data of kidney biopsies in Gansu Province also showed that the proportion of HSPN in SGD was the highest (26.1%) [28]. Therefore, it is reasonable to suggest that a high incidence of HSPN is a prominent feature in Northwest China. Intensive exposure to dust weather and other allergic factors in this area, which have a stronger pro-inflammatory effect and cause acute irritant effects on human health including some severe allergic reactions, may contribute to this epidemic character [28,29]. Although the number of HSPN cases remained at a high level, the composition ratio showed a downward trend. This may be related to the significant increase in other types of SGD. LN was the second most common form of SGD in our data and showed a downward trend. However, in Central, Eastern, and Southern China, LN remains the most common form of SGD [13,15,16]. The high incidence of LN is indeed a significant feature of SGD in China.

HTN had the highest growth rate in our study, which was in line with that of Japan and Korea [17]. China has approximately 270 million people with high blood pressure, making it the country with the largest absolute burden of hypertension in the world, and the prevalence rate is increasing year by year [30]. Currently, the proportion of HTN in patients with ESKD varies among different countries [31]. The incidence of HTN will undoubtedly increase in the future with the aging of the population and improvements in cardiovascular disease survival. However, the diagnosis of HTN at present is nonspecific and is mainly based on clinical manifestations [32]. Several studies that have compared biopsy-proven HTN with clinical HTN have found that the former could not be distinguished from renal damage caused by PGD, and the misdiagnosis rate was more than half [33,34]. To avoid misdiagnosis, the biopsy rate of HTN should be further improved. The diagnostic policy of DN is similar to that of HTN at present, and often only a kidney biopsy will make a differential diagnosis possible [35]. Although the detection rate of DN may be underestimated, we have found that it has become the most common type of SGD since 2018. According to the 2019 Scientific Report of Kidney Disease in China, DN has become the leading cause of CKD in hospitalized patients in China, as well as HTN [2]. In addition, in less-developed regions such as Northwest China, hypertension and diabetes are also highly prevalent and poorly managed due to low awareness, treatment, and control rates [36]. Therefore, there is much to be done in improving the diagnosis and management of hypertension and diabetes.

A change in the proportion of HBVN was also noted. The global prevalence of chronic hepatitis B is serious, particularly in certain developing countries, including China [37]. Western China has the highest incidence of hepatitis B in the country [38]. In a jointpoint analysis using hepatitis B morbidity and mortality data from the China Data Center for Public Health Sciences and demographic data from the National Bureau of Statistics, the reported incidence reached an inflection point in 2006 [37]. Our data reflects the epidemic profile of the disease from one side. The
proportion of HBVN showed a peak in 2013, which may be attributed to the control of hepatitis B infection and the widespread use of the hepatitis B vaccine in China. As expected, the population affected by and constituent ratio of HBVN should decrease further.

In our study, CNS was the most common clinical indication for kidney biopsy, followed by NS. Due to inconsistencies in clinical indication and variation in renal biopsy policies in different regions, the conclusions also varied, such as in Japan (CNS, followed by NS), South Korea (AUA, followed by NS), Turkey (NS, followed by AUA), and Central China (NS, followed by CNS) \[15,22\]. However, NS is generally considered to be the most important indicator for biopsy, and MCD is the most common form of NS in children whereas MN is the most common form in adults \[39\]. In Japan, approximately 40% of patients with primary NS are diagnosed with MCD \[5\]. In contrast to the management of NS in adults, a diagnostic kidney biopsy is generally not performed upon presentation in children to establish a diagnosis \[39\]. Therefore, the proportion of MCD in patients with NS may be underestimated.

The high incidence of hypertension in our study reflected a significant impact on prognosis. The Kidney Disease: Improving Global Outcomes (KDIGO) 2021 Clinical Practice Guideline for the Management of Blood Pressure in CKD reemphasizes the importance of blood pressure management \[40\]. Renal anemia is a common clinical manifestation of CKD, which increases the risk of ESKD, cardiovascular events, and death \[41\]. In addition to vascular inflammatory nephropathies such as SVARD and SCGN, DN is most associated with renal anemia. Anemia in DN not only occurs early and seriously but also promotes the progression of DN and induces and aggravates diabetic complications.

This study provides information about the latest epidemic spectrum of kidney disease in Northwest China, which is expected to provide a basis for public health prevention and therapeutic strategy. Generally, kidney disease is more common among the young and middle-aged populations, and the age of onset is increasing. PGD accounted for most cases of kidney disease in this group. The proportion of SGD increased while PGD declined. IgAN remained the most common pathological type, while MN significantly increased. HSPN was the most common pathological type of SGD and could be a hallmark of Northwest China. DN showed the most obvious increasing trend in types of SGD from the analysis, whereas the fastest growth since 2012 was in HTN. Moreover, HBVN showed a joinpoint from increase to decrease. The spectrum changes of kidney disease, especially the constituent ratio of SGD, pose a great challenge to public health.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: SS
Data curation: YQ, JZ, ZY
Formal analysis: YQ, YW, ZY, YZ
Funding acquisition: SS
Methodology: XW
Project administration: SS
Software: YQ, XW
Writing–original draft: YQ
Writing–review & editing: SS, JZ, ZY, YZ

All authors read and approved the final manuscript.

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Deep learning predicts the differentiation of kidney organoids derived from human induced pluripotent stem cells

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Background: Kidney organoids derived from human pluripotent stem cells (hPSCs) contain multilineage nephrogenic progenitor cells and can recapitulate the development of the kidney. Kidney organoids derived from hPSCs have the potential to be applied in regenerative medicine as well as renal disease modeling, drug screening, and nephrotoxicity testing. Despite biotechnological advances, individual differences in morphological and growth characteristics among kidney organoids need to be addressed before clinical and commercial application. In this study, we hypothesized that an automated noninvasive method based on deep learning of bright-field images of kidney organoids can predict their differentiation status.

Methods: Bright-field images of kidney organoids were collected on day 18 after differentiation. To train convolutional neural networks (CNNs), we utilized a transfer learning approach. CNNs were trained to predict the differentiation of kidney organoids on bright-field images based on the messenger RNA expression of renal tubular epithelial cells as well as podocytes.

Results: The best prediction model was DenseNet121 with a total Pearson correlation coefficient score of 0.783 on a test dataset. W classified the kidney organoids into two categories: organoids with above-average gene expression (Positive) and those with below-average gene expression (Negative). Comparing the best-performing CNN with human-based classifiers, the CNN algorithm had a receiver operating characteristic-area under the curve (AUC) score of 0.85, while the experts had an AUC score of 0.48.

Conclusion: These results confirmed our original hypothesis and demonstrated that our artificial intelligence algorithm can successfully recognize the differentiation status of kidney organoids.

Keywords: Deep learning, Gene expression, Kidney, Organoids
**Introduction**

Organoids are self-organizing three-dimensional (3D) aggregations of cells that represent the structure and function of organs and can be generated from human pluripotent stem cells (hPSCs) in vitro [1–5]. Kidney organoids derived from hPSCs contain multilineage nephrogenic progenitor cells and can recapitulate the development of the kidney [6]. A direct comparison of gene expression and localization between kidney organoids in vitro and human kidneys revealed that podocytes derived from hPSCs resemble podocytes in vivo at the capillary loop stage of glomerular development [6]. Kidney organoids derived from hPSCs can be used in regenerative medicine as well to model renal diseases, function in drug screening, and evaluate the nephrotoxicity of compounds [7–12].

Despite biotechnological advances, individual differences in the morphological and growth characteristics of kidney organoids, despite culture for the same time period and in the same well, have to be addressed prior to their clinical and commercial use [13]. A method for selecting highly matured kidney organoids is required to obtain reproducible and credible data from kidney organoid experiments.

To assess maturity based on the morphological and growth characteristics of kidney organoids, immunohistochemistry, immunofluorescence microscopy, and transcriptomic analysis using real-time (RT) polymerase chain reaction (PCR) or single-cell RNA sequencing analysis have been used [13,14]. However, these traditional analytic tools necessitate the destruction of cells within kidney organoids. Analysis of the morphological or growth characteristics of kidney organoids in a living state is essential.

In this study, we hypothesized that basic-contrast bright-field optical microscopy images could be used to assess the differentiation status of kidney organoids. Because manual selection under a microscope with bright-field imaging is subjective and results in variability between observers, a deep learning approach based on bright-field is required [13–17]. In this study, we demonstrated that an automated noninvasive method based on bright-field deep learning was able to predict the differentiation status of kidney organoids.

We used a convolutional neural network (CNN)-based approach to analyze organoid images. The CNN comprises convolutional layers that determine the relationships between spatially adjacent regions of the images. This approach has been used in a variety of fields in biology and medicine. For example, this approach has been applied to classify skin cancer and detect diabetic retinopathy in retinal fundus images [18,19]. Inspired by these examples, we hypothesized that a CNN would be able to extract sufficient information about tissue specification from bright-field images. Therefore, we utilized a CNN to predict the differentiation of kidney organoids and compare its classification performance with that of experts.

**Methods**

**Kidney organoid differentiation**

WTC11 induced pluripotent stem cell (iPSC) between passages 30 and 60 were used. Kidney organoid differentiation was induced as described previously [7]. In brief, iPSCs were plated at a density of 5,000 cells/well in a 24-well plate in mTeSR1 medium (Stem Cell Technologies) + 10 µM Y27632 (LC Laboratories) on plates (SPL Life Sciences) coated with 1% GelTrex (Thermo Fisher Scientific) (day -3). The medium was exchanged with 1.5% GelTrex in mTeSR1 (day -2), mTeSR1 (day -1), RPMI (Thermo Fisher Scientific) + 12 µM CHIR99021 (Tocris, Bristol, UK) (day 0), or RPMI + B27 supplement (Thermo Fisher Scientific) (day 1.5) and cells were fed every 2–3 days to promote kidney organoid differentiation. Organoids were analyzed on day 18.

**Immunofluorescence analysis**

For immunofluorescence, organoids were fixed on day 18 unless otherwise noted. For fixation, phosphate-buffered saline (PBS; Thermo Fisher Scientific) + 4% paraformaldehyde (Electron Microscopy Sciences) was added to the medium for 15 minutes, after which the samples were washed three times with PBS. Fixed organoid cultures were blocked in 5% donkey serum (Millipore, Burlington) + 0.3% Triton-X-100/PBS, incubated overnight in 3% bovine serum albumin (SigmaAldrich, St. Louis) + PBS with primary antibodies, washed, incubated with AlexaFluor secondary antibodies (Invitrogen), washed, and stained with DAPI or mounted in Vectashield H-1000. Images were acquired us-
ing a Zeiss LSM 700 confocal microscope (Carl Zeiss) and ZEN 3.1 software.

The following primary antibodies were used: anti-E-cadherin (ECAD) (1:100, ab11512; Abcam), anti-LTL (1:100, FL-1321; Vector Labs), and anti-nephrosis 1 (NPHS1) (1:100, AF4269; R&D System).

Quantitative real time polymerase chain reaction

Kidney organoid samples were harvested, and total RNA from each sample was isolated using an RNAiso Plus Kit (Takara) according to the manufacturer’s instructions. Complementary DNA (cDNA) was synthesized using a Maxima First Strand cDNA Synthesis kit for RT-qPCR (Thermo Fisher Scientific). Gene expression was analyzed with Power SYBR Green PCR Master Mix (Applied Biosystems) using real-time PCR (Applied Biosystems). Specific primers used were: human synaptopodin (SYNPO), F-5’ GCTGAGGAGGTGAGATGCAG and R-5’ CTCTGGAGAAGGTGCTGGT; NPHS1, F-5’ GGCTCCACGAGAAACTCTT and R-5’ CACAGACCAGAACTGCCTA; sodium-glucose cotransporter 2 (SGLT2), F-5’ GGGTTACGCCTTCCACGAG and R-5’ AGATGTTCACCGGCTGG; gamma-glutamyltransferase 1 (GGT1), F-5’ TGACCTTCAGGAGAACGAGA and R-5’ TCTTCTTCATGGCTCTGGT; ECAD, F-5’ CGAGAGCTACGTTCACCC and R-5’ GGGTTAGGAGGAAAATAGG; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), F-5’ AGGGCTGCTTTTAACTCTGGT and R-5’ CCCCACTTTGATTTCGAGGAG. All quantitative RT-PCR (qRT-PCR) reactions were performed in triplicate and relative messenger RNA (mRNA) expression levels were determined using the 2-ΔΔCt method.

Dataset preprocessing

We preprocessed the kidney organoid dataset as described below before introducing the bright-field images of kidney organoids into our proposed deep neural network model. Because the considered bright-field images of kidney organoids contain noisy regions, e.g., floating inclusions, we cropped all regions except the organoid region. We also introduced this cropping process to register the positions of the different organoids. For preprocessing of input dimensions, we used zero padding on cropped bright-field images of kidney organoids to avoid losing spatial information present for each image. Most typical methods, e.g., resizing, prevent the model from extracting spatially relevant features. Additionally, we applied min-max normalization to the bright-field images of the kidney organoid and qRT-PCR expressions. We also augmented the training images by flipping the images horizontally and vertically and rotating these images 90°.

Proposed prediction method

Differentiation of kidney organoids based on bright-field images was predicted using CNNs. We utilized a transfer learning-based approach with four well-performing models (ResNet50 [20], InceptionV3 [21], EfficientNetB5 [22], and DenseNet121 [23]) pretrained on ImageNet [24]. ResNet50 could solve the problem of vanishing or exploding gradients with skip connections. These add the input to the output after weight layers. InceptionV3 aims for less computational power by optimizing the network using a variety of strategies such as factorized convolutions and dimension reduction. EfficientNetB5 is a model that is constructed by efficiently balancing the layer scaling factors like width, depth, and image resolution. DenseNet121 contains densely connected layers and these connections create strong supervision, allowing the connections to obtain information related to the differentiation status of kidney organoids on bright-field images. While these models’ hyper-parameters are optimized to maximize their regression performance with regard to predicting the differentiation of kidney organoids, we performed comparison studies to determine which model exhibited the best predictive performance for different biomarkers of kidney organoids. To assess the predictive performance of the CNN models, we used Pearson correlation coefficient (PCC) scores. PCC scores can be used to measure the linear correlation between actual and predicted values and can be expressed as:

$$PCC = \frac{Cov(Y, \hat{Y})}{\sigma_Y \sigma_{\hat{Y}}},$$

where $Y$ and $\hat{Y}$ are the actual and predicted qRT-PCR expression values, respectively; $Cov$ is the covariance; and $\sigma_Y$ and $\sigma_{\hat{Y}}$ are the standard derivation of $Y$. We validate our method with five-fold cross-validation by splitting the dataset into five nonoverlapping subsets.
The above method of optimizing CNN models according to specific and various substructures of organoids was used as a biomarker selection criterion. We determined the optimal biomarkers in each substructure of kidney organoids, such as podocytes and proximal tubules, by comparing the performance of deep learning models. We evaluated two biomarkers for each substructure and selected one of these to determine whether the kidney organoids were highly differentiated using deep learning.

Last, we adopted a gradient-based class activation mapping (gradCAM) method to further evaluate the proposed model’s visual contributions to the prediction results \[25\]. To validate the gradCAM method, we implemented a qualitative comparison using immunofluorescence images of kidney organoids. As long as the substructure of kidney organoids was correctly highlighted, we were able to determine their differentiation status in a noninvasive manner using gradCAM.

Proposed classification method

To verify that our proposed method can potentially be utilized to guide the selection of biomarkers for predicting the differentiation of kidney organoids, we compared its performance to those of experts. Organoid images were used; images with above-average quantitative PCR (qPCR) expression were labeled as Positive, and images with below-average expression were labeled as Negative.

Accuracy, sensitivity, specificity, F1 score, receiver operating characteristic (ROC)-area under the curve (AUC), and the time required to perform the task were compared between our algorithm and experts. Accuracy is a statistical measure that refers to the proportion of correct determinations divided by the total number of images in the dataset. Sensitivity is defined as the true positive rate of all images with a condition, and specificity is defined as the true negative rate of all images that did not have a condition. The F1 score is the harmonic mean of the sensitivity and precision, which in this study referred to the proportion of correct positive predictions divided by the number of total images that were positive. The AUC score is calculated as the area under ROC curves where the false positive rate versus the true positive rate was plotted for different threshold values. The Student t test was used in the between-group analysis. The tested null hypothesis was that two independent samples would have identical averages and the populations would have identical variance. The p-value indicates the probability of observation above the extreme values if the hypothesis is true.

Results

Differentiation of human induced pluripotent stem cell-derived kidney organoids and the collection of the training dataset

To generate kidney organoids derived from human iPSCs, we applied an adherent culture differentiation protocol (Fig. 1A). On day 18 of differentiation, human iPSC-derived kidney organoids had discrete nephron-like structures consisting of podocytes, proximal tubules, and distal tubules (Fig. 1B). Bright-field microscopy showed that the kidney organoids had different morphologies (distribution of podocytes, proximal tubules, and distal tubules) from one another despite differentiation for the same amount of time (18 days) (Fig. 2A). Given our hypothesis that the morphology of kidney organoids obtained by bright-field microscopy reflects the gene expression of podocytes, proximal tubules, and distal tubules, we performed a preliminary experiment. An expert-selected 15 kidney organoids with “good morphology” and 15 kidney organoids with “unsatisfactory morphology” according to bright-field microscopy morphology (Fig. 2B); and qPCR was performed using primers targeting podocyte-, proximal tubule-, and distal tubule-specific genes (Fig. 2C). Fig. 2C shows that the gene expression of NPHS1 and SYNPO (podocyte markers), SGLT2 and GGT1 (proximal tubular markers), and ECAD (distal tubular marker) were significantly increased in kidney organoids with a “good morphology” compared to those with an “unsatisfactory morphology.” These findings suggest that accurate analysis of morphology by bright-field microscopy could predict the degree of differentiation of kidney organoids.

For an objective analysis of the bright-field images of the kidney organoids, approximately 150 kidney organoids were differentiated and analyzed. To train our model and label the dataset, we collected bright-field images for each kidney organoid on day 18 of differentiation and performed qPCR using podocyte-, proximal tubule-, and distal tubule-specific primers.
Convolutional neural network can predict the differentiation of kidney organoids

We conducted experiments with several CNN models to predict the differentiation of kidney organoids. As the feature extractors, four CNN models, i.e., ResNet50, InceptionV3, EfficientNetB5, and DenseNet121, were trained using the kidney organoid dataset. To compare the prediction performance of the above models, we used the PCC; +1 indicates a complete positive linear correlation; and the closer the value is to +1, the better the performance of the deep learning model. Furthermore, we employed a five-fold cross-validation method for each model to evaluate its predictive ability by averaging the prediction results for each data fold set to improve the reliability of the results. We predicted the qPCR expressions of the following biomarkers: NPHS1, SYNPO, SGLT2, GGT1, and ECAD. Quantitative results, namely the average and standard deviation values of PCC scores for estimating the qPCR expression values of kidney organoids on the testing set, are provided in Table 1. DenseNet121 achieved remarkable performance for all biomarkers except NPHS1, and EfficientNetB5 slightly outperformed DenseNet121 by 0.022 with regard to the qPCR expression of NPHS1. DenseNet121 was the optimal model for predicting the differentiation of kidney organoids and extracting features from bright-field images of the organoids. We confirmed that the total PCC score of DenseNet121 was 0.783; this indicates a strong positive relationship between actual and predicted values. These results suggest that a deep learning method overcomes...
Table 1. Comparison of Pearson correlation coefficient scores among convolutional neural network models

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Deep learning</th>
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<tbody>
<tr>
<td></td>
<td>ResNet50</td>
<td>InceptionV3</td>
<td>EfficientNetB5</td>
<td>DenseNet121</td>
<td></td>
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<tr>
<td></td>
<td>Score</td>
<td>p-value</td>
<td>Score</td>
<td>p-value</td>
<td>Score</td>
</tr>
<tr>
<td>Podocyte</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NPHS1</td>
<td>0.637 ± 0.295</td>
<td>0.12</td>
<td>0.605 ± 0.237</td>
<td>0.05</td>
<td>0.778 ± 0.075</td>
</tr>
<tr>
<td>SYNPO</td>
<td>0.524 ± 0.257</td>
<td>0.11</td>
<td>0.550 ± 0.161</td>
<td>&lt;0.05</td>
<td>0.671 ± 0.199</td>
</tr>
<tr>
<td>Proximal tubule</td>
<td></td>
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<tr>
<td>SGLT2</td>
<td>0.792 ± 0.045</td>
<td>&lt;0.05</td>
<td>0.691 ± 0.115</td>
<td>&lt;0.05</td>
<td>0.786 ± 0.100</td>
</tr>
<tr>
<td>GGT1</td>
<td>0.786 ± 0.087</td>
<td>&lt;0.05</td>
<td>0.436 ± 0.109</td>
<td>0.08</td>
<td>0.602 ± 0.174</td>
</tr>
<tr>
<td>Distal tubule</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECAD</td>
<td>0.648 ± 0.078</td>
<td>&lt;0.05</td>
<td>0.681 ± 0.081</td>
<td>&lt;0.05</td>
<td>0.644 ± 0.153</td>
</tr>
<tr>
<td>Total</td>
<td>0.677 ± 0.113</td>
<td>0.593 ± 0.105</td>
<td>0.696 ± 0.082</td>
<td>0.783 ± 0.059</td>
<td></td>
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</tbody>
</table>

Data are expressed as mean ± standard deviation.

ECAD, E-cadherin; NPHS1, nephrosis 1; SGLT2, sodium-glucose cotransporter 2; SYNPO, synaptopodin.

The limitation of predicting qPCR expression using only two-dimensional (2D) microscopic image data of kidney organoids.

In addition, there was a high correlation between actual and predicted values when SGLT2 was predicted using DenseNet121; the average PCC score was 0.874. In this way, we developed an appropriate deep learning model (DenseNet121) to analyze the differentiation of kidney or-
ganoids.

As several biomarkers can be used to evaluate the differentiation of kidney organoid substructures, we developed criteria for selecting the most suitable biomarkers using deep learning. As shown in Fig. 3A, prediction performance was compared by assessing the correlations between the actual and predicted qPCR expression levels of the biomarkers NPHS1 and SYNPO, which are expressed in podocytes. All CNN models showed a better ability to predict the expression of NPHS1 than of SYNPO. For example, the PCC score of EfficientNetB5 for predicting the expressions of NPHS1 was 0.778, while the PCC of the best model for SYNPO, i.e., DenseNet121, was 0.719. Similarly, all CNN models better predicted the expressions of SGLT2 than of GGT1 (biomarkers indicating the degree of differentiation of the proximal tubule), with DenseNet121 yielding a PCC score of 0.874 for SGLT2 (Fig. 3B). These results indicate that the expression of NPHS1, SGLT2, and ECAD can be utilized to analyze the differentiation of kidney organoid substructures, i.e., podocytes, proximal tubules, and distal tubules, respectively, in a noninvasive manner.

We further compared immunofluorescence images with activation maps to predict qPCR expressions. We employed gradCAM, which highlights regions on the CNN model’s activation map to predict expression. In other words, gradCAM, utilizing the gradient information of parameters and feature maps in the internal layers of the network, can be used to interpret the decision-making for predicting the qPCR expressions. Immunofluorescence images of kidney organoids and the activation maps of the proposed model for predicting the expression of selected biomarkers, i.e., NPHS1, SGLT2, and ECAD, are shown in Fig. 4. Red, white, and green regions in the immunofluorescence images correspond to NPHS1, SGLT2, and ECAD expression, respectively; these images demonstrate that the activation maps focused attention on the appropriate regions. This highlights the importance of activation maps in accurately predicting qPCR expression.

Convolutional neural network is more beneficial for classification of kidney organoids compared to human classifiers

To compare the performance of our DenseNet121 model with that of experts, we requested that two independent experts assign the following labels to kidney organoids: “useful organoids (Positive)” or “nonuseful organoids (Negative).” As shown in Fig. 5A, we employed metrics of accuracy, sensitivity, specificity, F1 score, and AUC to evaluate the classification performance of the experts and the proposed model. Comparing the best-performing CNN with human-based classifiers, the CNN algorithm had an accuracy of 76.67%, while the experts had an accuracy of 48.94% in classifying the organoids. DenseNet121 had an AUC average score of 0.85, while the experts had an AUC score of 0.48.

Times needed by the experts and our CNN to classify organoids are shown in Fig. 5B. The experts required 1.04
seconds to empirically judge the differentiation of kidney organoids using morphological information from bright-field images. In contrast, the deep learning model required 0.014 seconds to generate highly accurate classification results. This indicates that our noninvasive analysis technique is suitable for assessing the differentiation of kidney organoids in real-time.

**Discussion**

Despite the advances in differentiating kidney organoids from hPSCs, these organoids are still immature compared with human adult kidneys. Kim et al. [6] reported that hPSC-podocytes of kidney organoids have junction-rich basal membranes with junctional migration and microvillus-rich apical membranes but do not form bona fide foot processes with tertiary interdigitations seen in the capillary loop stage of glomerular development in the human kidney. Using single-cell transcriptomic analysis, Wu et al. [26] demonstrated that kidney organoid cells are immature compared with fetal and adult human kidneys, and 10% to 20% of kidney organoid cells are nonrenal, “off-target” cells. Kim et al. [27] showed that the hPSC-proximal tubule of kidney organoids has a resorption function similar to that of in vivo, but the barrier function of tubular structures is still immature.

To overcome the immaturity and the clinical application of kidney organoids for nephrotoxicity testing or regenerative medicine, an advanced protocol to generate highly matured kidney organoids similar to adult human kidneys is required. Predicting the maturity and selecting matured kidney organoids may also be an attractive option for their clinical use [28].

In this study, we proposed a deep learning-based noninvasive method for accurate and rapid prediction of kidney organoid differentiation.

We first utilized different CNN models as feature extractors to predict the mRNA expressions of specific kidney biomarkers using morphological information present in bright-field images of kidney organoids. We employed the...
gradCAM method to highlight regions in immunofluorescence images in which to predict gene expression and identified an optimal CNN model suitable for identifying well-differentiated kidney organoids. Our proposed CNN model was more accurate and faster at classification than were the experts.

Previous studies reported the utilization of deep learning in the field of organoid technology [13–17]. To predict the differentiation status of retina organoids, Kegeles et al. [13] trained CNNs on bright-field images of retina organoids labeled with RxGFP and divided organoids into retina and non-retina based on fluorescent reporter gene expression. Their deep learning-based computer algorithm to predict retinal differentiation in stem cell-derived organoids based on bright-field imaging, performed better than the expert in predicting retina organoid fate [13].

We predicted the differentiation level of kidney organoids based on mRNA expression levels rather than 2D confocal images as in a previous study. In kidney organoids, the prediction of differentiation based on 2D confocal images has some disadvantages. Because kidney organoids are 3D structures, 2D confocal images might not accurately reflect tubular structures or podocytes and vascular networks and can be unsuitable for obtaining quantitative biomarker expression data. Furthermore, the predictive power of deep learning based on 2D confocal images can be weakened by the lack of a clear consensus of criteria that can be used to assess the differentiation of kidney organoids from 2D confocal images.

However, prediction based on mRNA expression levels has several advantages. mRNA expression levels represent the levels in the entire kidney organoids. Quantitative data for 6 to 10 genes can be obtained simultaneously for one kidney organoid, which facilitates the comparison of various biomarkers among kidney organoids. For this reason, we chose to assess the differentiation level of kidney organoids based on mRNA expression levels rather than 2D confocal images.

However, prediction based on mRNA expression levels does have some limitations. First, protein expression and

Figure 5. Comparison of a human-based classifier and CNN-based classifier. (A) Evaluation of the classification performance with accuracy, sensitivity, specificity, F1 score, and AUC. (B) Times needed by the experts and our CNN to classify organoids. CNN classified organoids about 74 times faster than experts. AUC, the area under the receiver operating characteristic curve; CNN, convolutional neural network.

* p < 0.05, **p < 0.01.
the structural development stage of the kidney organoids are not necessarily correlated with mRNA expression levels. The development of CNN models based on the combination of mRNA expression levels and 2D confocal images can increase the accuracy of prediction of the differentiation status of kidney organoids. Second, to assess the precise maturity of kidney organoids, analysis of cell-to-cell interaction and function of kidney organoids is essential. However, the CNN model based on mRNA expression in this study is limited to predicting the cell-to-cell interaction and function of kidney organoids. In addition, in terms of the CNN-based approach, there is a limit to analyzing 3D-shaped organoids only in 2D bright-field images. The advanced CNN models to predict the cell-to-cell interaction based on single-cell RNA sequencing analysis as well as predict the functionality of kidney organoids are needed.

In conclusion, we demonstrated that a CNN model could accurately predict kidney organoid differentiation based on the analysis of simple bright-field images of kidney organoids. This noninvasive and nondestructive prediction method may accelerate the transition of kidney organoid technology “from the bench to the bedside.”

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization: KHP, JYL, SL, YKK
Data curation, Formal analysis, Visualization: KHP, JYL
Funding acquisition, Supervision: HWP, YKK, SCL
Investigation, Methodology, Resources: All authors
Project administration: KHP, JYL, HWP, YKK, SCL
Software: KHP
Writing–original draft: KHP, JYL, HWP, YKK, SCL
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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References


Weight change and risk of depression in patients with diabetic kidney disease: a nationwide population-based study

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Background: Several studies have reported that depression is prevalent in patients with diabetes or chronic kidney disease. However, the relationship between weight changes and the risk of depression has not been elucidated in patients with diabetic kidney disease (DKD).

Methods: From the Korean National Health Insurance Service database, we selected 67,866 patients with DKD and body weight data from two consecutive health examinations with a 2-year interval between 2009 and 2012. Weight change over 2 years was categorized into five groups: ≥–10%, <–10% to ≥–5%, <–5% to <5%, ≥5% to <10%, and ≥10%. The occurrence of depression was monitored via the codes of International Statistical Classification of Diseases, 10th revision through the end of 2018.

Results: During the 5.24-year follow-up, 17,023 patients with DKD developed depression. Weight change and the risk of depression had a U-shaped relationship: patients with ≥–10% weight change (hazard ratio [HR], 1.12) and those with ≥10% weight change (HR, 1.11) showed higher HRs for depression than those with <–5% to <5% weight change, even after adjusting for several confounding factors. In the subgroup analyses, the risk of depression tended to increase as weight gain or weight loss increased in all subgroups.

Conclusion: Both weight loss and weight gain increased the risk of depression in patients with DKD.

Keywords: Depression, Diabetic nephropathies, Risk, Weight change

Introduction

Diabetes is one of the leading causes of chronic kidney disease (CKD) and end-stage kidney disease worldwide [1,2]. Because diabetic kidney disease (DKD) is a heterogeneous disease with various overlapping etiologic pathophysiologies [3], it is well recognized that DKD has a poorer prognosis than CKD without diabetes. Anemia develops earlier in the course of disease progression in patients with DKD than in those with nondiabetic CKD [4]. In addition, the rates of mortality from cardiovascular disease and infection are higher in patients with DKD than in those with CKD.
from other causes [5,6]. In Korea and the United States, end-stage kidney disease is attributed to diabetes in nearly 50% of cases [1,2].

Patients with chronic illnesses such as diabetes and CKD are more likely to have or develop mental health problems than people without such conditions. In particular, patients with chronic diseases have a high risk of experiencing depression [7]. Alternatively, depression is a common complication of chronic diseases. Diabetes and CKD, which are representative chronic diseases, are also associated with an increased risk of depression. Depression is highly prevalent in patients with CKD [8-12], and even those with early-stage CKD have a high risk of depression [13]. The prevalence of depression in patients with CKD undergoing dialysis is 20% to 30%. Because depression is also common in patients with diabetes, the two diseases need to be managed together [14]. In particular, DKD, as a combination of two common chronic diseases, can be expected to cause not only a poor prognosis but also adverse effects on mental health. Therefore, early detection, treatment, and management of risk factors are necessary.

Several studies have reported that being overweight is a risk factor for depression [15]. Only a few studies have reported that being underweight might also increase the risk of depression [16]. In addition, although many studies have examined depression-related weight changes, few studies have considered whether weight changes can increase the risk of depression [17]. A recent study reported that body weight variability is related to the risk of depression in patients with type 2 diabetes, but it is unclear whether an increase or decrease in body weight is related to the risk of depression in DKD patients [18]. Therefore, we investigated the association between body weight change and the risk of depression in patients with DKD. To better elucidate the relationship between weight change and depression, we analyzed large-scale, nationally representative data from the Korean National Health Insurance System (NHIS).

Methods

Data source and study population

The Korean NHIS, which keeps an eligibility database (containing information on age, sex, socioeconomic variables, type of eligibility, and income level), a medical treatment database (based on claims submitted by medical service providers for medical expenses), a health examination database (containing the results of general health examinations and questionnaire surveys on lifestyle and behavior), and a medical care institution database (containing information on the types of medical care institutions and their locations, equipment, and number of physicians), makes a complete set of health information for 50 million Koreans available to researchers. The NHIS is managed by the government of Korea and enrolls approximately 97% of the Korean population, with the remaining 3% being covered by the Medical Aid program. The total claim rate for medical expenses is 100%. Therefore, only a few individuals from the Korean population are missing from the NHIS cohort. The Korean NHIS provides regular health examinations to the public. Those enrolled in the health insurance service are recommended to undergo health examinations at least biennially.

Identification of study subjects with diabetes and chronic kidney disease

In this study, we included participants with diabetes who underwent a health examination between 2009 and 2012 and also underwent a follow-up health examination 2 years later (Fig. 1). The index date was the date of the second health examination. Individuals aged <20 years were excluded. Subjects with a CKD diagnosis at both of the health examinations (with a 2-year interval between them) were recruited, and those with a history of malignancy or depression were excluded. After excluding participants with missing data, we enrolled 67,866 patients with DKD in this study. A detailed flowchart of the recruitment of study participants is provided in Fig. 1. The participants were followed up until they were diagnosed with depression, lost their health insurance eligibility, or the follow-up period ended (December 31, 2018). Diabetes was defined as follows: 1) having at least one claim per year for a prescription for antidiabetic medication with International Statistical Classification of Diseases, 10th revision (ICD-10) codes E11-E14 in the insurance claims data or 2) having a fasting plasma glucose level of ≥126 mg/dL in the health examination without a prescription for antidiabetic medication [19]. The following antidiabetic medications were considered diagnostic: sulfonylureas, metformin, dipeptidyl pepti-
dase-4 inhibitors, thiazolidinediones, alpha-glucosidase inhibitors, meglitinides, and insulin. Patients with type 1 diabetes mellitus (ICD-10 code E10) were excluded from this study. CKD was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m², as calculated by the Modification of Diet in Renal Disease (MDRD) formula.

**Definitions of depression and other variables**

Patients whose records contained ICD-10 code F32 or F33 during the follow-up period were defined as having a diagnosis of depression. The variables included in the health examinations, such as questionnaire items, laboratory data, and disease history, were described in our previous study [20]. Those variables were extracted from the results of the biennial health examinations provided to health insurance participants by the NHIS [21]. The hospitals that perform these health examinations are certified by the NHIS and subjected to regular quality control evaluations. The definitions of hypertension and dyslipidemia were taken from a previous study [20]. Data about smoking, alcohol consumption, and physical activity were obtained from the health examination questionnaires. Standardized self-reported questionnaires were used for alcohol consumption (none; mild, <30 g of alcohol/day; and heavy, ≥30 g of alcohol/day), and smoking status (never, former, and current). Regular physical exercise was defined as high-intensity activity ≥1 time/week or moderate-intensity activity ≥1 time/wk. Income level was divided into quartiles, and the lowest quartile was defined as low-income. Malabsorption disorders were identified by the ICD-10 codes for Crohn disease (K50), ulcerative colitis (K51), and intestinal malabsorption (K90). Weight change was estimated as the difference in weight between the consecutive health examinations with a 2-year interval and divided into the following five groups, as reported in previous studies: ≥–10%, <–10% to ≥–5%, <–5% to <5%, ≥5% to <10%, and ≥10% [22].

**Statistical analyses**

Continuous variables are presented as the mean ± standard deviation, and categorical variables are presented as number (percentage). Intergroup differences were estimated using the chi-square test or analysis of variance, as appropriate. The incidence rates of depression are presented per 1,000 person-years. A multivariable Cox proportional hazards regression analysis was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of depression associated with body mass index (BMI) and weight change, with adjustment for age, sex, smoking, alcohol consumption, regular exercise, low-income status, use of insulin, number of oral hypoglycemic agents, duration of diabetes, previous history of hypertension or dyslipidemia, and previous body weight. A sensitivity analysis was performed by excluding patients diagnosed with depression within the first year of follow-up. Subgroup analyses were performed to assess the effect of modification on the risk of depression in patients with DKD according to age (<65 and ≥65 years), sex, history of hypertension, duration of diabetes (<5 and ≥5 years), presence of proteinuria, BMI (<25 and ≥25 kg/m²), use of insulin, number of oral hypoglycemic agents used (≥3), CKD stage (stage 3a with a glomerular filtration rate [GFR] of ≥45 to <60 mL/min/1.73 m², and disease duration (≤5 and ≥5 years).
stage 3b with a GFR of ≥30 to <45 mL/min/1.73 m², stage 4 with a GFR of ≥15 to <30 mL/min/1.73 m², and stage 5 with a GFR of <15 mL/min/1.73 m², as calculated by the MDRD formula), and history of hospitalization. Interaction terms were added to test for effect modification across subgroups. Statistical analyses were performed using SAS (version 9.3; SAS Institute), and p < 0.05 was considered to indicate statistical significance.

Ethical approval

The requirement for ethical approval of this study was waived by the Institutional Review Board of Chonnam National University Hospital (No. CNUH-EXP-2021-321). The requirement for obtaining informed consent was also waived because the participants’ records and information were anonymized and de-identified before analysis. We received permission to use the database from the National Health Insurance Sharing Service (application No. REQ202103172-007).

Results

Baseline characteristics of the study population

The baseline characteristics of the participants are shown in Table 1 according to weight change status. At baseline, the mean age of all patients (54.6% males) was 67.8 ± 9.7 years. The mean eGFR was 45.2 ± 14.4 mL/min/1.73 m², and the mean BMI was 25.0 ± 3.3 kg/m². The distribution of patients according to weight change during the 2-year period was as follows: 70.5% had <5% weight change (stable weight group); 4.4% had ≥–10% weight change; 13.8% had <–10% to ≥–5% weight change; 8.3% had ≥5% to <10% weight change; and 3.0% had ≥10% weight change. Participants in the stable weight group were younger, more likely to be smokers and consume alcohol, more likely to exercise regularly, and had a lower income and higher baseline eGFR than those in the other weight loss and gain groups. Participants in the stable weight group also tended to have a lower prevalence of hypertension and dyslipidemia. BMI, waist circumference, total cholesterol, and triglyceride levels all tended to decrease with weight loss and increase with weight gain.

Risk of depression by body mass index, weight change status in diabetic kidney disease

During the follow-up period (median, 5.24 years; interquartile range, 4.01–6.65 years), a total of 17,023 incident cases of depression were diagnosed. The associations between baseline BMI and the incidence and risk of depression in participants with DKD are described in Table 2. We did not find a significant relationship between baseline BMI and the risk of depression. Adjusting for available confounding factors did not alter that result.

The associations between weight change and the incidence and risk of depression in participants with DKD are described in Table 3 and Fig. 2. Compared with the group with a <5% weight change in either direction over 2 years, the incidence rate of depression increased as weight loss or gain increased in the other groups. The risk of depression increased as weight change increased, and both weight loss and weight gain were significantly associated with an increased risk of depression. This trend did not change even after adjusting for various confounding factors. After adjusting for the participants’ age, sex, smoking or alcohol consumption, comorbidities, duration of diabetes, anti-diabetic medications, and baseline body weight (only in model 3), the adjusted HRs for depression increased to 1.07 (95% CI, 1.03−1.12) and 1.12 (95% CI, 1.04−1.20) in those with <–10% to ≥–5% weight change and ≥–10% weight change, respectively. Participants who gained weight also showed a significantly increased risk of depression (adjusted HR, 1.07 [95% CI, 1.01–1.13] in the ≥5% to <10% weight change group and 1.11 [95% CI, 1.02–1.21] in the ≥10% weight change group).

We performed a sensitivity analysis to account for the possibility of reverse causality. The risk of depression in patients with DKD was analyzed after excluding participants diagnosed with depression within the first year of follow-up (Table 4). Compared with a group with <–5% to <5% weight change in 2 years, both weight gain and weight loss were still significantly associated with an increased risk of depression in patients with DKD.

Subgroup analyses of the risk of depression in diabetic kidney disease

We further analyzed the association between weight
Table 1. Baseline characteristics of the study population according to weight change status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 67,866)</th>
<th>≥−10% (n = 2,975)</th>
<th>&lt;-10% to ≥−5% (n = 9,375)</th>
<th>&lt;−5% to &lt;5% (n = 47,865)</th>
<th>≥5% to &lt;10% (n = 5,646)</th>
<th>≥10% (n = 2,005)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>67.75 ± 6.65</td>
<td>70.98 ± 9.96</td>
<td>69.07 ± 9.4</td>
<td>67.3 ± 9.54</td>
<td>67.3 ± 9.89</td>
<td>68.8 ± 10.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>37.046 (54.6)</td>
<td>1,168 (39.3)</td>
<td>4,536 (48.4)</td>
<td>27,452 (57.4)</td>
<td>2,972 (52.7)</td>
<td>919 (45.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former</td>
<td>43,903 (64.7)</td>
<td>2,252 (75.7)</td>
<td>6,462 (68.9)</td>
<td>30,073 (62.8)</td>
<td>3,708 (65.7)</td>
<td>1,408 (70.2)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14,929 (22.0)</td>
<td>421 (14.2)</td>
<td>1,744 (18.6)</td>
<td>11,134 (23.3)</td>
<td>1,259 (22.3)</td>
<td>371 (18.5)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>9,034 (13.3)</td>
<td>302 (10.2)</td>
<td>1,169 (12.5)</td>
<td>6,688 (13.9)</td>
<td>679 (12.0)</td>
<td>226 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.03 ± 3.26</td>
<td>22.51 ± 3.26</td>
<td>24 ± 3.13</td>
<td>25.23 ± 3.13</td>
<td>25.89 ± 3.44</td>
<td>26.32 ± 3.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131.11 ± 16.46</td>
<td>129.54 ± 17.11</td>
<td>129.65 ± 16.41</td>
<td>131.22 ± 16.28</td>
<td>132.64 ± 16.69</td>
<td>133.36 ± 17.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.16 ± 10.35</td>
<td>76.26 ± 10.98</td>
<td>76.46 ± 10.41</td>
<td>77.25 ± 10.25</td>
<td>77.69 ± 10.51</td>
<td>78.27 ± 10.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>45.18 ± 14.43</td>
<td>43.62 ± 14.49</td>
<td>45.26 ± 14.03</td>
<td>45.45 ± 14.51</td>
<td>44.42 ± 14.19</td>
<td>42.84 ± 14.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>131.16 ± 47.69</td>
<td>132.31 ± 63.78</td>
<td>129.44 ± 52.04</td>
<td>131.42 ± 45.62</td>
<td>130.78 ± 45.81</td>
<td>132.25 ± 51.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>180.78 ± 45.04</td>
<td>177.53 ± 42.77</td>
<td>179.13 ± 42.26</td>
<td>181.11 ± 45.18</td>
<td>181.55 ± 49.61</td>
<td>183.1 ± 43.66</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>47.35 ± 14.11</td>
<td>48.25 ± 16.97</td>
<td>48.12 ± 14.23</td>
<td>47.19 ± 13.46</td>
<td>47.12 ± 15.84</td>
<td>46.89 ± 17.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>101.77 ± 44.95</td>
<td>101.25 ± 57.13</td>
<td>101.04 ± 56.78</td>
<td>101.81 ± 44.59</td>
<td>102.07 ± 52.36</td>
<td>104.09 ± 45.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>140.98</td>
<td>128.44</td>
<td>131.66</td>
<td>142.97</td>
<td>145.84</td>
<td>145.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>23.57 (23.5–23.64)</td>
<td>22.13 (21.81–22.45)</td>
<td>22.81 (22.63–22.99)</td>
<td>23.82 (23.74–23.91)</td>
<td>23.72 (23.48–23.97)</td>
<td>22.96 (22.58–23.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>20.46 (20.38–20.54)</td>
<td>17.34 (17.01–17.68)</td>
<td>18.78 (18.59–18.97)</td>
<td>21.06 (20.96–21.15)</td>
<td>20.64 (20.36–20.93)</td>
<td>19.29 (18.85–19.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>γ-GTP (IU/L)</td>
<td>27.51 (27.37–27.65)</td>
<td>23.97 (23.25–24.58)</td>
<td>25.73 (25.37–26.09)</td>
<td>28.15 (27.98–28.32)</td>
<td>27.64 (27.15–28.15)</td>
<td>26.2 (25.44–26.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin use</td>
<td>16,049 (23.6)</td>
<td>932 (31.3)</td>
<td>2,309 (24.6)</td>
<td>10,393 (21.7)</td>
<td>1,687 (29.9)</td>
<td>728 (36.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>42,679 (62.9)</td>
<td>1,926 (64.7)</td>
<td>5,869 (62.6)</td>
<td>29,946 (62.6)</td>
<td>3,579 (63.4)</td>
<td>1,359 (67.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metformin</td>
<td>45,679 (67.3)</td>
<td>2,128 (71.5)</td>
<td>6,518 (69.8)</td>
<td>31,995 (68.6)</td>
<td>3,729 (66.0)</td>
<td>1,309 (65.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Meglitinides</td>
<td>3,510 (5.2)</td>
<td>218 (7.3)</td>
<td>482 (5.1)</td>
<td>2,255 (4.7)</td>
<td>381 (6.7)</td>
<td>174 (8.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thiazolidinedione</td>
<td>6,774 (10.0)</td>
<td>306 (10.3)</td>
<td>881 (9.4)</td>
<td>4,408 (9.2)</td>
<td>858 (15.2)</td>
<td>321 (16.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DPP-4 inhibitor</td>
<td>16,149 (23.8)</td>
<td>736 (24.7)</td>
<td>2,403 (25.6)</td>
<td>11,086 (23.2)</td>
<td>1,449 (25.7)</td>
<td>475 (23.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-Glucosidase inhibitors</td>
<td>14,985 (22.1)</td>
<td>745 (25.0)</td>
<td>2,105 (22.5)</td>
<td>10,245 (21.4)</td>
<td>1,352 (24.0)</td>
<td>538 (26.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of OHA ≥ 3</td>
<td>22,106 (32.6)</td>
<td>1,100 (37.0)</td>
<td>3,185 (34.0)</td>
<td>15,002 (31.3)</td>
<td>2,040 (36.1)</td>
<td>779 (38.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
ALT, alanine transaminase; AST, aspartate transaminase; DBP, diastolic blood pressure; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OHA, oral hypoglycemic agents; SBP, systolic blood pressure; γ-GTP, gamma-glutamyl transpeptidase.
Table 2. Incidence rates and HRs for depression according to BMI category

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>No. of patients</th>
<th>No. of patients with depression</th>
<th>Follow-up duration (person-years)</th>
<th>Incidence rate per 1,000 person-years</th>
<th>HR (95% CI) Model 1a</th>
<th>HR (95% CI) Model 2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5</td>
<td>882</td>
<td>206</td>
<td>3,651.83</td>
<td>56.4101</td>
<td>1.04 (0.90–1.19)</td>
<td>1.04 (0.90–1.19)</td>
</tr>
<tr>
<td>≥18.5 to &lt;23</td>
<td>16,789</td>
<td>4,151</td>
<td>81,195.01</td>
<td>51.1238</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>≥23 to &lt;25</td>
<td>17,386</td>
<td>4,328</td>
<td>87,461.58</td>
<td>49.4846</td>
<td>0.99 (0.95–1.03)</td>
<td>1.00 (0.95–1.04)</td>
</tr>
<tr>
<td>≥25 to &lt;30</td>
<td>28,083</td>
<td>7,164</td>
<td>141,580.41</td>
<td>50.6002</td>
<td>1.02 (0.98–1.06)</td>
<td>1.02 (0.99–1.06)</td>
</tr>
<tr>
<td>≥30</td>
<td>4,726</td>
<td>1,174</td>
<td>23,815.04</td>
<td>49.2966</td>
<td>0.98 (0.92–1.04)</td>
<td>0.98 (0.92–1.04)</td>
</tr>
</tbody>
</table>

BMI, body mass index; CI, confidence interval; HR, hazard ratio.

*aAdjusted for age and sex. *bAdjusted for age, sex, smoking, alcohol consumption, regular exercise, low-income status, use of insulin, number of oral hypoglycemic agents, diabetes duration, and previous history of hypertension or dyslipidemia.

Table 3. Incidence rates and HRs for depression according to weight change status

<table>
<thead>
<tr>
<th>Weight change (%)</th>
<th>No. of patients</th>
<th>No. of patients with depression</th>
<th>Follow-up duration (person-years)</th>
<th>Incidence rate per 1,000 person-years</th>
<th>HR (95% CI) Model 1a</th>
<th>HR (95% CI) Model 2b</th>
<th>HR (95% CI) Model 3c</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥–10</td>
<td>2,975</td>
<td>837</td>
<td>13,052</td>
<td>64.1257</td>
<td>1.16 (1.08–1.25)</td>
<td>1.11 (1.04–1.20)</td>
<td>1.116 (1.04–1.20)</td>
</tr>
<tr>
<td>&lt;–10 to ≥–5</td>
<td>9,375</td>
<td>2,532</td>
<td>45,249</td>
<td>55.9567</td>
<td>1.09 (1.04–1.14)</td>
<td>1.07 (1.03–1.12)</td>
<td>1.07 (1.03–1.12)</td>
</tr>
<tr>
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<td>47,865</td>
<td>11,595</td>
<td>242,654</td>
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<td>1 (Reference)</td>
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<td>≥5 to &lt;10</td>
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<td>1,495</td>
<td>27,625</td>
<td>54.1184</td>
<td>1.12 (1.06–1.18)</td>
<td>1.07 (1.01–1.13)</td>
<td>1.07 (1.01–1.13)</td>
</tr>
<tr>
<td>≥10</td>
<td>2,005</td>
<td>564</td>
<td>9,123</td>
<td>61.8218</td>
<td>1.2 (1.10–1.31)</td>
<td>1.12 (1.03–1.22)</td>
<td>1.11 (1.02–1.12)</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio.

*aAdjusted for age and sex. *bAdjusted for age, sex, smoking, alcohol consumption, regular exercise, low-income status, use of insulin, number of oral hypoglycemic agents, diabetes duration, and previous history of hypertension or dyslipidemia. *cAdjusted for age, sex, smoking, alcohol consumption, regular exercise, low-income status, use of insulin, number of oral hypoglycemic agents, duration of diabetes, previous history of hypertension or dyslipidemia, and baseline body weight.

change and the risk of depression according to subgroups stratified by age, sex, BMI, presence of proteinuria, hypertension, diabetes duration, number of oral hypoglycemic agents, insulin use, and CKD stage (Fig. 3). In all subgroups, the risk of depression tended to increase as weight gain or loss increased. The status of obesity, based on a BMI of 25 kg/m², had a significant effect on the relationship between weight change and the risk of depression (p for interaction = 0.02). In patients without obesity (BMI, <25 kg/m²), only weight loss showed a significant association with an increased risk of depression (adjusted HR, 1.09 [95% CI, 1.03–1.15] for –10% to ≥–5% weight change and 1.12 [95% CI, 1.03–1.22] for ≥10% weight change). Weight gain was not associated with an increased risk of depression in patients without obesity. In obese patients (BMI, ≥25 kg/m²), both ≥–5% and ≥10% weight changes were significantly associated with an increased risk of depression (adjusted HR, 1.08 [95% CI, 1.01–1.16] for ≤–10% to ≥–5% weight change, 1.18 [95% CI, 1.02–1.37] for ≥–10% weight change, and 1.17 [95% CI, 1.05–1.30] for ≥10% weight change). CKD stage also had a significant effect on the relationship between...
weight change and the risk of depression (p for interaction = 0.001). In CKD stage 4 and 5 patients, weight gain tended to be associated with an increased risk of depression (adjusted HR, 1.38 [95% CI, 1.08–1.75] for ≥10% weight change in CKD stage 4 and 1.28 [95% CI, 0.93–1.77] for ≥10% weight change in CKD stage 5). In CKD stage 3b, the effect of weight gain on the risk of depression was less than that in stages 4 or 5. On the other hand, ≥-10% weight change significantly increased the risk of depression. The risk of depression associated with weight change did not differ according to the rest of the subgroups. Multicollinearity was not observed between the use of insulin or number of oral hypoglycemic agents and the duration of diabetes (Supplementary Table 1, available online).

We analyzed whether weight change affects the risk of depression in DKD patients according to the presence or absence of hospitalization. In DKD patients without a history of hospitalization, a weight gain or loss of 5% to 10% correlated with a significant increase in the risk of depression (Supplementary Table 2, available online). Patients with a weight gain or loss of 10% or more showed a tendency toward an increased risk of depression, but that finding was not statistically significant. In addition, comorbidities such as malabsorption disorders might have changed the effect of weight change on the risk of depression, so we analyzed that possibility. Although about 1,400 malabsorption patients were excluded from that analysis, the effect of weight change on the risk of depression did not change (Supplementary Table 3, available online).

### Discussion

In this nationwide population-based study, we investigated the relationship between BMI or weight change and the risk of depression. Our results show a U-shaped association between weight change and the risk of depression. Weight change of <–10% to ≥-5% and ≥-10% over 2 years increased the risk of depression by approximately 7% and 12%, respectively, compared with maintaining a stable weight (within 5%). Similarly, a weight change of ≥5% to <10% and ≥10% over 2 years also increased the risk of depression by approximately 7% and 11%, respectively. On the other hand, baseline BMI did not show any significant association with the risk of depression.

Although numerous studies have investigated the relationship between body weight and physical health, relatively few studies have examined the relationship between body weight and mental health. Several studies have confirmed a strong correlation between obesity and depression [15,23–26]. A meta-analysis of longitudinal studies showed that not only obesity (BMI, >30 kg/m²) but also being overweight (BMI, 25–30 kg/m²) increased the risk of depression (odds ratio [OR], 1.55 for BMI of ≥30 kg/m² and 1.27 for BMI of 25 to <30 kg/m²). Conversely, depression was found to predict the development of obesity (OR, 1.58). Several researchers have hypothesized about the mechanisms underlying the pathophysiological link between obesity and depression. The first possible mechanism is the psychological effects of obesity. Given the cultural equation of a lean body with beauty, obesity can lower the self-esteem of obese individuals [27]. Similarly, the stigma toward individuals with obesity could contribute to the de-
Figure 3. HRs (95% confidence intervals) for depression according to weight change status in subgroups.
BMI, body mass index; DM, diabetes mellitus; HR, hazard ratio; OHA, oral hypoglycemic agents.
velopment of depression. Studies have shown that persons with obesity are treated differently in many social situations, including in marriage relationships [28]. Therefore, obesity can increase psychological distress, which can lead to depression. Another possible mechanism is the adverse effects of obesity on physical health. Obesity is a strong risk factor for diabetes and cardiovascular diseases [29,30].

Those diseases lead to a decrease in physical ability and quality of life. Furthermore, the associated increase in medical costs can cause economic problems [31]. Obesity induces an inflammatory state [32,33], and inflammation has been reported to be associated with depression in [34–36]. In addition, obesity causes dysregulation of the hypothalamic–pituitary–adrenal axis [37,38], which is associated with the development of depression [39,40].

Although obesity and depression are strongly correlated, contrasting results have also been reported. However, results from studies about the relationship between underweight and the risk of depression are scarce and inconsistent. de Wit et al. [41] revealed a significant U-shaped association between BMI categories and depression in the general population of the Netherlands. In a meta-analysis of 76 longitudinal studies, both underweight and obesity increased the risk of depression [16]. In recent reports, underweight adolescents showed a tendency toward a depressed mood compared with their normal-weight peers [42,43]. In a study by McCrea et al. [44], the relationship between BMI and the risk of depression showed different patterns according to sex and age. In young women, the probability of having a mental disorder, including depression, increased with BMI. In contrast, in young men, the relationship was U-shaped, with high probabilities for both underweight and obese men. These associations diminished in the older age group. In another study, neither underweight nor obesity conferred a higher risk of depression than normal weight [45]. In this study, we evaluated the risk of depression according to baseline BMI and found that neither overweight nor underweight had a significant relationship with the risk of depression.

Therefore, we attempted to determine whether weight change, rather than BMI itself, is related to the risk of depression. The effect of dynamic weight changes on the occurrence of depression has been less studied than the effect of static weight status on depression. Singh et al. [17] reported that a weight gain of >2.5% of the baseline weight increased the risk of incident depression (OR, 1.30; 95% CI, 1.14–1.49) in middle-aged women. In that study, a loss of >2.5% in the baseline weight was significantly associated with an increased risk of incident depression in the age-adjusted analysis (OR, 1.25; 95% CI, 1.06–1.48); however, that result was not maintained after adjusting for confounding factors. A more recently published meta-analysis revealed that neither weight gain nor weight loss was associated with an increased risk of depression [16]. However, that meta-analysis included less than 10 studies in its analysis of the effect of weight change, and the study populations were rather heterogeneous. Our study showed somewhat different results from those previous studies. We found that both weight gain and weight loss were associated with an increased risk of depression. In addition, our analysis with a 1-year lag performed to consider the effect of reverse causality, produced consistent results. We presume that our results differ from those of previous studies because our study population was limited to patients with DKD. As mentioned above, patients with DKD are expected to have a poor prognosis because DKD is a combination of two chronic diseases and is highly likely to be accompanied by various comorbidities. Furthermore, DKD progresses more quickly than other CKDs because its pathophysiology involves diverse pathways and extensive ranges. Therefore, weight changes are more likely to affect the mental health of patients with DKD than in the general population.

Recently, An et al. [18] reported that an increase in body weight variability in a Korean population with type 2 diabetes was associated with an increased risk of depression. Specifically, they found that high body weight variability in either direction was associated with an increased risk of depression in a CKD subpopulation similar to our study population. In this study, we examined whether a decrease or increase in body weight increased the risk of depression and further tested the effects of the width of body weight changes. As described above, we found that both decreases and increases in body weight were associated with an increase in the risk of depression, such that a greater weight change carried a greater risk of depression. Although it is difficult to directly compare the results of our study with those of An et al. [18], it seems true that changes in body weight have a negative effect on mental health in DKD patients.

We analyzed the association between weight changes and the risk of depression according to subgroups stratified
by CKD stages and found the CKD stage had a significant effect on the relationship between weight change and the risk of depression. In advanced CKD patients (stages 4 and 5), weight gain tended to be associated with an increased risk of depression. In CKD stage 3b, a ≥10% weight change was found to significantly increase the risk of depression, but the effect of weight gain on the risk of depression was less than that in stages 4 or 5. Overall, weight gain appears to further increase the risk of depression as CKD advances. It can be assumed that the risk of developing depression increases when edema or weight gain occurs due to fluid retention in patients with poor renal function.

We also analyzed whether weight change in DKD patients affects the risk of depression according to hospitalization history because weight changes can occur rapidly in the hospital. In DKD patients without a history of hospitalization, a weight gain or loss of 5% to 10% correlated with a significant increase in the risk of depression. However, patients without a history of hospitalization who experienced a weight gain or loss of 10% or more showed a tendency toward an increased risk of depression but without statistical significance. For that reason, we estimate that patients who experienced a weight gain or loss of 10% or more were likely to have other physical conditions not counted in our data, and we assume that those diseases might have influenced this outcome.

Although this study provides important information about weight change and the risk of depression, it has several limitations. First, it was unclear whether the weight change in our patients, especially weight loss, was intentional. Intentional weight loss for health-related reasons, especially in patients with obesity, might have influenced the results of our study. Second, we identified the occurrence of depression using the relevant ICD-10 codes. However, that might have overestimated or underestimated the actual incidence of depression. Due to data limitations, we could not include the use of an antidepressant in the diagnostic criteria. In addition, among patients with diabetic neuropathy, there was a concern about over-diagnosing depression because tricyclic antidepressants are often used in the absence of depression. Similarly, we diagnosed DKD as diabetic patients with CKD (eGFR, <60 mL/min/1.73 m²). But without renal function decline, physicians do not usually enter a separate CKD diagnosis code for patients who have only proteinuria or those in the hyperfiltration phase of DKD, so they might have been excluded from the database when we chose our study population. Also, the operative definition of diabetes that we used here might have unintentionally excluded diabetes patients whose fasting blood glucose was below 126 mg/dL. Third, the effect of long-term weight change on the occurrence of depression might be different because the observation period for weight change in our study was relatively short. In addition, information about weight changes after the 2 years between health examinations was not considered in our analyses. Fourth, this study included only Koreans. Body weight is closely associated with socially accepted aesthetic standards, which could affect the incidence of depression. Therefore, the results of this study cannot be extended to other countries or races. Despite those limitations, our study has some strengths. This study included the largest ever sample size, although we limited the target population to patients with DKD, which is a group at high risk of depression. In addition, our study had a longitudinal design. Also, we minimized the possibility of reverse causality by performing an analysis with a 1-year lag. To our knowledge, this is the first study to examine the relationship between weight change and depression in a nationwide population.

In conclusion, both weight loss and weight gain increased the risk of depression in patients with DKD. But only weight loss, not weight gain, increased the risk of depression in nonobese DKD patients. Therefore, patients with DKD who experience weight loss or weight gain need to be informed about the possibility of depression. In addition, not only medical treatment but also psychological support should be provided to those patients.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

Anonymized data are publicly available from the National Health Insurance Sharing Service and can be accessed at https://nhiss.nhis.or.kr/bd/ab/bdaba000eng.do.

Authors’ contributions

Conceptualization: HSC, TRO, SHS, MK, CSK, EHB, SKM, SWK
Data curation, Formal analysis: BK, KDH
Funding acquisition: HSC, SWK
Supervision: SWK
Writing—original draft: HSC
Writing—review & editing: HSC, TRO, SHS, MK, CSK, EHB, SKM, SWK
All authors read and approved the final manuscript.

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Comparison of dominant and nondominant C3 deposition in primary glomerulonephritis

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Background: Alternative complement pathway dysregulation plays a key role in glomerulonephritis (GN) and is associated with C3 deposition. Herein, we examined pathological and clinical differences between cases of primary GN with C3-dominant (C3D-GN) and nondominant (C3ND-GN) deposition.

Methods: We extracted primary GN data from the Korean GlomeruloNEphritis sTudy (KoGNET). C3D-GN was defined as C3 staining two grades greater than C1q, C4, and immunoglobulin via immunofluorescence analysis. To overcome a large difference in the number of patients between the C3D-GN and C3ND-GN groups (31 vs. 9,689), permutation testing was used for analysis.

Results: The C3D-GN group exhibited higher serum creatinine (p ≤ 0.001), a greater prevalence of estimated glomerular filtration rate of <60 mL/min/1.72 m² (p ≤ 0.001), higher (but not significantly so) C-reactive protein level, and lower serum C3 level (p ≤ 0.001). Serum albumin, urine protein/creatinine ratio, number of patients who progressed to end-stage renal disease, and all-cause mortality were comparable between groups. Interstitial fibrosis and mesangial cellularity were greater in the C3D-GN group (p = 0.04 and p = 0.01, respectively) than in the C3ND-GN group. C3 deposition was dominant in the former group (p < 0.001), in parallel with increased subendothelial deposition (p ≤ 0.001).

Conclusion: Greater progression of renal injury and higher mortality occurred in patients with C3D-GN than with C3ND-GN, along with pathologic differences in interstitial and mesangial changes.

Keywords: Glomerulonephritis, Complement system C3 convertase, Alternative pathway
Introduction

The complement system is a major host defense mechanism of innate and adaptive immunity, with complement proteins synthesized and secreted in response to various stimuli [1]. The kidney is susceptible to complement-associated injury, which is most frequently triggered by immune complex (IC) deposition and classic complement pathway activation but can also be induced via activation of the alternative pathway (AP) in the absence of IC deposition [2]. Deficiency of specific components or defective complement regulation at different sites results in various manifestations of disease, clinical features, and outcomes [3].

Recently, C3 glomerulopathy (C3G) was designated as a unique pathological entity that includes a spectrum of diseases with predominant glomerular C3 fragment deposition in the near absence of C1q, C4, and immunoglobulins (Ig) under immunofluorescence (IF) analysis, with the underlying pathogenesis being driven by overactivation of the AP system [4–6]. The C3 protein plays a central role in C3G pathophysiology through its enzymatic cleavage into C3a and C3b. Of these, C3a is an anaphylatoxin and cytokine precursor, while C3b forms AP C3 convertase and amplifies the C3 activation loop. Through C5 convertase activation by AP C3 convertase, C5a and C5b are generated, with the latter leading to formation of the membrane attack complex. C3G can be subdivided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) based on electron microscopy (EM) findings. In DDD, EM reveals linear, hyperosmophilic deposits along the glomerular basement membrane and in the mesangium, while mesangial and/or subendothelial, intramembranous, and subepithelial deposits are present in C3GN. Additionally, DDD is associated with more aggressive clinical outcomes, such as end-stage renal disease (ESRD) [7,8].

Although C3G is confirmed by pathological findings, different features may appear under light microscopy (LM), and variable amounts of Ig can be detected via IF: C3G is considered a disease process rather than just a result of biopsy analysis [4]. Thus, we retrospectively redefined glomerulonephritis (GN) with C3-dominant deposition (C3D-GN) based on IF findings among primary GN cases from a multicenter database in Korea. Our aim was to investigate and compare the clinical characteristics, pathological findings, and long-term outcomes of C3D-GN in primary GN with those of non-C3 dominant GN (C3ND-GN).

Methods

This study was conducted in accordance with the 1964 Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Seoul National University Hospital (No. B-1707/408-106). The need for informed written consent was waived by the IRB because of the retrospective nature of the study and the minimal risk to participants.

Patients and data collection

This retrospective study used information from the Korean GlomeruloNephritis sTudy (KoGNET), which is a database containing information from 21,697 patients who underwent renal biopsies at 18 centers across Korea between 1979 and 2018. In all cases, routine analyses including LM, IF, and EM were performed, and a renal pathologist established the diagnosis at each hospital. IF findings were graded on a scale of 0–4, as follows: 0, trace; 1+; 2+; 3+; and 4+. All clinical data at the time of biopsy and the last follow-up were saved in the hospital information system. We retrieved medical records to obtain data on demographic and clinical features of age, sex, body mass index (BMI), underlying disease, renal function, proteinuria, serum C3, serum C4, and outcomes (ESRD and all-cause mortality).

Proteinuria was evaluated by a 24-hour quantitative measurement. The estimated glomerular filtration rate (eGFR) was calculated based on the original Modification of Diet in Renal Disease equation. Data on ESRD and mortality were extracted from each hospital’s information system and the ESRD registry of the Korean Society of Nephrology and the Statistics Korea, respectively. The median duration of follow-up was 99.03 ± 116.33 months for ESRD and 105.46 ± 116.16 months for mortality.

Definition of C3D-GN

Pathological findings were reviewed based on the evaluation findings of the pathologist at each center. Cases for which complete IF findings were not available were excluded. We extracted cases diagnosed with primary GN based on histologic findings in renal biopsy. Primary GN was categorized as minimal change disease (MCD), IgA
nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS), membranous GN (MN), and membranoproliferative GN (MPGN) without an evident cause such as viral disease (hepatitis B and C) or systemic disease. Except for all ambiguous or overlapping cases and missing data, only those diagnosed with primary GN were sorted. Both MCD and FSGS were excluded from the C3D-GN reclassification because the diagnosis is highly likely to change if C3 is the dominant deposit in those diseases. If the authors were unable to review the pathology slides anew, those diagnoses were excluded.

In principle, C3G can be defined based on IF and EM findings [9]. For a more precise definition, genetic testing or a complement system evaluation is needed; however, we could not obtain such data. Therefore, C3D-GN was defined as C3-dominant when C3 staining was at least two grades stronger than any combination of C1q, C4, IgG, IgM, and IgA by IF; similar to the definition of C3G. Owing to the large difference in patient counts between groups (50 C3D-GN patients vs. 13,070 C3ND-GN patients), we performed permutation tests for analyses (Fig. 1).

### Definition of histologic findings

Glomerular findings included sclerosis, crescent formation, ischemic injury, and mesangium cellularity. Sclerosis and crescent formation were defined as positive if the findings were greater than 10% of glomeruli. Ischemic injury was defined as positive if it was noted in LM findings. Interstitial fibrosis, inflammation, and tubular changes were graded, while vascular changes were defined as positive if atherosclerosis and intimal thickening were noted in LM findings. EM findings were defined as positive if mesangial, subendothelial, and subepithelial deposition were noted.

### Statistical analysis

For demographic, clinical, and laboratory findings, continuous variables are expressed as mean values, while categorical variables are expressed as prevalence rates. There was a large difference in patient count between the groups, and we used permutation testing to evaluate whether the differences between the study groups were significant. We

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**Figure 1. The selection of patients between C3D-GN and C3ND-GN cases.**

GN, glomerulonephritis; C3, complement component 3; C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; DDD, dense deposit disease.
performed a permutation test of the differences in baseline characteristics of C3ND-GN patient groups. The permutation test was performed on 10,000 permutations. In the permutation test, the two groups were assumed to be identical under the null hypothesis. Therefore, a random sample of 100 patients was selected among the permutations and randomly divided into two groups as many as 31 patients (C3ND-GN). For continuous variables, the averages are computed, and their differences are recorded. Then, p-values were calculated as the proportion of permutations with an absolute difference larger than that of our whole data. The permutation test for categorical variables was conducted in a similar way except that the difference in proportions for each category was computed and averaged over the categories. All analyses were conducted using R version 4.0.5 (R Foundation for Statistical Computing).

Results

The mean age of the C3ND-GN group was $41.41 \pm 16.27$ years, and that of the C3D-GN group was $38.92 \pm 20.31$ years. The proportion of males was 54.2% in the C3ND-GN and 58.1% in the C3D-GN group. The BMI was comparable between groups at approximately 24 kg/m$^2$. There were more diabetic patients and fewer cancer patients in the C3D-GN group than in the C3ND-GN group (16.1% vs. 9.0% and 0% vs. 8.3%, respectively) (Table 1).

Clinical differences

Systolic blood pressure was significantly higher in the C3D-GN group than in the C3ND-GN group ($135.37 \pm 23.90$ mmHg vs. $125.80 \pm 18.16$ mmHg, $p = 0.005$). Hemoglobin

### Table 1. Baseline characteristics of C3D-GN and C3ND-GN patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C3ND-GN group</th>
<th>C3D-GN group</th>
<th>p-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>9,689</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>41.41 ± 16.27</td>
<td>38.92 ± 20.31</td>
<td>0.34</td>
</tr>
<tr>
<td>Male sex</td>
<td>5,247 (54.2)</td>
<td>18 (58.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>845 (9.0)</td>
<td>5 (16.1)</td>
<td>0.09</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4,922 (51.5)</td>
<td>21 (67.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>265 (3.1)</td>
<td>0 (0)</td>
<td>0.64</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>211 (2.5)</td>
<td>1 (3.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Cancer</td>
<td>737 (8.3)</td>
<td>0 (0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Current smoker</td>
<td>745 (9.3)</td>
<td>1 (5.6)</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>23.82 ± 3.93</td>
<td>23.62 ± 3.89</td>
<td>0.82</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.80 ± 18.16</td>
<td>135.37 ± 23.90</td>
<td>0.005</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.12 ± 12.84</td>
<td>80.23 ± 15.19</td>
<td>0.37</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.99 ± 1.99</td>
<td>12.02 ± 2.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.58 ± 0.83</td>
<td>3.56 ± 0.91</td>
<td>0.92</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
<td>212.89 ± 80.44</td>
<td>203.88 ± 64.87</td>
<td>0.58</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>130.20 ± 64.87</td>
<td>127.75 ± 64.15</td>
<td>0.92</td>
</tr>
<tr>
<td>C3 (mg/dL)</td>
<td>108.13 ± 26.86</td>
<td>76.73 ± 40.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C4 (mg/dL)</td>
<td>28.29 ± 11.70</td>
<td>23.99 ± 11.64</td>
<td>0.06</td>
</tr>
<tr>
<td>ANCA</td>
<td>105 (2.0)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>ANA</td>
<td>923 (16.9)</td>
<td>1 (4.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>dsDNA</td>
<td>4 (0.1)</td>
<td>0 (0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Follow-up for ESRD (mo)</td>
<td>103.11 ± 91.41</td>
<td>123.00 ± 131.67</td>
<td>0.22</td>
</tr>
<tr>
<td>Follow-up for mortality (mo)</td>
<td>113.18 ± 96.34</td>
<td>137.55 ± 132.11</td>
<td>0.16</td>
</tr>
</tbody>
</table>

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

ANNA, antinuclear antibody > 1:320 titer; ANCA, antineutrophil cytoplasmic antibodies; BMI, body mass index; C3, complement component 3; C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; C4, complement component 4; DBP, diastolic blood pressure; dsDNA, double-stranded DNA; LDL, low-density lipoprotein; SBP, systolic blood pressure.

$^a$Continuous variables were assessed by the independent t test using permutation testing; categorical variables were assessed by the chi-square test using permutation testing.
Complement C3 level was within the normal range in both groups but lower in the C3D-GN group than in the C3ND-GN group (76.73 ± 40.69 mg/dL vs. 108.13 ± 26.86 mg/dL, p < 0.001). There was no difference in C4 level between groups. The level of C-reactive protein, a marker of inflammation, tended to be higher in the C3D-GN group than in the C3ND-GN group (2.02 ± 5.17 mg/dL vs. 1.07 ± 4.25 mg/dL, p = 0.097) (Table 2).

With regard to renal injury, serum creatinine (Cr) level in the C3D-GN group suggested the presence of greater damage than in the C3ND-GN group (2.17 ± 3.52 mg/dL vs. 1.20 ± 1.01 mg/dL, p < 0.001). The eGFR tended to be low and there were significantly more patients with eGFR of <60 mL/min/1.73 m² in the C3D-GN group than in the C3ND-GN group (43.3% vs. 26.8%, p ≤ 0.001). There was no significant difference between the groups in terms of number of patients with proteinuria of >3.5 g/Cr (Table 2).

For ESRD and all-cause mortality, the number of C3GN patients who progressed to ESRD (19.4%) and the all-cause mortality rate (6.5%) tended to be higher in the C3D-GN group (vs. 11.2% and 1.5% in the C3ND-GN group), although the difference was not significant.

### Histological differences

Among all primary GN cases, GN defined as C3-GN was most frequent among cases of MPGN (61.3%), followed by IgAN (25.8%) and MN (12.9%). (Table 3).

### Pathological findings in C3D-GN and C3ND-GN cases

<table>
<thead>
<tr>
<th>Pathological finding</th>
<th>C3D-GN</th>
<th>C3ND-GN</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global sclerosisc</td>
<td>4,838 (51.9)</td>
<td>4,478 (48.1)</td>
<td>0.46</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>18 (60.0)</td>
<td>12 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Segmental sclerosisb</td>
<td>5,517 (59.2)</td>
<td>3,801 (40.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>21 (70.0)</td>
<td>9 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Crescentc</td>
<td>7,875 (84.5)</td>
<td>1,448 (15.5)</td>
<td>0.61</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>24 (80.0)</td>
<td>6 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Ischemic injuryd</td>
<td>None</td>
<td>Present</td>
<td>0.64</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>9,120 (97.6)</td>
<td>223 (2.4)</td>
<td></td>
</tr>
<tr>
<td>C3D-GN</td>
<td>223 (2.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; UPCR, urine protein/creatinine ratio.

### Table 2. Clinical outcome comparison between C3D-GN and C3ND-GN groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C3ND-GN group (n = 9,689)</th>
<th>C3D-GN group (n = 31)</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.20 ± 1.01</td>
<td>2.17 ± 3.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>83.34 ± 70.83</td>
<td>74.85 ± 51.63</td>
<td>0.22</td>
</tr>
<tr>
<td>&gt;60</td>
<td>6,940 (73.2)</td>
<td>17 (56.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;60</td>
<td>2,537 (26.8)</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td>UPCR (g/g)</td>
<td>2.71 ± 3.46</td>
<td>2.71 ± 2.66</td>
<td>0.99</td>
</tr>
<tr>
<td>&lt;3.5</td>
<td>5,935 (75.4)</td>
<td>18 (75.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>&gt;3.5</td>
<td>1,934 (20.0)</td>
<td>6 (25.0)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>1.07 ± 4.25</td>
<td>2.02 ± 5.17</td>
<td>0.097</td>
</tr>
<tr>
<td>ESRD</td>
<td>1,082 (11.2)</td>
<td>6 (19.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>142 (1.5)</td>
<td>2 (6.5)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; UPCR, urine protein/creatinine ratio.

aContinuous variables were assessed by the independent t test using permutation testing; categorical variables were assessed by the chi-square test using permutation testing.

---

[Table 2. Clinical outcome comparison between C3D-GN and C3ND-GN groups]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C3ND-GN group (n = 9,689)</th>
<th>C3D-GN group (n = 31)</th>
<th>p-valuea</th>
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<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.20 ± 1.01</td>
<td>2.17 ± 3.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>83.34 ± 70.83</td>
<td>74.85 ± 51.63</td>
<td>0.22</td>
</tr>
<tr>
<td>&gt;60</td>
<td>6,940 (73.2)</td>
<td>17 (56.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;60</td>
<td>2,537 (26.8)</td>
<td>13 (43.3)</td>
<td></td>
</tr>
<tr>
<td>UPCR (g/g)</td>
<td>2.71 ± 3.46</td>
<td>2.71 ± 2.66</td>
<td>0.99</td>
</tr>
<tr>
<td>&lt;3.5</td>
<td>5,935 (75.4)</td>
<td>18 (75.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>&gt;3.5</td>
<td>1,934 (20.0)</td>
<td>6 (25.0)</td>
<td></td>
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</tr>
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<td>ESRD</td>
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</tr>
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<td>142 (1.5)</td>
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<td>0.08</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%).
C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; UPCR, urine protein/creatinine ratio.

aContinuous variables were assessed by the independent t test using permutation testing; categorical variables were assessed by the chi-square test using permutation testing.

---

[Table 3. Proportions of C3D-GN and C3ND-GN among primary GN cases]

<table>
<thead>
<tr>
<th>Variable</th>
<th>C3D-GN</th>
<th>C3ND-GN</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPGN</td>
<td>19 (61.3)</td>
<td>523 (5.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgAN</td>
<td>8 (25.8)</td>
<td>7,264 (75.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MN</td>
<td>4 (12.9)</td>
<td>1,902 (19.6)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; IgAN, immunoglobulin A nephropathy; MN, membranous nephropathy; MPGN, membranous proliferative GN.

aAssessed by the chi-square test using permutation testing.

---

[Table 4. Glomerular change in light microscopy findings in C3D-GN and C3ND-GN cases]

<table>
<thead>
<tr>
<th>Pathological finding</th>
<th>&lt;10%</th>
<th>&gt;10%</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global sclerosisc</td>
<td>4,838 (51.9)</td>
<td>4,478 (48.1)</td>
<td>0.46</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>18 (60.0)</td>
<td>12 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Segmental sclerosisb</td>
<td>5,517 (59.2)</td>
<td>3,801 (40.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>21 (70.0)</td>
<td>9 (30.0)</td>
<td></td>
</tr>
<tr>
<td>Crescentc</td>
<td>7,875 (84.5)</td>
<td>1,448 (15.5)</td>
<td>0.61</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>24 (80.0)</td>
<td>6 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Ischemic injuryd</td>
<td>None</td>
<td>Present</td>
<td>0.64</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>9,120 (97.6)</td>
<td>223 (2.4)</td>
<td></td>
</tr>
<tr>
<td>C3D-GN</td>
<td>223 (2.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN.

aAssessed by permutation testing.
Within 10% of glomeruli in light microscopy.
cases of IgAN (25.8%) and MN (12.9%) (Table 3, 4).

There was no significant difference in glomerular change between groups. Rates of global sclerosis, crescent formation, and ischemic injury tended to be similar in the C3D-GN and C3ND-GN groups. However, there was a significant pattern of greater proliferation of mesangial cellularity in the C3D-GN group (Table 5, 6).

Compared to the C3ND-GN group, the C3D-GN group exhibited a significantly greater rate of interstitial fibrosis (above moderate, 8.0% vs. 10.0%; p = 0.04). Although not significant, vascular atherosclerosis and intimal thickening were more severe in the C3D-GN group (Table 7).

Regarding complement and Ig deposition as evaluated by IF, notable C3 deposition was observed in the C3D-GN group. IgA was more frequent in the C3ND-GN group, but the C3D-GN group also exhibited trace and grade 1 deposition of IgA. IgM and IgG were deposited up to grades 1 and 2.

### Table 5. Cellularity change in light microscopy findings in C3D-GN and C3ND-GN cases

<table>
<thead>
<tr>
<th>Pathological characteristic</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Moderate to severe</th>
<th>Severe</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellularity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>5,495 (58.8)</td>
<td>2,731 (29.2)</td>
<td>847 (9.1)</td>
<td>54 (0.6)</td>
<td>216 (2.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>18 (60.0)</td>
<td>6 (20.0)</td>
<td>3 (10.0)</td>
<td>0 (0)</td>
<td>3 (10.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Mesangial matrix</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>5,675 (60.7)</td>
<td>2,518 (27.0)</td>
<td>765 (8.2)</td>
<td>147 (1.6)</td>
<td>238 (2.5)</td>
<td>0.78</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>19 (63.3)</td>
<td>10 (33.3)</td>
<td>1 (3.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Mesangial cellularity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>3,678 (39.4)</td>
<td>4,016 (43.0)</td>
<td>1,092 (11.7)</td>
<td>174 (1.9)</td>
<td>383 (4.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>10 (33.3)</td>
<td>9 (30.0)</td>
<td>5 (16.7)</td>
<td>1 (3.3)</td>
<td>5 (16.7)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%). C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN.
<sup>a</sup>Assessed by permutation testing.

### Table 6. Pathological light microscopy findings in the interstitium and tubule of C3D-GN and C3ND-GN patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Moderate to severe</th>
<th>Severe</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interstitium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>3,315 (35.7)</td>
<td>3,769 (40.6)</td>
<td>1,451 (15.6)</td>
<td>150 (1.6)</td>
<td>589 (6.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>18 (60.0)</td>
<td>5 (16.7)</td>
<td>4 (13.3)</td>
<td>0 (0)</td>
<td>3 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>3,522 (38.0)</td>
<td>3,874 (41.8)</td>
<td>1,290 (13.9)</td>
<td>103 (1.1)</td>
<td>473 (5.1)</td>
<td>0.20</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>14 (46.7)</td>
<td>8 (26.7)</td>
<td>4 (13.3)</td>
<td>0 (0)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Tubules</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>2,739 (29.6)</td>
<td>4,215 (45.5)</td>
<td>1,518 (16.4)</td>
<td>158 (1.7)</td>
<td>626 (6.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>14 (46.7)</td>
<td>8 (26.7)</td>
<td>3 (10.0)</td>
<td>0 (0)</td>
<td>5 (16.7)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%). C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN.
<sup>a</sup>Assessed by the Pearson chi-square test using permutation testing.

### Table 7. Pathological light microscopy findings in the vessels of C3D-GN and C3ND-GN patients

<table>
<thead>
<tr>
<th>Vessel</th>
<th>None</th>
<th>Present</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atherosclerosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>8,198 (87.7)</td>
<td>1,145 (12.3)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>26 (86.7)</td>
<td>4 (13.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Intimal thickening</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3ND-GN</td>
<td>7,445 (79.7)</td>
<td>1,898 (20.3)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>24 (80.0)</td>
<td>6 (20.0)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number (%). C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN.
<sup>a</sup>Assessed by the Pearson chi-square test using permutation testing.
Table 8. IF pathological findings in C3D-GN and C3ND-GN

<table>
<thead>
<tr>
<th>Variable</th>
<th>None</th>
<th>Trace</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3ND-GN</td>
<td>3,829 (39.5)</td>
<td>1,001 (10.3)</td>
<td>2,351 (24.3)</td>
<td>1,653 (17.1)</td>
<td>779 (8.0)</td>
<td>76 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (16.1)</td>
<td>18 (58.1)</td>
<td>8 (25.8)</td>
<td>0.23</td>
</tr>
<tr>
<td>C3</td>
<td>&lt;0.001</td>
<td>8,337 (86.0)</td>
<td>561 (5.8)</td>
<td>547 (5.6)</td>
<td>168 (1.7)</td>
<td>61 (0.6)</td>
<td>15 (0.2)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>23 (74.2)</td>
<td>4 (12.9)</td>
<td>3 (9.7)</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C1q</td>
<td>0.23</td>
<td>3,552 (36.7)</td>
<td>492 (5.1)</td>
<td>1,839 (19.0)</td>
<td>1,827 (18.9)</td>
<td>1,667 (17.2)</td>
<td>331 (3.2)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>21 (67.7)</td>
<td>4 (12.9)</td>
<td>6 (19.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.35</td>
</tr>
<tr>
<td>IgA</td>
<td>0.23</td>
<td>6,476 (66.8)</td>
<td>708 (7.3)</td>
<td>1,232 (12.7)</td>
<td>625 (6.5)</td>
<td>569 (5.9)</td>
<td>79 (0.8)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>22 (71.0)</td>
<td>2 (6.5)</td>
<td>7 (22.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.67</td>
</tr>
<tr>
<td>IgG</td>
<td>0.35</td>
<td>5,533 (57.1)</td>
<td>1,459 (15.1)</td>
<td>2,061 (21.3)</td>
<td>531 (5.5)</td>
<td>79 (0.8)</td>
<td>26 (0.3)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>19 (61.3)</td>
<td>2 (6.5)</td>
<td>9 (29.0)</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IgM</td>
<td>0.73</td>
<td>7,363 (76.0)</td>
<td>357 (3.7)</td>
<td>863 (8.9)</td>
<td>681 (7.0)</td>
<td>399 (4.1)</td>
<td>26 (0.3)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>22 (71.0)</td>
<td>3 (9.7)</td>
<td>3 (9.7)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Kappa</td>
<td>0.73</td>
<td>6,920 (71.4)</td>
<td>291 (3.0)</td>
<td>726 (7.5)</td>
<td>988 (10.2)</td>
<td>699 (7.2)</td>
<td>65 (0.7)</td>
</tr>
<tr>
<td>C3D-GN</td>
<td>22 (71.0)</td>
<td>2 (6.5)</td>
<td>4 (12.9)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
C3; complement C3, C1q; complement 1q, C3D-GN, C3-dominant glomerulonephritis (GN); C3ND-GN, non-C3 dominant GN; IF; immunofluorescence; Ig; immunoglobulin.
*Assessed by the Pearson chi-square test using permutation testing.

2 in the C3D-GN group, which was comparable to observations in the C3ND-GN group. Light chains were deposited at various grades in both groups (Table 8).

EM revealed that the C3D-GN group had greater subendothelial deposition than the C3ND-GN group (48.4% vs. 12.0%, p < 0.001). Additionally, subepithelial deposition and podocyte effacement were more pronounced in the C3D-GN group, but the difference was not significant (Table 9).

Acute changes, such as crescent formation, mesangial proliferation, vascular wall thickening, and interstitial inflammation, seemed more pronounced in the C3D-GN group than in the C3ND-GN group. Interstitial fibrosis, considered a chronic change, was significantly more frequent in the C3D-GN group.

Discussion

Our study revealed a significant prevalence of C3-domi-
nant deposition in primary GN cases with differences in clinical and histological findings compared to cases of non-dominant C3 deposition. We defined C3D-GN as C3 accumulation at least two grades greater than any other immune-reactant deposition as determined by IF, as previously proposed by Hou et al. [9]. That definition was used to reclassify C3G according to the degree of deposition in primary GN. However, as no evaluation such as pathologi-
cal slide review or AP system analysis was performed, it was deemed more appropriate to record such cases as C3D-GN rather than C3G. Hou et al. [9] tested several IF criteria with varying stringencies for a more precise C3G definition, proposing that a strict definition, such as “C3 only,” is imprac-
tical. Thus, “C3 dominance and at least two grades more in-
tense than any immune reactant (IgG, IgM, IgA, and C1q)” was proposed as a more useful classification. Accordingly, we established our definition of C3D-GN.

Based on our definition, cases of MPGN (61.3%) were most commonly reclassified as C3D-GN, with IgAN (25.8%) and MN (12.9%) cases also often reclassified. MPGN exhibited similar LM findings to those in C3G. Accordingly, our data indicate that C3D-GN occurred mostly in MPGN cases.

In our study comparing C3D-GN and C3ND-GN, LM findings revealed significantly more frequent severe mesangial proliferation in C3D-GN. Also, interstitial fibrosis, inflammation, and tubular atrophy were more progressive in C3D-GN; in particular, interstitial fibrosis was signific-
tantly more severe, in agreement with the histological findings of C3G [8]. However, these findings should be interpreted with caution as both acute inflammation and chronicity are more advanced in C3D-GN [10].

Although the C3D-GN definition is different from the strict C3G definition, C3-dominant deposition could be associated with AP abnormalities. AP dysregulation is central to the pathogenesis of C3G, which is related to gen-
etic deficiencies, such as those of complement factor H (CFH), CFHR1-5, complement factor I, and CD46, or to auto-antibodies against factor H, factor B, and C convers-
tase [4,7,9,11]. A strict definition of C3G with no or scarce deposition of immune factors derived from the classic pathway has been extensively adopted. However, if C3G is con-
sidered part of the disease process, deposition of other mediators may also occur during C3G [4].

IF analysis of C3D-GN revealed multiple immune-reactant depositions. C3 deposition was more prominent than that of other mediators, yet C1q, IgG, IgM, and IgA deposi-
tions were observed up to grade 1. C1q often initiates the classic pathway, and it can interfere with the AP by binding to C3b. Activation of CP via C1q can occur through deposits of IgG1 or IgG3, which have been reported to precede IgG4 deposition early in MN [12–14]. IgM staining could be attributed to nonspecific trapping in areas of sclerosis or cap-
illary wall thickening [9]. IgA was deposited up to grade 1, as observed in cases where IgAN or MPGN was the primary diagnosis. In IgAN, IgA deposition may be attributed to the nature of the disease. With regard to MPGN, a review of the biopsy specimens from some cases revealed IgA deposition in previously diagnosed MPGN. Furthermore, in rare cases, IgA deposition was more dominant than that of other immu-
nee reactants [15,16]. Taken together, the findings indi-
cate that other forms of immunologic injury may occur in the glomeruli under C3G, and immune reactions, such as CP activation, that induce the deposition of IgM, IgG, and other mediators cannot be excluded. In addition to MPGN, C3 may be deposited in IgAN or MN. When C3 deposition was accompanied by IgAN and MN, the renal outcome was worse in cases with above-moderate C3 dominance [17–19]. Therefore, it is necessary to review and reclassify the pri-
mary diagnosis; due to practical problems, this should be considered a limitation of the present study.

The serum C3 level in the C3D-GN group was within normal limits but lower than that of the C3ND-GN group. Increased renal injury rates and a greater tendency for ele-
vated C-reactive protein level were observed in the C3D-
GN group. These observations may be a result of AP system dysregulation. Some previous studies have documented a decrease in renal function in C3G patients at diagnosis [20,21]. Considering these findings together with our current results, we hypothesize that a similar clinical pattern would be present as a result of AP dysregulation—that is, renal injury and inflammation would be attributed to dam-
age caused by the already advanced CP activation as well as additional damage due to AP dysregulation. Regardless of which pathway is activated first, the amplification loop of complement activation can occur through a cascade that primarily involves proteins of the AP, with AP activation stimulating other complement systems to induce further tissue inflammation [22]. As our study did not evaluate AP dysregulation, we cannot conclude whether this was the driving mechanism. Nevertheless, we consider the pos-
sibility of AP dysregulation contributing to the observed findings.

With regard to clinical outcome, in the C3D-GN group, more patients progressed to ESRD, but this difference was not significant. At the time of diagnosis, acute injury was more prevalent, mesangial proliferation and interstitial inflammation indicating acute changes in pathological findings were severe, and interstitial fibrosis indicating chronicity showed significant progress in C3D-GN patients, which suggests that ESRD patients tended to be more prevalent in the C3D-GN group. However, the small number of patients likely limited our results. In addition, ESRD progression is influenced by the patient’s response to medications and other treatments and the presence of other diseases. However, this study did not evaluate those factors, so it is possible that they may have influenced the occurrence of ESRD. C3D-GN patients showed a higher rate of all-cause mortality, but the difference was not significant. In terms of C3G and mortality, a previous study documented lower survival with C3GN [20]. That study recorded fewer deaths and patients who died of sepsis and cancer and did not confirm how C3GN affected mortality. Other studies have determined that the mortality rate is high or better in C3G, although the relationship between C3G and mortality is uncertain [23,24]. However, a lower C3 level or the AP, which is assumed to be the main pathology in this study, has an effect on mortality and allowed hypothesis about the increase in mortality [25,26]. Nonsignificant differences may also have had an impact due to the small number of patients. The results of this study suggest the importance of reassessment of C3-dominant deposition if renal injury has progressed or acute inflammation is severe. In addition, the effects of APs in clinical manifestations can be considered if C3 is dominant in pathology and serum C3 level is low. If further studies show that dominant C3 deposition and lower C3 level are directly related to APs and if an acute-phase inflammatory marker or kidney injury is concurrent with a difference in C3 level, it is necessary to change or reclassify the diagnosis considering the treatment and prognosis of APs.

This study’s relevance lies in the reclassification of primary GN as C3-dominant and the evaluation of its clinical and histological characteristics. Studies on the classification and evaluation of primary GN based on C3 dominance are limited. By analyzing KoGNET data, we discovered that most GN cases in Republic of Korea were IgAN (34.17%), MN (9.17%), MCD (9.13%), and FSGS (7.65%), and all these conditions were more prevalent than MPGN (2.63%) [27]. As other primary GNs may also exhibit complement system abnormalities, we sought to determine the present aspects of C3 deposition.

The present study has some limitations. First, we reviewed IF data based on biopsy reports rather than through reevaluation of the original IF glass slides. Thus, the precise characteristics and distribution of staining for each factor could not be evaluated. Discrepancies in diagnosis and grading systems between hospitals can exist, so review of pathologic slides by a single pathologist is necessary. However, there were no data to identify patients, and it was difficult to retrieve and review all pathological slides in 18 centers. This is the major limitation of our study. Second, the primary diagnoses were not reviewed by a second pathologist. Such review might have resulted in different primary diagnosis through slide reinterpretation according to the complements and Ig deposition. If there was a change in the primary diagnosis after review, patient characteristics or outcomes may also have differed. However, as mentioned earlier, not conducting a slide review is also a limitation. In future research, we will perform a pathology slide review. Third, we did not evaluate AP dysregulation and could not determine whether it was an underlying cause of C3D-GN. If the AP system had been evaluated, the association with C3-dominant deposition and explanations of clinical and pathological differences may have been supplemented.

The current study showed that C3D-GN could constitute an additional category of primary GN. C3D-GN patients exhibited different pathologic and clinical features, highlighting the importance of considering complement deposition. With regard to C3-dominant deposition, additional studies are necessary to evaluate molecular and genetic abnormalities at each step and to assess AP system involvement. Taken together, the observed differences between C3-dominant and nondominant deposition in primary GN emphasize the importance of complement dysregulation in the pathophysiology of GN.

**Conflicts of interest**

All authors have no conflicts of interest to declare.
Funding

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Data sharing statement

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

Authors’ contributions

Conceptualization: JR, HJC
Methodology: JR, JCJ
Software: EB, HES
Validation: SK, KYN
Formal analysis: JR, HES, JYR
Investigation: JR, SK
Data curation: EB, HES, SPK, SHK
Writing–original draft preparation: JR
Writing–review and editing: JR, HJC
Visualization: JCJ, JHJ, SK, SPK, SHK
Supervision: HJC, DWC
Project administration: TIC, BSC
All authors have read and agreed to the published version of the manuscript.

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References


Hemodialysis facility star rating affects mortality in chronic hemodialysis patients: a longitudinal observational cohort study

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Background: Many countries have their own hemodialysis (HD) quality assurance programs and star rating systems for HD facilities. However, the effects of HD quality assurance programs on patient mortality are not well understood. Therefore, in the present study, the effects of the Korean HD facility star rating on patient mortality in maintenance HD patients were evaluated.

Methods: This longitudinal, observational cohort study included 35,271 patients receiving HD treatment from 741 facilities. The five-star ratings of HD facilities were determined based on HD quality assessment data from 2015, which includes 12 quality measures in structural, procedural, and outcome domains. The patients were grouped into high (three to five stars) and low (one or two stars) groups based on HD facility star rating. Cox proportional hazards model was used to evaluate the effects of star rating on patient mortality during the mean follow-up duration of 3 years.

Results: The patient ratio between high and low HD facility star rating groups was 82.0% vs. 18.0%. The patients in the low star rating group showed lower single-pool Kt/V and higher calcium and phosphorus levels compared with subjects in the high star rating group. After adjusting for sociodemographic and clinical parameters, the HD facility star rating independently increased the mortality risk (hazard ratio, 1.11; 95% confidence interval, 1.04–1.18; p = 0.002).

Conclusion: The HD facilities with low star rating showed higher patient mortality.

Keywords: Health care, Health care quality assurance, Mortality, Outcome assessment, Renal dialysis
Introduction

The number of hemodialysis (HD) patients increases every year and health expenditures for their treatment continue to expand [1]. The main reasons for the recent increase in the number of HD patients are the increased prevalence of underlying comorbidities such as diabetes and hypertension as well as the increasing geriatric population [2]. Because mortality and morbidity rates are higher in HD patients than in the general population and subsequent healthcare cost is exponentially increasing, evaluating adequacy and quality of HD service is important to improve outcomes in HD patients as well as to reduce medical cost [3,4].

Korea is a country with a rapidly rising prevalence of HD patients [5], increasing nearly 50% from 42,596 in 2011 to 62,634 in 2015. Accordingly, the number of HD centers in Korea has increased by 20% from 770 in 2011 to 917 in 2015 [6]. Therefore, an HD quality assessment tool was developed by the Health Insurance Review and Assessment (HIRA) Service to control HD quality and reduce medical costs [7]. After a pilot survey in 2008, the HIRA has performed HD quality assessment regularly since 2009 and provided HD facilities with five-star ratings based on assessment results.

The HD facility star rating helps each HD center improve the quality of service to their patients. In addition, the rating provides useful information regarding HD facilities to the patients in a recognizable format [8]. Many countries have their own HD quality assurance programs and star rating system for HD facilities [3,9–12]. In 2014, an international group of experts gathered to develop recommendations on how to develop and implement quality assurance measures among HD facilities [13].

Although items included in HD quality assessment tools differ by country and are continuously amended yearly, the effects of HD quality assurance programs on patient mortality are poorly understood. Therefore, in the present study, the effects of HD facility star rating developed by the Korean HIRA on patient mortality among maintenance HD patients were evaluated.

Methods

This study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board of Ewha University Medical Center approved the study protocol (No. EUMC 2018-12-025) and written informed consent was waived due to the retrospective study design.

Study design

This was a longitudinal, observational cohort study among Korean maintenance HD patients. The baseline data including HD facility star rating were collected from HD quality assessment data starting in 2015 and mortality data collected through June 2019.

Hemodialysis facility star rating method

The HD quality assessment tool includes 12 quality measures in three domains including structural, procedural, and outcome (Supplementary Table 1, available online). The five-star rating was determined based on the sum of weighted scores from 12 measures of HD quality (Supplementary Table 2, available online). A total score summed up to 100. The weight was applied from 0.5 to 2.0 based on the importance of the measures. The star rating ranged from one-star to five-star based on the absolute sum of weighted scores: one-star, <65; two-star, 65 to 75; three-star, 75 to 85; four-star, 85 to 95; and five-star, ≥95.

Data source and study population

The target patients were 18 years of age or older who underwent HD at least twice weekly as outpatients at a single HD center during the assessment period. Subjects who were admitted to the hospital during the assessment period, received HD less than twice weekly, or transferred to another HD unit were excluded from the analysis. The HD centers selected were facilities in which HD services were performed with HD equipment and claims submitted for HD fees. The HD facilities with less than five measurements in either procedural or outcome domains were excluded from the star rating.

The HD service providers who submitted fee claims in 2015 were screened and the 12 measures in three domains (structure, process, and outcome) were assessed from October 2015 to December 2015. The assessment data were collected using a web-based data collection system. Each
HD facility entered the general information regarding HD facilities, number of HD treatments, medical expenses, and number of HD equipment. In addition, information regarding the seven measures in the structural domain were entered such as personnel, availability of isolated HD equipment and emergency equipment, and satisfaction of the minimum required frequency of water quality testing. Lastly, the following patient factors in procedural and outcome domains were entered: frequency and satisfaction rate of HD adequacy, vascular access stenosis monitoring, frequency of regular laboratory tests, and satisfaction rate of calcium and phosphorus control. Data retrieved from the web-based database were compared with the data from electronic medical records to confirm the accuracy and reliability.

Sociodemographic and clinical data were obtained from the HIRA database. The sociodemographic factors collected included age, sex, dialysis vintage, cause of end-stage renal disease, body mass index, and health insurance status. The medical comorbidities of the subjects were identified by reviewing the medical history 1 year before the initiation of dialysis therapy. The International Classification of Disease (ICD-10) codes were used to extract the following comorbidities: ischemic heart disease (I20–25), congestive heart failure (I50), cerebrovascular disease (I60–64, I69), diabetes mellitus (E10–14), hypertension (I10–13, I15), and atrial fibrillation (I48). Predialysis systolic and diastolic blood pressures were measured. The laboratory parameters including plasma hemoglobin, serum albumin, calcium, and phosphorus, were collected every month during the assessment period. The single-pool Kt/V was measured as an indicator of HD adequacy. The date of the patient’s death was estimated from the date of insurance loss.

Statistical analyses

Statistical analyses were performed using SAS Enterprise Guide 6.1 (SAS Institute). The subjects were divided into two groups based on star rating: low star rating (one or two stars) and high star rating (three to five stars). The chi-square test was used to compare categorical variables and independent t test was used to compare continuous variables between groups. Kaplan-Meier survival curves were used to compare the risk of mortality between groups. The patients who received kidney transplantation after HD quality assessment were censored in survival analysis. Multivariable-adjusted Cox proportional hazards model was used to evaluate star rating as an independent variable for mortality. Model 1 was adjusted for age, sex, dialysis vintage, and body mass index. Model 2 was adjusted for medical comorbidities in addition to the factors included in model 1. Model 3 was adjusted for all sociodemographic and clinical factors including health insurance status and laboratory parameters. Finally, subgroup analyses were used to define the relative risk of mortality based on star rating.

Results

Baseline characteristics of the subjects based on hemodialysis facility star rating

A total of 35,271 HD patients from 741 HD centers were included in the analysis. The average star rating score was 83.1 ± 11.2. Based on the five-star rating system, 82 centers (11.1%) received five stars, 298 (40.2%) received four stars, 208 (28.1%) received three stars, 104 (14.0%) received two stars, and 49 (6.6%) received one star.

Baseline characteristics of the subjects based on star rating groups are presented in Table 1. A total of 28,907 patients from 588 HD facilities were included in the high star rating group and 6,364 patients from 153 HD facilities were included in the low star rating group. The patients in the low star rating group had higher serum calcium and phosphorus levels, higher diastolic blood pressure but a lower proportion of chronic heart failure and lower single poor Kt/V compared with patients in the high star rating group.

Crude rate of all-cause mortality based on hemodialysis facility star rating

A total of 7,630 deaths (21.6%) occurred during 36.2 ± 11.1 months. The crude death rate was 72 patients per 1,000 person-years. The crude mortality rate ratio was lower in the high star rating group than in the low star rating group (69 patients vs. 82 patients per 1,000 person-years, p < 0.001). However, minimal difference was found between five-star and four-star ratings (Supplementary Table 3, available online). After censoring 2,033 cases (5.8%) who received kidney transplantation, Kaplan-Meier survival
curve showed a lower risk of patient mortality in the high star rating group than in the low star rating group (Fig. 1).

Hemodialysis facility star rating independently increases mortality risk

Cox proportional hazards model was used to determine risk factors associated with patient mortality (Table 2). In univariate analysis, older age, male sex, lower body mass index, higher systolic blood pressure, lower diastolic blood pressure, presence of comorbidities (diabetes mellitus, ischemic heart disease, heart failure, and cerebrovascular accident), lower plasma hemoglobin, lower serum albumin, and National Health Insurance status were associated with higher mortality risk. In addition, the low star rating group was associated with higher mortality (hazard ratio [HR], 1.18; 95% confidence interval [CI], 1.12–1.25; p < 0.001). After adjusting for age, sex, dialysis vintage, and body mass index (model 1), the low star rating group remained an independent predictor for patient mortality (HR, 1.12; 95% CI, 1.05–1.20; p < 0.001). After adjusting for comorbidities in addition to factors included in model 1 (model 2), the low star rating group remained an independent risk factor for patient mortality (HR, 1.10; 95% CI, 1.03–1.17; p = 0.004). After adjusting for sociodemographic and clinical factors found significantly associated with mortality in univariate analysis (model 3), the low star rating group remained an independent risk factor for patient mortality (HR, 1.11; 95% CI, 1.04–1.18; p = 0.002).

Patients in the low star rating group showed poorer patient survival across different subgroups except those with cerebrovascular disease (Fig. 2). Patients younger than 65 years of age and shorter dialysis vintage (<5 years) showed a higher benefit from selecting a high-star-rating HD facility.

Discussion

In this prospective cohort study using nationwide HD quality assessment data, the effects of HD facility star rating on patient mortality were evaluated. The patients in the HD facilities with low star ratings (one or two stars) showed lower HD adequacy, higher serum calcium and phosphorus levels as well as higher diastolic blood pressure. The HD facilities with low star ratings had poorer patient survival compared with high-star-rating facilities. Multivariable Cox regression analysis showed that low HD facility star rating

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**Table 1. Baseline characteristics of the subjects based on HD facility star rating**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 35,271)</th>
<th>1–2 Stars (n = 6,364)</th>
<th>3–5 Stars (n = 28,907)</th>
<th>Standardized means difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HD facilities</td>
<td>741</td>
<td>153</td>
<td>588</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>60.1 ± 12.8</td>
<td>60.8 ± 12.3</td>
<td>59.9 ± 12.9</td>
<td>0.070</td>
</tr>
<tr>
<td>Male sex</td>
<td>20,758 (58.9)</td>
<td>3,801 (59.7)</td>
<td>16,957 (58.7)</td>
<td>0.020</td>
</tr>
<tr>
<td>Dialysis vintage (yr)</td>
<td>5.7 ± 5.2</td>
<td>6.1 ± 5.4</td>
<td>5.7 ± 5.2</td>
<td>0.077</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20,539 (58.2)</td>
<td>3,870 (60.8)</td>
<td>16,669 (57.7)</td>
<td>0.063</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28,584 (81.0)</td>
<td>5,296 (83.2)</td>
<td>23,288 (80.6)</td>
<td>0.068</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>11,311 (32.1)</td>
<td>2,192 (34.4)</td>
<td>9,119 (31.6)</td>
<td>0.060</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4,691 (13.3)</td>
<td>673 (10.6)</td>
<td>4,018 (13.9)</td>
<td>0.101</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>2,942 (8.3)</td>
<td>564 (8.9)</td>
<td>2,378 (8.2)</td>
<td>0.025</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.7 ± 0.9</td>
<td>10.7 ± 0.9</td>
<td>10.7 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.99 ± 0.35</td>
<td>4.0 ± 0.36</td>
<td>3.98 ± 0.34</td>
<td>0.057</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>8.99 ± 0.81</td>
<td>9.15 ± 0.82</td>
<td>8.95 ± 0.8</td>
<td>0.247</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.94 ± 1.33</td>
<td>5.12 ± 1.39</td>
<td>4.9 ± 1.31</td>
<td>0.165</td>
</tr>
<tr>
<td>Single-pool Kt/V</td>
<td>1.55 ± 0.28</td>
<td>1.50 ± 0.28</td>
<td>1.56 ± 0.28</td>
<td>0.214</td>
</tr>
<tr>
<td>Medical Aid</td>
<td>7,053 (20.0)</td>
<td>1,298 (20.4)</td>
<td>5,722 (19.8)</td>
<td>0.015</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.4 ± 3.4</td>
<td>22.1 ± 3.2</td>
<td>22.4 ± 3.4</td>
<td>0.088</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141.2 ± 15.5</td>
<td>142.0 ± 15.6</td>
<td>141.0 ± 15.5</td>
<td>0.065</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.6 ± 9.6</td>
<td>79.3 ± 8.8</td>
<td>77.2 ± 9.7</td>
<td>0.219</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%). HD, hemodialysis; Kt/V, hemodialysis adequacy.
increased patient mortality risk by approximately 11%.

In the United States, the Centers for Medicare and Medicaid Services launched the end-stage renal disease Quality Incentive Program (QIP) in 2012 to pay for performance based on quality improvement [14]. In addition, the Centers for Medicare and Medicaid Services launched the Dialysis Facility Compare Star Program in 2015 with the purpose of presenting differences in quality of care among dialysis facilities based on the reported quality measures [12]. Since then, the distribution of HD facility star rating has shifted upward showing an improvement in quality of HD care [8]. The QIP and Dialysis Facility Compare Star Program is similar to the Korean HD quality assessment and HD facility five-star rating systems. Although the HD quality assessment tool and HD facility star rating system were developed to improve patient health outcome, minimal research has been conducted regarding their effect on patient outcome. In addition, discussing the effects of the QIP program or star rating system on patient outcome has been difficult because many indicators of quality assessment have changed over time and each version has not yet been compared.

This is the first study in which the effects of HD quality assessment and HD facility star rating system on patient mortality among prevalent HD patients were reported. Recently, Ajmal et al. [15] reported the dialysis facilities with

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**Table 2. Multivariable Cox regression analysis of patient mortality based on HD facility star ratings**

<table>
<thead>
<tr>
<th>Star rating</th>
<th>Unadjusted</th>
<th>Model 1(^a)</th>
<th>Model 2(^b)</th>
<th>Model 3(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI) p-value</td>
<td>HR (95% CI) p-value</td>
</tr>
<tr>
<td>3–5 Stars</td>
<td>(Reference)</td>
<td></td>
<td>(Reference)</td>
<td></td>
</tr>
<tr>
<td>1–2 Stars</td>
<td>1.18 (1.12–1.25)</td>
<td>&lt;0.001</td>
<td>1.12 (1.05–1.20)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI, confidence interval; HD, hemodialysis; HR, hazard ratio.

low QIP scores were associated with a higher mortality rate within 1 year of beginning HD among incident patients. However, the effects of HD facility star rating system on long-term patient mortality have not been investigated in any other study. We prospectively collected survival data during a mean follow-up of 3 years. The results showed HD facility star rating is also independently associated with long-term patient mortality.

Significant attention has been given to patient characteristics to improve clinical outcome among HD patients. Patient characteristics such as age, presence of diabetes mellitus, previous cardiovascular disease, and low level of serum albumin are associated with higher mortality risk in HD patients [16]. In addition, increasing HD efficacy and treating anemia and mineral bone disorders may be important for improving patient outcome. However, increasing HD dose/frequency in previous large-scale clinical trials failed to reduce all-cause mortality [17–19] or manage anemia [20] and mineral bone disease [21]. Conversely, minimal attention has been given to the effects of structural and procedural components of HD service on patient outcome. In a recent study by Ajmal et al. [15], the clinical effects of QIP measures on patient outcome were evaluated. The United States QIP data includes percentage of waste removed during HD (HD adequacy), percentage of anemia overcorrection (plasma hemoglobin > 12.0 g/dL), vascular access type, infection rate, In-Center HD Consumer Assessment of Healthcare Providers and Systems, monthly reporting of calcium and phosphorus levels, monthly dosage of erythropoietin-stimulating agents, and monthly reporting of hemoglobin and hematocrit levels [12]. However, QIP has been criticized for including easily obtained laboratory measures with a limited evaluation regarding patient outcome [22–24]. To improve the quality of HD care, decreasing the workload of HD personnel, improving the water treatment process, reducing events associated with vascular access, and regularly monitoring patient-related outcomes are essential. The strength of the Korean HD facility star rating system is the inclusion of structural and procedural indicators. For example, evaluating the percentage of vascular access (catheter vs. fistula) and the satisfaction rate of regular monitoring for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subgroup</th>
<th>No. of patients</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
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<tr>
<td>Age group</td>
<td>&lt;65 yr</td>
<td>21,710</td>
<td>1.30 (1.18–1.43)</td>
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<tr>
<td></td>
<td>≥65 yr</td>
<td>13,727</td>
<td>1.08 (1.00–1.15)</td>
<td>0.04</td>
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<tr>
<td>Sex</td>
<td>Female</td>
<td>14,592</td>
<td>1.16 (1.06–1.27)</td>
<td>0.002</td>
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<td></td>
<td>Male</td>
<td>20,845</td>
<td>1.19 (1.11–1.28)</td>
<td>&lt;0.001</td>
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<tr>
<td>Vintage group</td>
<td>&lt;5 yr</td>
<td>17,796</td>
<td>1.22 (1.12–1.33)</td>
<td>&lt;0.001</td>
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<tr>
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<td>≥5 yr</td>
<td>13,037</td>
<td>1.11 (1.01–1.22)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>No</td>
<td>14,784</td>
<td>1.16 (1.04–1.29)</td>
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<tr>
<td></td>
<td>Yes</td>
<td>20,653</td>
<td>1.16 (1.09–1.24)</td>
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<tr>
<td>Cerebrovascular disease</td>
<td>No</td>
<td>32,478</td>
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<td>Yes</td>
<td>2,959</td>
<td>1.07 (0.92–1.24)</td>
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<tr>
<td>Heart disease (IHD+CHF)</td>
<td>No</td>
<td>22,081</td>
<td>1.21 (1.12–1.30)</td>
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<td>Yes</td>
<td>13,356</td>
<td>1.14 (1.06–1.24)</td>
<td>0.001</td>
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</tbody>
</table>

**Figure 2.** Forest plot depicting the relative risk of patient mortality based on HD facility star rating in different subgroups. The patients in the high HD facility star rating group showed better patient survival across all subgroups except subjects with cerebrovascular disease.

CHF, congestive heart failure; CI, confidence interval; HD, hemodialysis; HR, hazard ratio; IHD, ischemic heart disease.
the stenosis of arteriovenous fistula is part of the system. In addition, physician-to-patient ratio and proportion of experienced personnel in each HD unit are monitored. In a recent study by Harley et al. [25], high nephrology case-load was reportedly associated with poor patient outcome. Therefore, structural components in addition to laboratory measures may affect patient mortality.

In the present study, patients from low-star-rating HD facilities had more comorbidities and poorer clinical indices such as higher blood pressure and lower hemoglobin and albumin levels. However, whether this is due to the large portion of elderly patients or poor patient management in low-star-rating HD facilities is unclear. However, HD facility star rating remained an independent risk factor for patient mortality after adjusting for known risk factors including older age, male sex, medical comorbidities, and health insurance status.

The present study had several limitations. Because this study was from a single country with data from a single assessment year, the results may not be generalized. The baseline covariates between two HD facility star rating groups were not balanced before analysis using propensity score matching. Each component of star rating was not analyzed, therefore, which component of HD quality assessment mainly affected patient mortality could not be determined. In addition, the cause of death was not analyzed. In addition, disease-specific mortality was not compared between groups. Further studies should be performed to evaluate the importance of each indicator (facility personnel or procedural indicator) or underlying disease for all-cause mortality and specific patient outcomes. Patients admitted to the hospital were excluded from the analysis, therefore, patients with severe comorbidities or those admitted to nursing hospitals may have been excluded from the analysis. Next, this study was performed with only prevalent HD patients. Therefore, the effect of HD quality assessment on incident HD patients cannot be determined from this study. In addition, whether the current HD facility star rating scoring system is optimal is beyond the scope of this study. Further studies are needed to validate the current scoring system and determine whether the star rating system is optimal. Lastly, whether star rating of HD facilities improves patient outcome cannot be concluded from the results and may be deduced in another study with sequential HD quality assessment data.

In conclusion, the low HD facility star rating based on HD quality assessment may result in higher patient mortality. Further prospective studies are needed to prove whether improvement in star rating reduces patient mortality.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

The raw data were generated at the Health Insurance Review and Assessment Service. The database can be requested from the Health Insurance Review and Assessment Service by sending a study proposal including the purpose of the study, study design, and duration of analysis through an e-mail (turtle52@hira.or.kr) or at the portal site (https://www.hira.or.kr/bbsDummy.do?pgmid=HIRAA020002000100&brdSclBltno=4&brdBltno=9025&pageIndex=1#none). The authors cannot distribute the data without permission.

Authors’ contributions

Conceptualization: YEK, DRR, YKL
Data curation: YEK, DRR, KHY, EMW, JHS
Formal analysis: HCP, HYC, JK
Methodology: DHK, AC
Writing—original draft: HCP, HYC
Writing—review & editing: DHK, AC, YEK, DRR, KHY, JHS, JK, YKL
All authors read and approved the final manuscript.

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References

Impact of needle type on substitution volume during online hemodiafiltration: plastic cannulae versus metal needles

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**Background:** Plastic cannulae have attracted increasing interest as an alternative to traditional metal needles with the aim of reducing cannulation-related complications. We investigated whether the substitution volumes during hemodiafiltration differ using these two types of needles in dialysis patients.

**Methods:** An intervention study involving 26 hemodialysis patients was conducted in Korea between March and September in 2021. Patients first received online hemodiafiltration using traditional metal needles, and thereafter plastic cannulae were used in a stepwise protocol. Repeated-measures design and linear mixed-effect models were used to compare substitution volumes between the two needle types with the same inner diameter.

**Results:** The mean patient age was 62.7 years, and their mean dialysis vintage was 95.2 months. Most patients (92.3%) had an arteriovenous fistula as the vascular access. The substitution volume increased as blood flow and needle size increased for both plastic cannulae and metal needles. The substitution volume was significantly higher with 17-gauge (G) plastic cannulae than with 16-G metal needles at blood flow rates of 280, 300, and 330 mL/min. Similar results were obtained for 15-G metal needles and 16-G plastic cannulae at a blood flow rate of 330 mL/min. However, the patient ratings of pain on a visual analogue scale were higher for plastic cannulae.

**Conclusion:** Higher substitution volumes were obtained at the same prescribed blood flow rate with plastic cannulae than with metal needles during online hemodiafiltration. Plastic cannulae are an option for achieving high-volume hemodiafiltration for patients with low blood flow rates.

**Keywords:** Hemodiafiltration, Hemodialysis, Ultrafiltration
**Introduction**

Mortality remains high for hemodialysis (HD) patients despite continuous improvements in HD devices and membrane biocompatibility [1]. HD is based on diffusion across a semipermeable membrane, which allows adequate clearance of low-molecular-weight particles. However, simply increasing the HD dose to remove more of the small solutes does not improve survival [2]. Online hemodiafiltration (OL-HDF) provides additional clearance of larger toxins compared with standard HD. OL-HDF also offers effective removal of uremic substances over a wider range of molecular sizes, which has potential clinical advantages [3–5].

During the past few years, several prospective, randomized clinical trials (RCTs) have compared survival outcomes in patients receiving conventional HD and OL-HDF [6–9]. None of these RCTs have shown statistically significant beneficial effects of OL-HDF on mortality. However, in all of these RCTs, post hoc analyses showed that patients with the highest delivered convection volume had considerably lower risk of all-cause mortality than those receiving HD [10]. Achieving a high convection volume is not easy in older patients and those with fragile vessels, especially Asians. We previously conducted a study of the stepwise achievement of high convection volumes in patients receiving OL-HDF by changing the needle size and dialyzer surface area and found that high convection volume was feasible by increasing the needle size and dialyzer surface areas in patients with a low blood flow rate [11].

Two main types of needles are commercially available and used for HD: metal needles and plastic cannulae [12–14]. Metal needles are made of stainless steel and are either sharp or blunt [15]. Plastic needles are designed specifically for HD cannulation and contain a sharp metal needle housed within a flexible plastic sheath. The metal needle is used to access an arteriovenous fistula (AVF) and to guide the insertion of the plastic sheath into the vessel. Previous studies have reported that plastic cannulae have a lower risk of causing vascular injury, needle infiltration during cannulation, and hematoma compared with traditional metal needles [16–18].

There is some concern that high blood flow may have a negative effect on vascular access survival [19]. A recent study demonstrated that patients treated with plastic cannulae showed less negative arterial pre-pump pressures and lower venous pressures than those treated with metal needles at all prescribed blood flow rates [20]. In that study, the plastic cannulae had stable arterial and venous pressures at the prescribed blood pump flow rates in patients undergoing HD. Therefore, we assumed that patients treated with plastic cannulae can achieve higher substitution volumes (SV) than those treated with metal needles at the same pump speed when applied during OL-HDF. In this study, we investigated the impact of needle type on SV in patients using different needle types during OL-HDF.

**Methods**

**Study design and population**

We conducted an intervention study between March and September 2021. This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Hallym University Kangnam Sacred Heart Hospital (No. 2020-03-023). Written informed consent was obtained from all patients before enrollment.

Patients with end-stage renal disease, aged >18 years undergoing chronic intermittent HD for ≥3 months in two dialysis centers of Kangnam and Chuncheon Sacred Heart Hospital in South Korea were included. Patients were eligible for inclusion if they were being treated three times per week with high-flux HD and were able to understand the study procedures and provide informed consent. The exclusion criteria were severe nonadherence regarding frequency and/or duration of HD treatment and a life expectancy of <3 months because of nonrenal disease.

**Procedures**

Blood flow rate and needle size and type were determined for all patients (Fig. 1). Metal needles (15-gauge [G] and 16-G with a needle length of 25 mm; JMS Singapore Ltd) and plastic cannula (Supercath Clampcath 16-G and 17-G with a cannula length of 25 mm; Togo Medikit) were used in this study. The needle size of the plastic cannula indicates the size of the introducer needle, which serves as an introducer for the cannula into the vessel. After removal of the introducer needle, the inner diameter of plastic cannula is the same as that of a metal needle that is one
gauge larger; e.g., a 16-G metal needle has the same inner diameter as a 17-G plastic cannula (Supplementary Table 1, available online). We compared SVs obtained using 15-G and 16-G needles. Metal needles were used at the start of the study protocol. The blood flow rate and needle size were increased in a stepwise manner. Each step was performed three times, and when a patient was able to tolerate one step, the next step was started. After reaching the last step with a metal needle, patients moved to the steps with a plastic cannula. The treatment times were fixed at 240 minutes. During the study, training of nursing staff on the study protocol was not required.

Postdilution HDF was performed using a 5008 CorDiax HDF machine (Fresenius Medical Care) with the AutoSub plus function. High-flux polysulfone dialyzers (surface area, 1.8 m$^2$, FX80; Fresenius Medical Care) were used. The dialysis and substitution fluid composition were standardized as follows: sodium, 140 mEq/L; potassium, 2 mEq/L; calcium, 3.0 mEq/L; and bicarbonate, 32 mEq/L. Unfractionated heparin was used for anticoagulation. Sterile and nonpyrogenic substitution fluids were produced by ultrafiltration of the ultrapure dialysate. Ultrapure quality was defined as a bacterial count of <0.1 colony forming unit/mL and endotoxin level of <0.025 endotoxin unit/mL [21].

Measurements

Demographic (age and sex), medical history (diabetes, hypertension, and dialysis duration), and clinical data were collected at baseline. Biochemical parameters were assessed before HD. Single-pool Kt/V was determined using two-point urea modeling based on the intradialytic decrease in blood urea concentration and intradialytic weight loss [22]. The reduction ratio of β2-microglobulin was calculated using the plasma concentrations of the solute before and at the end of HD [23]. The concentration at the end of HD was corrected for ultrafiltration. The SVs were automatically adapted and obtained based on pressure pulse attenuation and cross-membrane pressure assessment by the signal analysis, known as the AutoSub plus function [24]. A visual analogue scale (VAS) was used to assess the perception of pain caused by using plastic cannulae and metal needles. VAS is a validated, subjective measure for acute and chronic pain [25]. Scores are recorded by making a handwritten mark on a 10 cm line that represents a continuum between “no pain” (0 cm) and “worst pain” (10 cm) [26].

Statistical analyses

Data are expressed as mean (±standard deviation, SD) for continuous variables and as numbers of patients and percentages for categorical variables. Linear mixed-effect models were used to analyze associations between the increase in steps and SV, factors related to SV, and comparison of SV according to the needle type and size. Differences in VAS, Kt/V, and the reduction ratio of β2-microglobulin for each step were also analyzed using linear mixed-effect
models. A post hoc Tukey test was used to compare differences in the response variables between the groups. Each subject in the models was used as a random factor. The model to identify factors related to SV was adjusted for serum albumin level and hematocrit, which are factors used to estimate convection volume in patients during OL-HDF [27]. Statistical analyses were performed using R software (version 4.0.5; R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/). All p-values were two-sided, and p < 0.05 was considered to be significant.

**Results**

**Baseline characteristics**

A total of 26 patients were included in the study, and all patients completed each step. Patient characteristics are summarized in Table 1. The mean age was 62.7 years, 23.1% were women, the average dialysis vintage was 95.2 months, and 57.7% had diabetes. The mean hematocrit was 31.5%, and the mean serum albumin concentration was 3.9 g/dL. A high percentage (92.3%) of patients had an AVF as the vascular access. The mean blood flow was 285 mL/min.

**Substitution volumes at each step**

The mean (SD) values for each step and trends for the changes in SV in the different steps are shown in Fig. 2. Positive linear trends could be seen for each step. As blood flow and needle size increased, the SV increased using both plastic cannulas (β coefficient, 0.80; 95% confidential interval [CI], 0.61–0.97; p < 0.001) and metal needles (β coefficient, 1.14; 95% CI, 0.95–1.33; p < 0.001).

Next, we used a linear mixed-effect model to identify factors associated with the SV. After adjusting for serum albumin concentration and hematocrit, high SV was significantly associated with the use of plastic needles (vs. metal needle: β coefficient, 1.69; 95% CI, 1.33–2.05; p < 0.001), blood flow rates of 280 mL/min (vs. 280 mL/min: β coefficient, 1.29; 95% CI, 0.79–1.78; p < 0.001) and 330 mL/min (vs. 280 mL/min: β coefficient, 2.64; 95% CI, 2.14–3.13; p < 0.001), and use of 15-G needles (vs. 16-G needles: β coefficient, 0.67; 95% CI, 0.26–1.08; p < 0.001).

**Comparisons of substitution volumes**

The comparison of SVs for plastic cannulae and metal needles is shown in Fig. 3. For 16-G needles, the SVs were significantly higher when using plastic cannulae than metal needles at blood flow rates of 280 (estimated difference, 1.39; standard error [SE], 0.37; p < 0.001), 300 (estimated difference, 1.88; SE, 0.38; p < 0.001), and 330 (estimated difference, 2.32; SE, 0.38; p < 0.001) mL/min. For 15-G needles, the SVs were higher for plastic cannulae than for metal needles, but the significance was borderline (estimated difference, 1.17; SE, 0.38; p = 0.05).

**Visual analogue scale of perceived pain according to needle type**

The comparison of pain VAS scores according to needle type is shown in Fig. 4. For 15-G needles, the VAS score for pain was higher for plastic cannulae than for metal needles (3.76 ± 1.46 vs. 2.62 ± 1.53; p < 0.001). For 16-G needles, the VAS score was also higher for plastic cannulae than for metal needles (3.94 ± 1.73 vs. 2.73 ± 1.40; p < 0.001).

---

**Table 1. Baseline characteristics of the study subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>26</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>62.7 ± 11.1</td>
</tr>
<tr>
<td>Male sex</td>
<td>20 (76.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.6 ± 3.7</td>
</tr>
<tr>
<td>Predialysis SBP (mmHg)</td>
<td>148.4 ± 22.0</td>
</tr>
<tr>
<td>Predialysis DBP (mmHg)</td>
<td>70.5 ± 14.1</td>
</tr>
<tr>
<td>Duration of dialysis (mo)</td>
<td>95.2 ± 57.8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15 (57.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (65.4)</td>
</tr>
<tr>
<td>Vascular access, AVF</td>
<td>24 (92.3)</td>
</tr>
<tr>
<td>Blood flow rate (mL/min)</td>
<td>285 ± 16.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.4 ± 0.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>31.5 ± 2.3</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>3.9 ± 0.3</td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>8.8 ± 0.7</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>5.1 ± 1.1</td>
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<tr>
<td>Single-pool Kt/V</td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, or number (%). AVF, arteriovenous fistula; DBP, diastolic blood pressure; SBP, systolic blood pressure.
Figure 2. Spaghetti plot of SVs plotted separated for metal needles (A) and plastic cannulae (B). The smooth lines (blue) show the linear trend of SVs using locally estimated scatterplot smoothing analysis. SD, standard deviation; SV, substitution volume.

Figure 3. SVs by needle size and type. (A) 16-Gauge (G) metal needle vs. 17-G plastic cannula. (B) 15-G metal needle vs. 16-G plastic cannula. SV, substitution volume.
Kt/V and β2-microglobulin reduction ratio

We compared the changes in Kt/V and β2-microglobulin reduction ratio between needle types (Table 2). For metal needles, the Kt/V and β2-microglobulin reduction ratio tended to be higher for step 4 compared with step 1 (p < 0.001). For plastic cannulae, the Kt/V was significantly higher for step 5 compared with step 1. The change in the β2-microglobulin reduction ratio did not change significantly between steps 1 and 5 (p = 0.70). In step 1, plastic cannulae showed higher Kt/V (p = 0.01) and β2-microglobulin reduction ratio (p = 0.01) than metal needles. Between step 4 of metal needles and step 5 of plastic cannulae, the difference in Kt/V was not significant (p = 0.05), and β2-microglobulin reduction ratio with metal needle was significantly higher than with plastic cannula (p = 0.003).

Discussion

In this study, we found that the SVs were higher using plastic cannulae than metal needles in patients undergoing OL-HDF. With both types of needles, the SVs increased with higher blood flow rates and needle size. However, plastic cannula insertion was perceived by the patients to be more painful than metal needle puncturing. We used a stepwise protocol to the adjust blood flow rate and needle size. The first step involved a low blood flow rate and high needle gauge. Thereafter, the blood flow rate was increased and a lower gauge needle was used. All participants tolerated each step. In the protocol for the use of metal needles, the 15-G metal needle was not applied at a blood flow rate of 300 mL/min. The choice of gauge may be based on AVF vintage and expansion, patient tendency for bleeding, and patient preference [28]. Except for the initial cannulation, most guidelines do not recommend a specific gauge but instead recommend that the needle gauge matches the blood flow rate [29]. However, there is concern that larger needles are associated with complications of vascular access. We used 15-G metal needles only at the highest blood flow rate in our protocol.

Traditional sharp metal needles used to cannulate vascular access can harm the vessel or even infiltrate into the vessel wall during cannulation or during HD treatment. With plastic cannulae, the risk of vessel damage during HD or infiltration may be reduced because the cannula is soft and made of flexible material and the introducer needle is smaller than a metal needle. Studies have found that the

![Figure 4. VAS pain scores.](image)

G, gauge; VAS, visual analogue scale.

### Table 2. Kt/V and β2-microglobulin reduction ratio according to the needle type

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>Metal needle</th>
<th>Plastic cannula</th>
<th>p-value</th>
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<td>Step 1</td>
<td>Kt/V</td>
<td>1.64 ± 0.28</td>
<td>1.78 ± 0.31</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>β2-microglobulin reduction ratio (%)</td>
<td>70.56 ± 10.12</td>
<td>75.49 ± 8.31</td>
<td>0.01</td>
</tr>
<tr>
<td>Steps 4, 5</td>
<td>Kt/V</td>
<td>2.02 ± 0.44</td>
<td>1.92 ± 0.35</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>β2-microglobulin reduction ratio (%)</td>
<td>82.01 ± 3.81</td>
<td>75.14 ± 10.28</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation.
use of plastic cannulae results in lower rates of vessel damage, infiltration, and hematoma, and less stenosis of the vascular access [16,18,30]. Early cannulation with a plastic cannula, which means use of vascular access before 10 days from creation, did not affect vascular access patency in a retrospective cohort study in Japan [31].

OL-HDF is currently the most advanced and promising alternative to conventional HD. Previous large RCTs and observational studies have failed to show a consistent significant beneficial effect of OL-HDF on all-cause mortality [6–9,32,33]. However, the results of a pooled analysis of individual data from the RCTs and meta-analysis showed significant all-cause and cardiovascular survival benefits of HDF over HD when high convection volumes were achieved [10,34,35]. Treatment time, blood flow rate, and filtration fraction are stronger determinants of the convection volume than individual characteristics [36]. In feasibility studies, high-volume HDF was possible for >80% of HD sessions with modification of these factors [24,36]. However, the blood flow rate applied in these studies was >350 mL/min, which cannot be achieved easily in patients with fragile vascular access. In Asian patients, a low blood flow rate is the main obstacle to high-volume HDF. In this regard, our study suggests that the use of plastic cannulae may be an option for increasing SVs in patients whose blood flow rate is <350 mL/min.

In preliminary clinical observations, flow images at venous cannulation sites show distinct patterns for the two types of needles [37]. Images of metal needles show that the jet flow effect appears to be a solid stream projected toward the vessel wall. In contrast, images of plastic cannulae show that this effect appears more diffuse and extends from the side holes to the tip of the cannula toward the center of the vessel lumen. A study of hemodynamics found that the plastic cannula helps to maintain stable blood flow and reduces dynamic arterial and venous pressure despite the smaller diameter of the inner introducer needle compared with a metal needle [20]. There were lower negative arterial pre-pump pressures and lower venous pressures during HD with the use of plastic cannulae compared with the metal needles at all prescribed blood pump flow rates. The real blood flow rate is somewhat lower than the set value, and a higher the blood pump speed is correlated with a wider difference [38,39]. This phenomenon is explained by partial collapse of the tubes at more negative pre-pump pressures, which may be more prominent in HDF because it has more negative pre-pump pressure than conventional HD. These findings support our results showing that plastic cannulae can achieve higher SVs than metal needles. A plastic cannula has four sides with round holes all along the circumference of the tip, which improves steady blood flow during dialysis and prevents occlusion of the cannula by the vessel wall [30]. The VAS pain scores were higher for plastic cannulae than metal needles in this study. Previous studies have reported inconsistent results for the pain response during cannulation using plastic cannulae and metal needles [16,30]. Choi et al. [16] reported that plastic cannula insertion is more painful than metal needle puncturing. The larger outer diameter of the plastic cannula might be one of reasons for the high VAS pain scores of plastic cannulae. Furthermore, the insertion techniques differ between plastic cannulae and metal needles [17]. Because of the complicated cannulation technique, miscannulation can occur when trying to insert a plastic cannula, which might also cause pain. Adequate training of nursing staff is needed for the use of plastic needles in clinical practice. For example, Choi et al. [16] noted that nursing staff felt that plastic cannulae were much easier to use after a training period.

SVs with 16-G plastic cannulae were higher than ones with 15-G metal needles. However, the 15-G metal needles also give high SVs, so the difference in SVs by needle types was not significant (24.4 ± 3.3 with 16-G plastic cannula vs. 23.1 ± 3.3 with 15-G metal needle at blood flow rate of 330 mL/min). High convection volumes are advantageous to small molecule removal. We found that Kt/Vs were significantly improved in both metal needles and plastic cannulae as steps increased. However, β2-microglobulin reduction ratios were not significantly increased with plastic cannulae in higher step. The mean β2-microglobulin reduction ratios at the first step were higher with plastic cannulae than with metal needles, due to the high SVs of plastic cannulae. However, the β2-microglobulin reduction ratio with plastic cannulae at the fifth step did not significantly increase, despite high SVs, compared with the first step. Furthermore, the β2-microglobulin reduction ratio with plastic cannulae was lower than with metal needles at later steps. The relationship between convection volume and removal amount of middle molecules is unclear [40].
should be investigated in future studies. This study has several limitations. First, we used a repeated-measures design with the same participants for the response variable. There are several threats to the internal validity of this design because when patients are tested several times, their scores tend to regress toward the mean and may change during the course of the experiment. However, this study design helps to make a study more efficient and keeps the variability low while allowing for smaller-than-usual subject groups. Second, we enrolled a small number of patients from a single center, and therefore, our results are not generalizable. However, there are few data on the effects of needle type on SV during OL-HDF. Our findings suggest that plastic cannulae can be considered as a modifying factor for high-volume HDF. Third, we did not measure the dynamic venous pressure and effective blood flow rate at the cannulation site. Further studies are needed to compare the hemodynamic effects of these two types of needles.

In conclusion, SVs during OL-HDF differed between the two types of needles. Higher SVs were achieved with plastic cannulae than with metal needles. This may reflect the ability of plastic cannulae to maintain a stable blood flow rate with less negative pressure, but further studies are needed to confirm this result. Our findings suggest that plastic cannulae can be used for patients who cannot achieve high-volume HDF because of a low blood flow rate.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

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

Authors’ contributions

Conceptualization: AJC, HCP, YRS, JWY, YKL

Data curation: ShK

Formal analysis: AJC, JKK, YKL

Investigation: GC

Methodology: DHK, GHS

Project administration, Supervision YKL

Resources: HBC

Software: AJC

Visualization: HK

Writing–original draft: AJC

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Optimal peritoneal fluid white blood cell count for diagnosis of peritonitis in peritoneal dialysis patients

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**Background:** The diagnosis of peritonitis among peritoneal dialysis (PD) patients is based on clinical presentation, dialysis effluent white blood cell (WBC) count, and dialysis effluent culture. Peritoneal fluid WBC count is very important in the initial diagnosis of peritonitis. The purpose of this work was to determine the optimal number of peritoneal WBCs with different clinical presentations at admission to define PD-related peritonitis.

**Methods:** Medical records of chronic PD patients who underwent work-up for suspected peritonitis between 2008 and 2019 were reviewed retrospectively. Results of all peritoneal WBC count tests during this period were collected. Clinical manifestations and follow-up analysis of each peritoneal WBC count were performed.

**Results:** The peritoneal WBC count cutoff of 100/μL recommended by International Society for Peritoneal Dialysis provided specificity of only 35%. Increasing peritoneal WBC count cutoff to 150, 200, and 250/μL provided sensitivity around 98% and gradually increasing specificity. The chi-square automatic interaction detector model of statistical analysis determined that peritoneal WBC count below 230/μL combined with absence of inflammatory markers (fever, increased C-reactive protein) ruled out peritonitis with 99.8% sensitivity. Peritoneal fluid WBC count cutoff of 230/μL provided specificity of 89% and good positive and negative likelihood scores of 8.3 and 0.03, respectively. Peritoneal fluid polymorphonuclear count has lower discriminating ability for peritonitis compared to peritoneal fluid WBC count.

**Conclusion:** Increasing peritoneal fluid WBC count cutoff to 230/μL in suspected PD-related peritonitis could improve specificity without compromising the sensitivity of the test.

**Keywords:** Abdominal pain, Peritoneal dialysis, Peritonitis, White blood cells

**Introduction**

The prevalence of kidney failure requiring replacement therapy is increasing due to an aging population and increase in the incidence of diabetes and hypertension. The estimated number of patients needing renal replacement therapy by 2030 is to be between 4.9 and 9.7 million around the world [1]. Peritoneal dialysis (PD) is a commonly used treatment modality for kidney failure. PD-related peritonitis remains a major cause of technique failure as well as...
mortality in maintenance PD patients [2,3]. According to the International Society for Peritoneal Dialysis (ISPD), peritonitis in PD patients can be diagnosed when at least two of the following are present: 1) clinical features consistent with peritonitis, e.g., abdominal pain and/or cloudy dialysis effluent; 2) dialysis effluent WBC count of >100/μL or >0.1 × 10⁹/L (after a dwell time of at least 2 hours), with >50% polymorphonuclear leukocytes (PMNs); and 3) positive dialysis effluent culture [4]. Dialysis effluent WBC count cutoff of 100/μL was determined in 3–4 decades-old studies [5–8]. No study focusing on PD-related peritonitis diagnosis was performed in the last decades. In everyday practice, the clinical picture of peritonitis at presentation can be unclear. The severity and character of the associated abdominal pain are not specific and cannot be differentiated from those of other causes. Differential diagnosis of abdominal pain and other gastrointestinal symptoms includes such common diseases as urinary tract infection, gastroenteritis, and cholecystitis. Pancreatitis can cause cloudy peritoneal fluid with increased neutrophil count. There is also a variety of conditions causing cloudy dialysate without peritonitis [9]. In addition, patients undergoing automated PD (APD), compared with chronic ambulatory PD (CAPD), commonly present with no history of cloudy fluid [8]. Peritoneal fluid WBC count is very important in the diagnosis of peritonitis since peritoneal microbial culture results are not available at patient presentation. From our clinical experience, the great majority of PD-related peritonitis cases presented with much higher peritoneal WBC count than recommended by ISPD [4]. Misdiagnosis of the true source of infection, especially when assessed by less experienced medical staff and emergency department personal can result in false diagnosis of peritonitis and lack of necessary investigation for alternative diagnoses. When mistakenly diagnosed with peritonitis, patients receive improper or unnecessary antibiotics, and further investigation, for example, imaging such as ultrasonography or computed tomography, for an alternative diagnosis is delayed.

The purpose of this work was to determine the optimal number of peritoneal fluid WBCs at different clinical presentations at admission to define PD-related peritonitis.

**Methods**

Medical records of patients on chronic PD who underwent work-up for suspected peritonitis between 2008 and 2019 were reviewed retrospectively. Results of all peritoneal WBC count tests at presentation during this period of time were collected. Clinical status of each peritoneal fluid sample was analyzed at presentation and further follow-up, including bacteriological culture, antibiotics treatment, and laboratory tests (WBC count and culture of peritoneal fluid). Demographic data, cause of kidney disease, and PD modality were gathered.

Primary renal disease diagnosis was determined and recorded by an unbiased nephrologist. Concise criteria of major etiologies include: diabetic kidney disease diagnosis was based on long standing diabetes mellitus with albuminuria progressing to proteinuria, and progressive decline of kidney function, without significant hematuria or abnormalities on imaging, and after ruling out other etiologies through serologic testing. In cases where kidney biopsy was performed, the diagnosis of diabetic nephropathy was based on the consensus Renal Pathology Society criteria [10]. Patients with symptomatic congestive heart failure and renal failure having urine protein of <0.5 g/24 hours, normal urine microscopy and normal kidneys per sonography were classified as having cardiorenal syndrome. Chronic glomerulonephritis diagnosis was based on course of disease, previous urine sediments, and serology testing, usually accompanied by a diagnostic biopsy. Hypertensive nephrosclerosis was diagnosed in patients with kidney dysfunction, proteinuria, small kidneys on imaging, and after ruling out other etiologies based on urine sediment and serology testing. In cases where kidney biopsy was performed, the diagnosis of hypertensive nephrosclerosis was based on clinicopathologic criteria which include clinical hypertension associated with the histopathologic findings of vascular wall medial thickening, intimal fibrosis, arteriolar hyalinosis, and glomerular ischemic changes of capillary wall wrinkling [11].

The laboratory parameters collected during peritonitis investigation were peritoneal WBC count, peritoneal PMN percentage, blood WBC count, blood PMN percentage, and serum C-reactive protein (CRP) level. Information concerning antibiotics treatment and peritonitis course was also collected.
The final diagnosis of peritonitis was made when peritoneal culture results were available, usually 2 to 3 days after the first presentation, when at least two of the following were present: 1) clinical features consistent with peritonitis, i.e., abdominal pain and/or cloudy dialysis effluent; 2) dialysis effluent WBC of >100/μL after a dwell time of at least 2 hours; and 3) positive dialysis effluent culture [4]. Clinical features leading to the peritoneal fluid WBC examination were 1) abdominal pain; 2) cloudy dialysate; 3) fever or increased serum CRP; 4) exit site infection; 5) difficulties with peritoneal catheter flow; 6) gastrointestinal symptoms other than abdominal pain, such as diarrhea, vomiting, or nausea; 7) other symptoms. “Other symptoms” included complaints of weakness, chest pain, general deterioration, dyspnea, confusion, unexplained weight loss, drowsiness, hypotension, and syncope.

An exit-site infection was defined by the presence of purulent drainage, with or without erythema of the skin around the catheter. Relapsing peritonitis was defined as an episode with the same organism that occurred within 4 weeks of completion of therapy for a prior episode or one sterile episode.

The initial empirical antibiotic treatment included intraperitoneal vancomycin or cefamezin (based on severity of clinical presentation and previous microbiological results if available) with ceftazidime. After the culture results were available, antibiotic treatment was changed accordingly.

Statistical analysis

Categorical variables were described as frequency and percentage. Continuous variables were evaluated for normal distribution using histogram and Q-Q plot. These results were reported as mean and standard deviation if they were normally distributed or as median and interquartile range (IQR) if they were skewed. A generalized estimating equation using a binary logistic model was used to study the association between each of the input fields and the outcome and tests for significance using a chi-square independence test. If more than one of these relations is statistically significant, CHAID will select the input field that is the most significant. If an input has more than two categories, these are compared, and categories that show no differences in outcome are combined by successively joining the pair of categories showing the smallest significant difference. This category-merging process stops when all remaining categories differ at the specified testing level. For nominal input fields, any categories can be merged; for an ordinal set, only contiguous categories can be merged [13]. The following variables were included in the CHAID analysis: age at first dialysis; sex; etiology of renal disease; underlying diabetes mellitus; dialysis mode; clinical presentation including abdominal pain, cloudy fluid, inflammatory marker, or fever; exit site infection; catheter occlusion or sterility disruption; other gastrointestinal symptoms; and other symptoms, PD WBC, PD PMN, blood WBC, blood PMN, and CRP.

Maximum tree depth was organized into three levels, with the minimum number of required cases in parent and child nodes set as 100 and 50, respectively.

All statistical tests were two-sided, and p < 0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS version 24 (IBM Corp).

Table 1. Baseline parameters of peritoneal dialysis patients presenting with suspected peritonitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>147</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>65 (27–92)</td>
</tr>
<tr>
<td>Sex, female:male</td>
<td>35/112</td>
</tr>
<tr>
<td>Renal disease</td>
<td></td>
</tr>
<tr>
<td>Cardiorenal</td>
<td>41 (27.9)</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>41 (27.9)</td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>11 (7.5)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>35 (23.8)</td>
</tr>
<tr>
<td>Others</td>
<td>19 (12.9)</td>
</tr>
<tr>
<td>Prevalent diabetes mellitus</td>
<td>72 (49.0)</td>
</tr>
<tr>
<td>Peritoneal dialysis modality (APD)</td>
<td>63 (42.9)</td>
</tr>
</tbody>
</table>

Data are expressed as number only, value (range), or number (%). APD, automated peritoneal dialysis.
Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Sheba Medical Center (No. 6479-19-SMC) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, informed consent is not required.

Results

Patient baseline characteristics

A total of 176 chronic PD patients were treated in our unit between 2008 and 2019. Of them, 29 were never investigated.

Table 2. Peritoneal culture results of peritonitis cases in peritoneal dialysis patients (n = 165)

<table>
<thead>
<tr>
<th>Organism identified</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative Staphylococcus</td>
<td>38 (23.0)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>26 (15.8)</td>
</tr>
<tr>
<td>Streptococcus sp.</td>
<td>13 (7.9)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>13 (7.9)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12 (7.3)</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>10 (6.1)</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>8 (4.8)</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>5 (3.0)</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>5 (3.0)</td>
</tr>
<tr>
<td>Listeria</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Fungi</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (4.2)</td>
</tr>
<tr>
<td>Culture negative</td>
<td>18 (10.9)</td>
</tr>
</tbody>
</table>

Table 3. Probability of peritonitis in peritoneal dialysis patients according to clinical and laboratory parameters (n = 753)

<table>
<thead>
<tr>
<th>Variable</th>
<th>No (n = 588)</th>
<th>Yes (n = 165)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>65.6 ± 11.3</td>
<td>64.7 ± 12.1</td>
<td>0.63</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>Male</td>
<td>451 (78.4)</td>
<td>124 (21.6)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>137 (77.0)</td>
<td>41 (23.0)</td>
<td></td>
</tr>
<tr>
<td>Etiology of renal disease</td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>190 (78.5)</td>
<td>52 (21.5)</td>
<td></td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>130 (77.8)</td>
<td>37 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Cardiorenal syndrome</td>
<td>126 (84.0)</td>
<td>24 (16.0)</td>
<td></td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>65 (73.0)</td>
<td>24 (27.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>77 (73.3)</td>
<td>28 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>299 (78.9)</td>
<td>80 (21.1)</td>
<td>0.56</td>
</tr>
<tr>
<td>Dialysis mode</td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>CAPD</td>
<td>259 (80.4)</td>
<td>63 (19.6)</td>
<td></td>
</tr>
<tr>
<td>APD</td>
<td>329 (76.3)</td>
<td>102 (23.7)</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>93 (48.2)</td>
<td>100 (51.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cloudy fluid</td>
<td>26 (28.6)</td>
<td>65 (71.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fever/inflammation</td>
<td>126 (77.3)</td>
<td>37 (22.7)</td>
<td>0.59</td>
</tr>
<tr>
<td>Exit site infection</td>
<td>21 (91.3)</td>
<td>2 (8.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>Catheter occlusion</td>
<td>22 (84.6)</td>
<td>4 (15.4)</td>
<td>0.57</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>72 (72)</td>
<td>28 (28)</td>
<td>0.13</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>341 (93.9)</td>
<td>22 (6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peritoneal WBC (cells/μL)</td>
<td>138.7 ± 79.3</td>
<td>2,651.1 ± 3,285.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peritoneal PMN (%)</td>
<td>34.8 ± 15.2</td>
<td>64.6 ± 23.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood WBC (cells/μL)</td>
<td>10,070.1 ± 5,037.9</td>
<td>11,685.7 ± 6,173.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Blood PMN (%)</td>
<td>77.0 ± 9.6</td>
<td>82.0 ± 10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>75.0 ± 147.7</td>
<td>141.1 ± 128.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; PMN, polymorphonuclear leukocytes; WBC, white blood cells.
ed for peritonitis and were excluded from analysis. In the remaining 147 patients, 753 cases of suspected peritonitis were analyzed. The baseline characteristics of the patients are shown in Table 1. The median age of patients was 65 years (range, 27–92 years), and 76.2% were males. Diabetic nephropathy and cardiorenal syndrome were the most frequent causes of renal failure, at 27.9% each. Glomerulonephritis was the cause of renal failure in 23.8% of patients, nephrosclerosis in 7.5%, and other diseases in 12.9% of cases. Underlying diabetes mellitus was found in 49.0% of patients, and 42.9% of patients were on APD. Microbiological culture results of peritoneal culture are presented in Table 2. Peritoneal WBC count was measured in asymptomatic patients: the median peritoneal WBC count was 80 cells/mm$^3$ (range, 20–160 cells/mm$^3$), with a PMN cell percentage of 28.6% (range, 6.7%–52.1%).

Correlations with peritonitis diagnosis

The association of several clinical and laboratory param-

![Figure 1. Receiver operating characteristics curve of peritoneal fluid WBCs and peritoneal fluid PMN for diagnosis of peritonitis. The area under the curve for peritoneal fluid WBCs for predicting peritonitis was 0.989 (range, 0.979–0.998), and that for peritoneal fluid PMN was 0.842 (range, 0.803–0.881). PD, peritoneal dialysis; PMN, polymorphonuclear leukocytes; WBC, white blood cells.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dc (×10$^3$/µL)</th>
<th>Dc ≥100</th>
<th>Dc ≥150</th>
<th>Dc ≥200</th>
<th>Dc ≥230</th>
<th>Dc ≥250</th>
<th>Dc ≥300</th>
<th>Dc ≥350</th>
<th>Sensitivity</th>
<th>1-Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD WBC</td>
<td>0.986</td>
<td>0.998</td>
<td>0.986</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.948–0.999</td>
<td>0.979–0.998</td>
<td>0.289</td>
<td>0.995</td>
<td>0.010</td>
<td>0.654</td>
</tr>
<tr>
<td>PD PMN</td>
<td>0.328</td>
<td>0.572</td>
<td>0.288</td>
<td>0.247</td>
<td>0.237</td>
<td>0.228</td>
<td>0.258</td>
<td>0.237</td>
<td>0.777–0.942</td>
<td>0.741–0.914</td>
<td>0.533–0.652</td>
<td>0.397–0.562</td>
<td>0.694–0.887</td>
<td>0.729–0.971</td>
</tr>
<tr>
<td>Reference</td>
<td>1.000</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.976</td>
<td>0.979–0.993</td>
<td>0.979–0.998</td>
<td>0.986–0.999</td>
<td>0.986–0.999</td>
<td>0.986–0.999</td>
<td>0.986–0.999</td>
</tr>
</tbody>
</table>

Table 4. Peritoneal WBC counts for peritonitis diagnosis; diagnostic test results (n = 753)

Data are expressed as number (95% confidence interval). AUC, area under the curve; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; WBC, white blood cells.
eters with confirmed diagnosis of peritonitis was evaluated. In univariate analysis, abdominal pain and cloudy dialysate were positively correlated with peritonitis, while “other symptoms” correlated negatively (Table 3). Laboratory parameters of peritoneal fluid WBC count, peritoneal fluid PMN cell count, blood WBC count, blood PMN cell count, and CRP were positively correlated with peritonitis (Table 3).

**Peritonitis diagnosis based on peritoneal white blood cell count**

The ROC curves of peritoneal fluid WBCs and peritoneal fluid PMN percentage were used for diagnosis of peritonitis (Fig. 1). The area under the curve (AUC) for peritoneal fluid WBCs for predicting peritonitis was 0.989 (range, 0.979–0.998), indicating good discrimination ability of the biomarker. The AUC for peritoneal fluid PMN for predicting peritonitis was 0.842 (range, 0.803–0.881), a lower discrimination ability for peritonitis compared to peritoneal fluid WBCs. The AUC for peritoneal fluid WBC combined with peritoneal fluid PMN was 0.992, indicating that peritoneal fluid PMN did not improve significantly the discrimination ability of peritoneal fluid WBC count alone.

In order to identify accurate threshold levels of peritoneal fluid WBC count for diagnosis of peritonitis, sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of different peritoneal fluid WBC count values were analyzed (Table 4). While the sensitivity of peritoneal fluid WBC count of 100 cells/μL was 100%, specificity was only 35%. Increasing peritoneal fluid WBC count cutoff to 150, 200, and 250/μL produces a sensitivity around 98% and gradually increased specificity (Table 4).

The CHAID model was used for identification of peritonitis based on peritoneal fluid WBC count and clinical findings. According to the CHAID model, peritoneal fluid WBC count above 440 cells/μL was the best discriminator for peritonitis diagnosis (Fig. 2). Among those with peritoneal fluid WBC count above 440/μL, the next discriminator was abdominal pain at presentation. Patients on PD presenting with abdominal pain and peritoneal fluid WBC

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**Figure 2.** The chi-square automatic interaction detector model of decision tree to identify peritoneal dialysis-related peritonitis. WBC, white blood cells.

*Peritoneal WBC adjusted p-value <0.001, chi-square = 649.810, degree of freedom (df) = 2. *Clinical presentation of inflammatory markers or fever, adjusted p-value = 0.009, chi-square = 6.772, df = 1. *Clinical presentation of abdominal pain, adjusted p-value = 0.03, chi-square = 5.048, df = 1.
count above 440/μL had 100% probability of having peritonitis. The CHAID model identified peritoneal WBC count less than 230 cells/μL as the best discriminator to rule out peritonitis. After this peritoneal fluid WBC count, the next discriminator was absence of inflammatory markers (fever or increased serum CRP level). Patients on PD without fever or elevated serum CRP level and with peritoneal fluid WBC count less than 230 cells/μL had 99.8% probability of not having peritonitis. Based on the above two analyses, a threshold of peritoneal WBC count of 230 cells/μL will provide a better specificity for diagnosis of peritonitis compared to a cutoff of 100 cells/μL without compromising the sensitivity of the test. We also assessed the peritonitis rate in cases with low WBC count (WBC < 230 cells/μL) and high PMN count (PMN ≥ 50%). Of the 78 such patients identified, none had peritonitis.

Peritoneal white blood cell count and severity of peritonitis

The annual peritonitis rate in our unit was 0.29 cases per year. In 44 episodes of peritonitis (26.7%), patients were hospitalized. Peritoneal WBC count at presentation was significantly higher in episodes of peritonitis when patients were hospitalized compared to episodes treated ambulatorily. Median peritoneal WBC count was 2,325 cells/μL (range, 290–17,860 cells/μL) in episodes requiring hospitalization versus 1,270 cells/μL (range, 120–17,110 cells/μL) in episodes treated ambulatorily (p = 0.02). In 13 peritonitis episodes (7.9%), the Tenckhoff catheter was removed due to partial response to treatment or to subsequent development of fungal infection. There was no difference in peritoneal WBC count at presentation between peritonitis cases that responded to treatment and those who eventually needed Tenckhoff catheter removal—1,450 cells/μL (range, 120–17,860 cells/μL) and 1,610 cells/μL (range, 400–10,470 cells/μL), respectively (p = 0.40). In 17 episodes of peritonitis (10.3%), patients experienced relapse after initial improvement. There was no difference in peritoneal WBC count at presentation between peritonitis cases with and without relapse—1,950 cells/μL (range, 450–17,110 cells/μL) and 1,360 cells/μL (range, 120–17,860 cells/μL) cells, respectively (p = 0.29).

Peritoneal white blood cell count below 230 cells/μL

In only four episodes of established peritonitis in our study, patients presented with peritoneal WBC count above the cutoff suggested by the ISPD of 100 cells/μL but below our proposed cutoff level of 230 cells/μL. One of these four patients presented with the classical clinical symptom of peritonitis; abdominal pain. The three others presented with fever only or with fever and additional abdominal symptoms. In two of the patients, peritoneal WBC count increased later during follow-up. Peritoneal fluid culture returned positive several days after the initial presentation in all four patients. Because of their nonspecific presentation, patients did not receive intraperitoneal antibiotics immediately, but all eventually were treated with oral or intravenous antibiotics. Outcome was not compromised in any of these patients. One of these four patients (25.0%) with peritoneal WBC count below 230 cells/μL had classical presentation of abdominal pain, 126 patients (78.3%) with peritoneal WBC count above 230 cells/μL from the whole studied group had abdominal pain and/or cloudy peritoneal fluid (p = 0.04), typical presentation of peritonitis.

High peritoneal white blood cell count without peritonitis

In 57 cases, peritoneal WBC count was above 230 cells/μL (our suggested cutoff), but peritonitis was not diagnosed according to criteria and further follow-up. The median peritoneal WBC count in those cases was 290 cell/μL (range, 240–510 cells/μL). These cases were divided into groups based on final diagnosis: 40.4% had sepsis from origin other than peritonitis; 24.6% underwent WBC count for a peritonitis episode during follow-up, usually 2 weeks after antibiotic course completion; 12.3% presented with gastrointestinal symptoms such as diarrhea or vomiting and were suspected to have gastroenteritis; 10.5% were new PD patients not undergoing regular exchange; 8.8% were patients with history of ascites or peritoneal catheter occlusion; and 3.4% presented with unexplained transient episode of cloudy peritoneal fluid.

Validation study

Validation of our results was performed on a more recent
cohort of PD patients treated in our unit between 2020 and 2021. There were 116 cases of suspected peritonitis in 45 patients during this period. The median age of patients was 72 years (range, 29–92 years), and 64.4% were males. Diabetic nephropathy was the most frequent cause of renal failure, found in 44.4% of patients. Glomerulonephritis was a cause of renal failure in 24.4%, nephrosclerosis in 13.4%, cardiorenal syndrome in 4.4%, and other diseases in 13.4% of cases. Diabetes mellitus was found in 60.0% of patients, and 42.2% of patients were on APD. Using the peritoneal WBC cutoff of 230 cells/μL, peritonitis was excluded with 100% sensitivity and 97.9% specificity. The positive predictive value of this cutoff was 91.3%, negative predictive value was 100%, positive likelihood ratio was 47.5, and negative likelihood ratio was 0.024.

**Discussion**

Bacterial peritonitis is the most common complication of PD and is associated with significant morbidity, catheter loss, transfer to hemodialysis, transient loss of ultrafiltration, possible permanent membrane damage, and occasional death [14–16]. Treatment delay causes patient discomfort, peritoneal membrane damage, and catheter failure and contributes to mortality. However, unnecessary treatment should be avoided as it is costly and can lead to antimicrobial resistance, evolutionary selection of pathogenic organisms such as *Clostridioides* (formerly *Clostridium*) *difficile*, and drug toxicity [17]. Diagnosis of peritonitis is based on clinical presentation, dialysis effluent WBC count, and dialysis effluent culture [4]. However, at presentation, patient treatment can be started based only on clinical features and peritoneal fluid WBC count.

Cellular content of ascitic fluid has been used as an indicator of peritonitis since 1940 [18] when it was shown that nontuberculous peritonitis was associated with an ascites WBC count of ≥1,000/μL and a preponderance of PMN leukocytes. Tenckhoff [19] and Hurley et al. [20] used dialysate cell count and turbidity to diagnose PD-associated peritonitis, demonstrating that dialysate cell count above 500 WBC/μL is frequent in bacterial peritonitis. Hurley et al. [20] showed that peritoneal PMN leukocytosis accompanied peritonitis, while uninfected patients continued to have a predominance of macrophages in their peritoneal fluid. Rubin et al. [21] found that uninfected CAPD patients had dialysate WBC count less than 50/μL, while infected patients had PMN pleocytosis. Williams et al. [5] found that peritoneal WBC count ranged from 0 to 50 cells/mm³ in 38 control patients on CAPD without peritonitis, and the majority of the cells were mononuclear with a mean cell count of 11.6/mm³. At peritonitis presentation in 24 patients, the mean cell count was 2,585/mm³, with a range of 600 to 9,600/mm³. Their work [5] and the work of Rubin et al. [21] concluded that dialysate cell counts in asymptomatic patients are in the range of 501 cells/mm³. In a study by Tranæus et al. [6], the median value for all episodes was 1,032 cells/μL, with a median of 537 cells/μL in asymptomatic cases; 1,580 cells/μL in mild clinical cases; 1,470 cells/μL in moderately severe cases; and 1,110 cells/μL in severe clinical peritonitis. Flanigan et al. [8] measured peritoneal effluent cell count in 28 uninfected CAPD patients on 137 separate occasions. The cell count was 13 ± 2 cells/μL, with a range from 0 to 191 WBC/μL. The percentage of PMN cells was 11.97 ± 2.23. Uninfected patients based on APD systems had a WBC count of 72 ± 16/μL (range, 0–700/μL). In CAPD patients with peritonitis, peritoneal cell count was 2,311 ± 645 cells/μL, with 85.5% PMN; in APD patients with peritonitis, the count was 2,112 ± 761 cells/μL, with 84.8% PMN. In CAPD patients with peritonitis, WBC count generally exceeded 100/mm³ during infection, and PMN cells accounted for ~50% of the leukocytes [8].

While the average value of peritoneal WBC count for peritonitis diagnosis was high, mostly above 1,000 cells/μL [5–8], the minimum cutoff of 100 cells/μL was determined since peritoneal WBC count during peritonitis usually exceeded this value [8]. During the last two decades, the incidence of peritonitis declined substantially, particularly episodes caused by gram-positive organisms [22,23], due to the introduction of Y-set and double-bag disconnect systems. The introduction of “biocompatible” solutions with normal pH could improve peritoneal leukocyte function and further reduce peritonitis rate [24]. Moreover, patients tend to be more aware of the importance of early arrival with suspected peritonitis than in the past, and peritonitis diagnostic techniques have improved. Therefore, new updated studies regarding peritonitis diagnostics in PD patients are needed.

We demonstrated that peritoneal WBC count cutoff level higher than that recommended by the ISPD could improve the specificity of the test without compromising its sensi-
tivity. Peritoneal fluid WBC count below 230/μL effectively ruled out peritonitis given that inflammatory markers were not elevated. This peritoneal fluid WBC cutoff below 230/μL effectively ruled out peritonitis in a more recent PD patient validation group.

We found that severe peritonitis as defined by patient hospitalization was associated with significantly higher peritoneal WBC count. A count of 290 WBC/μL was the lowest limit demonstrated in peritonitis cases that required hospitalization. Two other potential features of severe peritonitis, need for catheter removal and peritonitis relapse, were not associated with higher peritoneal WBC count. These outcomes are probably more significantly related to the type of microbial pathogens, such as *Candida albicans* or *Pseudomonas aeruginosa*, rather than to the severity of inflammation, as represented by initial WBC count [25]. For example, it was demonstrated that *Corynebacterium* peritonitis often resulted in relapse or repeat episodes, catheter removal, permanent hemodialysis transfer, and death [26]. In addition, *Pseudomonas* peritonitis is associated with high rates of hospitalization, catheter removal, and permanent hemodialysis transfer [27,28].

Our patients with less than 230 cells/μL in peritoneal fluid who later were diagnosed as having peritonitis based on ISPD criteria and culture results did not have a classical presentation of PD-related peritonitis and were initially treated according to clinical judgment. In those cases, outcome was not compromised even though intraperitoneal antibiotics administration was delayed. It seems that there is a correlation between severity of peritonitis and peritoneal fluid WBC count. Patients with low peritoneal fluid WBC count at presentation have less severe peritonitis. Peritoneal PMN cell count was not more sensitive than peritoneal WBC in peritonitis diagnosis in our work. Peritoneal cell differential could be influenced by many factors. Resident peritoneal macrophages can multiply when dwell times are prolonged, while rapid exchanges and protracted handling result in decreased dialysate cell counts through dilution and clotting [5]. The number of leucocytes in the dialysate is influenced by the duration of dwell time [8], differences in dwell time from preceding measurements, and differences in the intensity of the inflammatory response between episodes. It is possible that a repeat peritoneal fluid cell count and differential after several hours from initial presentation could show higher peritoneal PMN percentage.

Our study demonstrates that it might be safe to increase the currently recommended peritoneal fluid WBC count cutoff of 100/μL for peritonitis diagnosis in PD-treated patients. This could decrease the possibility of unnecessary antibiotic treatment and allow a timely search for an alternative diagnosis, such as other intraabdominal pathologies. In this setting, clinical judgment is essential. In unclear cases, where peritoneal fluid WBC count is only slightly elevated, a repeat peritoneal fluid cell count and differential after several hours of observation can be beneficial. In many cases of peritonitis, the dialysate becomes cloudy on subsequent exchanges. In such cases, the patient should be closely monitored and antibiotics should be started if signs, symptoms, and repeat cell count are consistent with peritonitis.

Limitations of the study include a single-center performance and retrospective design. In addition, increasing peritoneal fluid WBC count above the currently recommended value as a diagnostic criterion for peritonitis could put the patient at risk of late diagnosis and delayed treatment. In this regard, in unclear cases, a repeat peritoneal fluid cell count and differential after several hours of observation can be beneficial. If this is not possible, antibiotic treatment should be administered immediately. Prospective studies should be performed to confirm our findings.

To conclude, it seems that the currently recommended cutoff peritoneal fluid WBC count for peritonitis diagnosis in PD patients can be safely increased provided close follow-up and clinical judgment.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

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

Authors’ contributions

Conceptualization, Formal analysis: MK
Investigation: MK, NAA
Supervision: PB
Validation: SM, NAA
Writing–original draft: MK
Writing–review & editing: SM, PB
All authors read and approved the final manuscript.

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References


Background: Generally, an induction agent is chosen based on the conditions of the deceased donor and the recipient. Antithymocyte globulin (ATG) is preferred in relatively high-risk conditions. No clear evidence indicates which induction agent is safer or more efficient for deceased donor kidney transplantation (DDKT). This study compares the efficacy and safety of basiliximab (BSX) and ATG according to donor characteristics in DDKT.

Methods: A total of 724 kidney transplant recipients from three transplant centers were enrolled, and propensity score matching was performed. Based on a donor age of 60 years, donor kidney with acute kidney injury (AKI), and Kidney Donor Profile Index (KDPI) score of 65%, we investigated how the choice of induction therapy agent affected the posttransplant clinical outcomes of delayed graft function (DGF), acute rejection (AR), infectious complications, and allograft and patient survival.

Results: AR and DGF did not differ significantly according to induction agent in elderly/young donor, AKI/non-AKI, and high-KDPI/low-KDPI subgroups. The infection rate did not show meaningful differences. The differences in death-censored allograft survival and patient survival rates between induction agents were not statistically significant.

Conclusion: Our study suggests that BSX can produce clinical outcomes similarly favorable to those of ATG even in DDKT cases with relatively poor donor conditions. Nonetheless, the donor and recipient conditions, immunological risk, and infection risk must be all taken into consideration when choosing an induction agent. Therefore, clinicians should carefully select the induction therapy agent for DDKT based on the risks and benefits in each DDKT case.

Keywords: Basiliximab, Delayed graft function, Graft rejection, Graft survival, Kidney transplantation, Thymoglobulin
**Introduction**

The incidence of end-stage renal disease (ESRD) is increasing worldwide. Kidney transplantation (KT) is one promising option for ESRD patients because it provides good quality of life after transplantation and a high survival rate. However, kidney donors are scarce, which leads to prolonged transplant waiting times and is associated with mortality among ESRD patients on the waiting list [1]. In December 2017, 19,807 patients were on the Korean Network for Organ Sharing waiting list for KT [2,3]. During the past decade in Korea, a 22.4% annual increase was recorded in the KT waiting list. The median wait time for KT from a deceased donor (DD) was 4.5 ± 2.7 years [3]. In Korea, 5.2 patients a day die while waiting for KT [4]. Using kidneys from donors with acute kidney injury (AKI) or elderly DDs is one attractive strategy to expand the donor pool. Although posttransplant clinical outcomes and prognosis in those cases are controversial, many previous studies have reported that KT from elderly DDs or DDs with AKI is a negative risk factor for delayed graft function (DGF), acute rejection (AR), and allograft survival [4,5]. The Kidney Donor Profile Index (KDPI) scoring system for DDs is widely used to predict postoperative graft function, and a high KDPI score is a well-known risk factor for allograft failure [6].

To compensate for those risk factors and enable successful KT, induction therapy is important. Antithymocyte globulin (ATG) and basiliximab (BSX) are the most widely used induction therapies in KT [7]. ATG is a lymphocyte depleting polyclonal antibody that targets multiple immunologic epitopes. BSX is a non-lymphocyte-depleting monoclonal antibody that targets interleukin-2 receptor (IL-2R). Previous studies have compared the efficacy and safety of ATG and BSX in KT in terms of clinical outcomes and complications. Webster et al. [8] reported no differences in allograft failure between BSX and ATG, but ATG showed a lower biopsy-proven AR (BPAR) rate 1 year after the transplant than did BSX. More recently, a prospective randomized study was performed to compare ATG (1.5 mg/kg from day 0 to day 4) and BSX (20 mg on day 1 and day 4) in deceased-donor KT (DDKT) patients at high risk for AR and DGF [9]. The ATG group had lower incidence and severity of AR. However, DGF and the graft survival and patient survival rates showed no statistically significant differences between the two induction therapies [10]. Furthermore, other studies found that patients who received BSX had a lower incidence of infection than those who received ATG [11,12]. Despite the various previous studies, recent randomized studies have failed to demonstrate which induction therapy is more efficient. Therefore, the purpose of this study was to compare the efficacy and safety of ATG and BSX as induction therapy for DDKT cases in which the donor characteristics are poor.

**Methods**

**Study population**

A total of 724 kidney transplant recipients (KTRs) who received KT at one of three transplant centers between October 1996 and July 2019 were enrolled, excluding 53 KTRs with no information about induction therapy or graft function after KT or with follow-up loss. The KTRs were divided into ATG-DDKT (252 KTRs) and BSX-DDKT (472 KTRs) groups. After propensity score (PS) matching, we used subgroups based on donor age of 60 years, donor kidney AKI, and KDPI score of 65% (elderly vs. young DDs, DDs with AKI vs. DDs without AKI, and DDs with high KDPI vs. DDs with low KDPI) to investigate how the induction therapy agent affected posttransplant clinical outcomes. We conducted re-matching within each subgroup based on cohort PS. The study population flow chart and patient distribution are presented in Fig. 1.

724 Patients were analyzed

472 in BSX group

252 in ATG group

Propensity score matching

218 in BSX group

218 in ATG group

**Figure 1. Flow chart of the study population.** A total of 724 kidney transplant recipients was divided into ATG-DDKT and BSX-DDKT groups, and propensity score matching was performed. ATG, antithymocyte globulin; BSX, basiliximab; DDKT, deceased donor kidney transplantation.
First, the ATG-DDKT and BSX-DDKT groups were subdivided into young-DDKT and elderly-DDKT subgroups based on a donor age of 60 years at the time of donation (<60 years vs. ≥60 years). When re-matching was conducted within each subgroup, the young-DDKT group contained 358 cases (83.3%), and the elderly-DDKT group contained 72 cases (16.7%).

Second, each group was subdivided into AKI-DDKT and non-AKI-DDKT subgroups based on whether the DD had AKI, defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria. The AKI-DDKT group contained 286 cases (68.8%), and the non-AKI-DDKT group contained 130 cases (31.2%).

Third, each group was subdivided into high KDPI-DDKT and low-KDPI-DDKT subgroups based on KDPI scores. We defined high KDPI score as that greater than 65%, the median KDPI score in this cohort. The KDPI scores were calculated using the KDPI calculator on the Organ Procurement and Transplantation Network website (https://optn.transplant.hrsa.gov/data/allocation-calculators/kdipi-calculator/). The high-KDPI-DDKT group contained 226 cases (57.7%), and the low-KDPI-DDKT group contained 166 cases (42.3%).

We then investigated the effects of choice of induction therapy agent in those groups in terms of both short- and long-term clinical outcomes.

In all patients in the BSX-DDKT group, 20 mg/day of BSX was administered on the operation day and postoperation day 4. In the ATG-DDKT group, ATG was given from the operation day to postoperation day 4. The standard dose of ATG was body weight × 1.25 mg/day, but that dose was halved when the white blood cell (WBC) count was 2,000/mm³ to 3,000/mm³ or the platelet count was lower than 75,000/mm³ but higher than 50,000/mm³. When the WBC count was less than 2,000/mm³ or the platelet count was lower than 50,000/mm³, ATG treatment was stopped.

Clinical parameters and outcomes

We retrospectively analyzed the medical records of both donors and recipients. The baseline donor data were age, sex, body mass index (BMI) (kg/m²), history of diabetes mellitus (DM) and hypertension (HTN), donor death due to cerebrovascular accident (CVA), cold ischemic time, KDPI, and serum creatinine (as an assessment of kidney function from the day of admission to the day of KT). The baseline and follow-up estimated glomerular filtration rates (eGFRs) were calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. We collected the following baseline recipient data: age, sex, BMI, history and duration of dialysis before KT, number of previous KTs, cause of ESRD, history of DM and HTN, number of human leukocyte antigen (HLA) mismatches, immunosuppressant type for induction and maintenance, and percentage of panel-reactive antibodies (PRAs). BPAR was diagnosed by the Banff classification. DGF was defined as the need for dialysis within the first week after KT because of unrecovered allograft function. Infection complications included BK virus nephropathy, *Pneumocystis jirovecii* pneumonia, CMV viremia, and other infections that could cause graft failure or patient death. The death-censored allograft survival rate was defined as the rate from KT to the return to dialysis except for allograft loss due to patient death. The patient survival rate was defined as the rate from KT to death by any cause.

The primary outcomes were a comparison of the death-censored allograft survival rate of KTRs according to induction therapy agent in subgroups formed according to donor age, presence of AKI, and KDPI score. The secondary outcomes were incidence of DGF and BPAR, infection rate, and patient survival rate between the ATG-DDKT and BSX-DDKT groups according to donor age, presence of AKI, and KDPI score. The causes of allograft failure included BPAR (both T-cell-mediated rejection and antibody-mediated rejection [AMR]), biopsy-proven chronic AMR, chronic allograft dysfunction, biopsy-proven BK virus-associated nephropathy, and biopsy-proven recurrent primary glomerulonephritis. Chronic allograft dysfunction was diagnosed when the allograft findings showed non-specific chronic tissue injury without evidence of rejection or when no allograft biopsy was performed within 1 year of allograft failure, and allograft function showed gradual deterioration for several years before allograft failure.

This study was approved by the Institutional Review Boards (IRBs) of Seoul St. Mary’s Hospital (No. XC15RI-MI0061K), Uijeongbu St. Mary’s Hospital (No. XC15RIM-I0061U), and Keimyung University Dongsan Hospital (No. 2021-07-041). The requirement for informed consent was waived by the IRBs of those three centers because the clinicians explained to all donor families and all recipients pri-
or to KT that personal data associated with the donor and recipient’s clinical course would be used for research purposes, and all information identifying individuals was protected. As a retrospective medical record study, our study did not use any distinguishable personal identification information. Furthermore, all methods were performed in accordance with the relevant guidelines and regulations. The three transplant centers involved in this study have never performed transplantation with kidneys procured from prisoners, and this study did not include them in this study population.

Statistical analysis

We applied a PS matching analysis to minimize the influence of potential confounding biases and increase comparability between the BSX and ATG groups. The following variables were used to calculate the PSs in a multivariate logistic regression model: donor and recipient age, sex, BMI, PRA class I + II >30%, DM, HTN, perioperative eGFR, KDPI score, donor kidney AKI, and CVA as the cause of donor death. PS re-matching within each subgroup was performed based on the cohort PS. A 1:1 PS matching method was applied based on the greedy 8-1-digit matching algorithm.

Continuous variables with normal distributions in the entire cohort are expressed as mean with standard deviation and were analyzed using independent t-test. Categorical variables in the entire cohort are expressed as number with percentage and were analyzed by chi-square test. The statistical analysis of the PS cohort used paired t-test and the McNemar test. After confirming the proportional hazard (PH) assumption, a conditional Cox PH regression analysis was used to investigate how the choice of induction therapy agent affected the clinical outcomes of DDKT and to find independent risk factors for allograft failure while considering confounding variables: transplant year (1996–2005 vs. 2006–2012 vs. 2013–2019), transplant center, recipient age, recipient sex, recipient BMI, donor age, donor sex, donor BMI, cold ischemic time, HLA mismatch number, PRA summation quantity, KDPI score, and donor kidney AKI. Any p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS version 19.0 (IBM Corp.), and the PS matching analysis was performed by the ‘MatchIt’ packages in R version 4.1.1 (R Foundation for Statistical Computing).

Results

Comparison of baseline characteristics according to induction therapy

The demographic and clinical data of the patients who underwent DDKT are shown in Table 1 according to the induction therapy agent used. The donors and recipients in the ATG group were older than those in the BSX group (p = 0.002). The donors in the ATG group had more frequent underlying DM than those in the BSX group (p = 0.02). The KDPI score (p = 0.04) and incidence of donors with AKI (p < 0.001) were higher in the ATG group, which also had more highly sensitized patients (PRA class I + II >30%) and lower donor preoperative eGFR than the BSX group (p < 0.001). On the other hand, the ratio of patients with previous KT was lower (p = 0.003) in BSX group. Among the operative characteristics, no difference was observed between the two groups in terms of mean cold ischemic time or HLA mismatch numbers, but brain death due to CVA was significantly higher in the BSX group (p = 0.001). The majority of renal disease in both groups was related to chronic glomerulonephritis. No significant differences between the two groups were observed in respect to donor sex, recipient BMI, dialysis duration, and etiology of recipient ESRD. Because the BSX and ATG groups differed significantly in various factors, we used PS matching to minimize the influence of potential confounding biases and increase the comparability of the groups.

Comparison of clinical outcomes according to induction therapy agent in all patients

Comparisons of postoperative patient survival and death-censored graft survival after induction with BSX versus ATG in the PS-matched cohort are shown in Fig. 2. Death-censored graft survival did not differ significantly after induction with BSX and ATG (p = 0.61). The patient survival rates with BSX and ATG were 93.3% and 94.2%, respectively (p = 0.86). In the PS-matched cohort, the incidence of AR (25.2%
### Table 1. Comparison of clinical and laboratory parameters according to induction therapy agent used in KT recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort (n = 724)</th>
<th>PS-matched cohort (n = 436)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Basiliximab KT (n = 472)</td>
<td>ATG KT (n = 252)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basiliximab KT (n = 218)</td>
<td>ATG KT (n = 218)</td>
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<tr>
<td>Donor</td>
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<td>Age at KT (yr)</td>
<td>44.9 ± 14.7</td>
<td>48.2 ± 12.7</td>
<td>0.002</td>
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<td>Sex, male:female</td>
<td>312:160</td>
<td>180:72</td>
<td>0.14</td>
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<td>Body mass index (kg/m²)</td>
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<td>23.9 ± 3.6</td>
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<td>Hypertension</td>
<td>100 (21.2)</td>
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<td>Diabetes mellitus</td>
<td>37 (7.8)</td>
<td>34 (13.5)</td>
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<td>Cause of donor death-CVA</td>
<td>343 (72.7)</td>
<td>154 (61.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Preoperative eGFR (mL/min/1.73 m²)</td>
<td>91.6 ± 44.1</td>
<td>70.2 ± 45.1</td>
<td>&lt;0.001</td>
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<td>KDPI score, ≥65</td>
<td>228 (48.3)</td>
<td>142 (56.3)</td>
<td>0.04</td>
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<td>Acute kidney injury</td>
<td>219 (46.4)</td>
<td>182 (72.2)</td>
<td>&lt;0.001</td>
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<td>Recipient</td>
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<td></td>
<td></td>
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<tr>
<td>Transplant year</td>
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<td></td>
<td>&lt;0.001</td>
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<tr>
<td>1999–2005</td>
<td>8 (1.7)</td>
<td>0 (0)</td>
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<td>2006–2012</td>
<td>208 (44.1)</td>
<td>10 (4.0)</td>
<td>94 (43.1)</td>
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<tr>
<td>2013–2019</td>
<td>256 (54.2)</td>
<td>242 (96.0)</td>
<td>124 (56.9)</td>
</tr>
<tr>
<td>Age at KT (yr)</td>
<td>49.0 ± 10.0</td>
<td>51.4 ± 9.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>290:182</td>
<td>134:118</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2 ± 3.8</td>
<td>23.3 ± 3.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Hypertension</td>
<td>401 (85.0)</td>
<td>193 (76.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>92 (19.5)</td>
<td>58 (23.0)</td>
<td>0.27</td>
</tr>
<tr>
<td>Dialysis duration (yr)</td>
<td>7.80 ± 6.78</td>
<td>9.77 ± 13.91</td>
<td>0.04</td>
</tr>
<tr>
<td>Previous KT</td>
<td>38 (8.1)</td>
<td>38 (15.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Cause of ESRD</td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>212 (44.9)</td>
<td>127 (50.4)</td>
<td>82 (37.6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>81 (17.2)</td>
<td>47 (18.7)</td>
<td>37 (17.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>86 (18.2)</td>
<td>38 (15.1)</td>
<td>49 (22.5)</td>
</tr>
<tr>
<td>Others</td>
<td>93 (19.7)</td>
<td>40 (15.9)</td>
<td>50 (22.9)</td>
</tr>
<tr>
<td>Cold ischemic time (min)</td>
<td>248.2 ± 118.5</td>
<td>257.4 ± 135.1</td>
<td>0.38</td>
</tr>
<tr>
<td>HLA mismatch number</td>
<td>3.57 ± 1.50</td>
<td>3.68 ± 1.57</td>
<td>0.36</td>
</tr>
<tr>
<td>PRA class I + II, &gt;30%</td>
<td>55 (11.7)</td>
<td>100 (39.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

ATG, antithymocyte globulin; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HLA, human leukocyte antigen; KDPI, Kidney Donor Profile Index; KT, kidney transplant; PRA, panel reactive antibody; PS, propensity score.

Comparison of clinical outcomes according to donor age

We compared postoperative patient survival and death-censored graft survival after induction with BSX or ATG according to donor age in the PS-matched subgroups. The elderly group (donor age of ≥60 years) contained 72 patients, and the young group (donor age of <60 years) contained 358 patients. In the elderly group, death-censored graft survival did not differ significantly between the BSX and ATG groups (p = 0.88). Likewise, in the young group, death-censored graft survival did not differ significantly between the BSX and ATG groups (p = 0.50). The patient survival rates in the elderly group after treatment with BSX and ATG were 93.9% and 92.6%, respectively (p = 0.97). Likewise, the patient survival rates in the young group
were 91.9% and 94.5% after treatment with BSX and ATG, respectively (p = 0.80). The incidence of AR (BSX, 27.8% vs. ATG, 22.2%; p = 0.79), the infection rate (BSX, 8.3% vs. ATG, 5.6%; p = 0.66), and DGF (BSX, 19.4% vs. ATG, 16.7%; p = 0.74) did not differ significantly in the elderly group. As in the elderly group, the incidence of AR (BSX, 24.0% vs. ATG, 24.0%; p > 0.99), infection rate (BSX, 3.9% vs. ATG, 5.6%; p = 0.61), and DGF (BSX, 20.7% vs. ATG, 23.5%; p = 0.61) did not differ significantly according to the induction therapy agent used in the young group (Table 3). The entire cohort analysis results are summarized in Supplementary Table 1 (available online).

**Table 2. Comparison of AR, infection rate, and delayed graft function according to induction therapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire cohort (n = 724)</th>
<th>PS-matched cohort (n = 436)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basiliximab (n = 472)</td>
<td>ATG (n = 252)</td>
</tr>
<tr>
<td>AR</td>
<td>109 (23.1)</td>
<td>60 (23.8)</td>
</tr>
<tr>
<td>Infection</td>
<td>24 (5.1)</td>
<td>13 (5.2)</td>
</tr>
<tr>
<td>DGF</td>
<td>90 (19.1)</td>
<td>59 (23.4)</td>
</tr>
</tbody>
</table>

Data are expressed as number (%).
AR, acute rejection; ATG, antithymocyte globulin; DGF, delayed graft function; PS, propensity score.

Comparison of clinical outcomes according to acute kidney injury in donor

We compared postoperative patient survival and death-censored graft survival after induction with BSX or ATG according to the presence of donor kidney AKI in the PS-matched subgroups. The AKI group contained 286 patients, and the non-AKI group contained 130 patients. In the AKI group, death-censored graft survival did not differ significantly between the patients who received BSX and those who received ATG induction therapy (p = 0.90). Likewise, in the non-AKI group, death-censored graft survival did not differ between BSX and ATG (p = 0.25). The patient survival rates in the AKI group after treatment with BSX and ATG
were 93.8% and 95.2%, respectively (p = 0.95). In the non-AKI group, the patient survival rates were 91.8% and 92.0% in patients treated with BSX and ATG, respectively (p = 0.67). The incidence of AR (BSX, 28.0% vs. ATG, 23.1%; p = 0.40), infection rate (BSX, 4.2% vs. ATG, 4.2%; p > 0.99), and DGF (BSX, 27.3% vs. ATG, 28.7%; p = 0.89) did not differ significantly by induction therapy in the AKI group. Likewise, the incidence of AR (BSX, 18.5% vs. ATG, 26.2%; p = 0.41), infection rate (BSX, 6.2% vs. ATG, 9.2%; p = 0.75), and DGF (BSX, 6.2% vs. ATG, 9.2%; p = 0.73) did not differ significantly in the non-AKI group (Table 4). The entire cohort analysis results are summarized in Supplementary Table 2 (available online).

### Comparison of clinical outcomes according to Kidney Donor Profile Index score

We compared postoperative patient survival and death-censored graft survival after induction with BSX and ATG according to KDPI score in the PS-matched subgroups. The high-KDPI group (KDPI score of >65%) contained 226 patients, and the low-KDPI group contained 166 patients. In the high-KDPI group, death-censored graft survival did not differ significantly between the patients who received BSX and those who received ATG induction therapy (p = 0.39). Likewise, in the low-KDPI group, death-censored graft survival did not differ significantly between patients who received BSX and those who received ATG (p = 0.55). The patient survival rates in patients treated with BSX and ATG were 92.7% and 95.1%, respectively, in the high-KDPI group (p = 0.46) and 96.2% and 95.8% in the low-KDPI group (p = 0.63). The incidence of AR (BSX, 25.7% vs. ATG, 28.3%; p = 0.77), infection rate (BSX, 6.2% vs. ATG, 5.3%; p = 0.78), and DGF (BSX, 23.0% vs. ATG, 26.6%; p = 0.64) did not differ between induction therapies in the high-KDPI group. Likewise in the low-KDPI group, the incidence of AR (BSX, 21.7% vs. ATG, 15.7%; p = 0.42), infection rate (BSX, 6.2% vs. ATG, 6.2%; p = 0.63), and DGF (BSX, 19.3% vs. ATG, 20.5%; p = 0.85) did not differ significantly between induction therapies (Table 5). The entire cohort analysis results are summarized in Supplementary Table 3 (available online).

### Discussion

Due to a shortage of organ donors and ethical issues, DDKT is a promising option for ESRD patients. Moreover, kidneys from donors with AKI, elderly DDs, and donors with high KDPI scores have recently become widely used to maximize...
the number of donor candidates. Due to improvements in KT strategies including induction therapy agents, the criteria for DDs have been expanded. Induction therapy plays an important role in transplantation by lowering the incidence of AR and thereby improving allograft survival [13]. Currently, almost 80% of KTRs in the United States receive induction therapy with either BSX or ATG [9]. In general, BSX is used for immunologically low-risk patients, and ATG is used for high-risk patients. However, the effects of selecting one induction immunosuppressant over the other in DDKT remain unclear, and the choice of induction agent remains controversial [14]. Therefore, we compared the efficacy and safety of ATG and BSX as induction therapy in DDKT patients whose donor condition was relatively poor.

A series of trials have demonstrated that induction therapy with ATG or BSX reduces the risk of early AR episodes after KT versus controls. In 2010, the Cochrane Collaboration published a meta-analysis of randomized controlled trials that compared BSX induction with placebo and with ATG [15]. Compared with placebo, the BPAR rates were 30% lower with BSX (1-year relative risk [RR], 0.72; 95% confidence interval [CI], 0.64–0.81), and graft loss was reduced (1-year RR, 0.75; 95% CI, 0.62–0.90). Brennan et al. [9] compared patients at high risk of AR or DGF who received DDKT with ATG (1.5 mg/kg from day 0 to day 4) with those who received BSX (20 mg on day 1 and day 4) as induction therapy. Their ATG patients had a lower incidence and lower severity of AR. A long-term follow-up study of those patients showed that the incidence of AR requiring antibody treatment in patients with ATG was lower than that in patients who received BSX [16].

The TAXI study compared DGF in high-risk DDKT recipients who received IL-2R antibody vs. ATG. Those ATG patients had a lower incidence of both rejection and DGF 1 year after transplant [17]. Jeong et al. [18] found that low-dose ATG (1 mg/kg on days 0, 1, and 2) significantly reduced the rates of DGF and AR compared with BSX in high-risk recipients (DGF, p = 0.035; AR, p = 0.004). Based on those previous studies, ATG is considered to have higher immunosuppressive effects than BSX and is expected to have clinical outcome benefits in DDKT with poor donor condition [19].

ATG is also considered to carry a higher risk of infection. Various previous studies have reported that patients who receive BSX have a lower incidence of infection than those who receive ATG [20–22]. About the cause for that difference, Liu et al. [23] said that it might be related to the different drug mechanisms. BSX is a monoclonal antibody that targets CD25 and binds to the α-chain of IL-2R, making it a potent inhibitor of IL-2-mediated T-cell proliferation. CD25 participates in lymphocyte differentiation, activation, and proliferation. CD8 T cells respond to viral infections and also participate in defense against bacterial and protozoal infections. Because most CD8 cells express IL-2R β and γ chains, CD25 therapy (BSX) might not impair the cytotoxic T-cells that contribute to the control of infection.

In previous studies, the death-censored graft and patient survival rates were similar between the two induction therapies [24–26]. Likewise, the differences in graft and patient survival rates between induction agents were not statistically significant in our study. We calculated a log minus log survival plot to test the assumption of PHs. Each risk factor graph curve had a constant vertical distance, indicating that our data satisfy the PH assumption. Furthermore, we conducted the Schoenfeld residual test to confirm that finding and found a Schoenfeld p-value of >0.05, which indicates that our study model meets the PH assumption. In our multicollinearity check, the variance inflation factor was less than 10 (range, 1.00–1.93). In our multivariable analysis using a conditional Cox regression hazard model,
induction therapy agent was not a significant risk factor for allograft failure (hazard ratio [HR], 1.73; 95% CI, 0.87–3.44; p = 0.12). Neither DD AKI (HR, 1.43; 95% CI, 0.63–3.23; p = 0.40) nor elderly DD (HR, 1.00; 95% CI, 0.96–1.05; p = 0.87) alone showed significance in the multivariable analysis, which agrees with previous reports. High KDPI score alone showed significance for allograft failure (HR, 4.15; 95% CI, 1.31–13.13; p = 0.02) (Table 6).

In addition to those results, which confirm those in previous studies, our results here provide interesting information that was not reported in previous studies. We found that induction therapy itself did not notably influence long-term clinical outcomes, including the incidence of AR and DGF. Neither AR nor DGF differed significantly by induction agent, not only in the total study population, but also in the elderly/young donor, AKI/non-AKI, and high-KDPI/low-KDPI subgroups. Our study suggests that BSX can produce clinical outcomes that are similarly favorable to those with ATG even in DDKT cases in which the donor condition is relatively poor. Therefore, using ATG induction therapy in high-risk DDKT as a matter of protocol or habit might not be an appropriate strategy.

Our study has some limitations, as suggested in our previous reports using this cohort. First, because it was a retrospective study, our study has a possibility of selection bias. To overcome that limitation and reduce the effects of confounding factors, we used PS matching and a relatively large number of KTRs from multiple centers. In addition, we adjusted our results to consider the transplant center and transplant year in the multivariable analysis. Second, not all KTRs corresponding to donors included in this study were included in our analysis because some organs were transferred to another institution according to the organ distribution rule in Korea, which could have induced bias during the analysis. Well-designed, stratified, prospective multicenter studies are required to overcome these issues.

In conclusion, clinicians should select an induction therapy agent carefully in DDKT. The donor and recipient conditions, immunological risk, and infection risk must all be considered. Selection of induction therapy agent should be individualized based on the risks and benefits in each DDKT case.

**Additional information**

Table 6. Risk factors for allograft failure in deceased donor KT (propensity score matched cohort)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Unadjusted HR (95% CI)</th>
<th>p-value</th>
<th>Adjusted HR(^a) (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG vs. basiliximab</td>
<td>1.70 (0.78–3.71)</td>
<td>0.18</td>
<td>1.73 (0.87–3.44)</td>
<td>0.12</td>
</tr>
<tr>
<td>Donor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.01 (0.95–1.07)</td>
<td>0.81</td>
<td>1.00 (0.96–1.05)</td>
<td>0.87</td>
</tr>
<tr>
<td>Sex, female vs. male</td>
<td>1.75 (0.51–5.98)</td>
<td>0.37</td>
<td>1.19 (0.59–2.41)</td>
<td>0.63</td>
</tr>
<tr>
<td>AKI by KDIGO, 1 vs. 0</td>
<td>0.33 (0.04–3.21)</td>
<td>0.34</td>
<td>1.43 (0.63–3.23)</td>
<td>0.40</td>
</tr>
<tr>
<td>KDPI score, ≥65 vs. &lt;65</td>
<td>5.00 (0.58–42.79)</td>
<td>0.14</td>
<td>4.15 (1.31–13.13)</td>
<td>0.02</td>
</tr>
<tr>
<td>Recipient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.94 (0.87–1.01)</td>
<td>0.09</td>
<td>1.00 (0.96–1.03)</td>
<td>0.85</td>
</tr>
<tr>
<td>Sex, female vs. male</td>
<td>1.75 (0.51–5.98)</td>
<td>0.37</td>
<td>1.19 (0.62–2.29)</td>
<td>0.60</td>
</tr>
<tr>
<td>Previous KT, yes vs. no</td>
<td>0.67 (0.11–3.99)</td>
<td>0.66</td>
<td>1.01 (0.30–3.44)</td>
<td>0.99</td>
</tr>
<tr>
<td>PRA class I + II, &gt;30% vs. ≤30%</td>
<td>0.40 (0.14–1.16)</td>
<td>0.09</td>
<td>0.47 (0.21–1.03)</td>
<td>0.06</td>
</tr>
<tr>
<td>HLA mismatch number</td>
<td>1.00 (0.68–1.47)</td>
<td>&gt;0.99</td>
<td>0.99 (0.79–1.25)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; ATG, antithymocyte globulin; CI, confidence interval; HLA, human leukocyte antigen; HR, hazard ratio; KDIGO, Kidney Disease Improving Global Outcomes; KDPI, Kidney Donor Profile Index; KT, kidney transplantation; PRA, panel reactive antibody.

\(^a\)Adjusted by recipient age, recipient sex, donor sex, donor age, HLA mismatch number, PRA summation quantity, donor kidney AKI, and KDPI score.
Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

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Data sharing statement

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

Authors’ contributions

Conceptualization: BHC, WYP
Data curation: SYH, YSK, KJ, SH
Funding acquisition: BHC
Formal analysis: SYH, YSK, KJ, WYP
Supervision: SH, CWY, BHC, WYP
Validation: SH, CWY
Writing–original draft: SYH, BHC, WYP
Writing–review & editing: SYH, WYP, BHC
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References

15. Hellemans R, Bosmans JL, Abramowicz D. Induction therapy for kidney transplant recipients: do we still need anti-IL2 receptor


A 40-year-old female patient with lupus-related end-stage renal disease received a preemptive living donor kidney transplant from her sister. Although graft function was well maintained under tacrolimus, prednisolone, and mycophenolate mofetil (MMF) maintenance therapy, the patient experienced an asymptomatic urinary tract infection. *Escherichia coli* was detected in urine culture. Oral third-generation cephalosporins were administered to the patient until discharge. Fourteen weeks after transplantation, the patient came to the emergency department complaining of right flank pain and fever. Laboratory tests showed hematuria, pyuria, bacteriuria, and leukocytosis, indicating a urinary tract infection. The patient was treated with antibiotics and discharged from the emergency department.

The patient revisited the emergency room with the same symptoms eighteen weeks after transplantation. At this time, there was no bacteriuria or pyuria on urinalysis. Computed tomography scan was performed to identify fever focus, and a huge infiltrative mass lesion was observed in the upper portion of the right native kidney, extending into the perinephric space and right psoas muscle (Fig. 1). For diagnosis and treatment, ultrasound-guided gun biopsy was performed and percutaneous catheter drainage (PCD) was inserted. *E. coli* was identified on PCD culture, but there was no bacteria growth on urine culture. On microscopy, cellular infiltrates were predominantly composed of large macrophages with foamy granular eosinophilic cytoplasm, prominent eccentric, hyperchromatic and round nuclei, indicating von Hansemann cells. In von Kossa staining, the cells contained some intracytoplasmic, concentrically laminated, round-ovoid, basophilic inclusions called Michaelis-Gutmann bodies. In addition, CD68 was positive on immunohistochemical staining (Fig. 2). These morphological and immunohistochemical characteristics were consistent with malakoplakia, not malignancy. Oral third-generation cephalosporin was administered to the patient for 4 months, and MMF was discontinued for 7 months. The patient recovered from malakoplakia without graft dysfunction or other complications.

Malakoplakia is an uncommon granulomatous inflammatory disorder that typically occurs in immunocompromised individuals. Malakoplakia affects mainly the genitourinary tract, although it involves nearly every organ. Malakoplakia of the graft kidney is more frequent in women with a history of bacterial urinary infection; *E. coli* is the most common pathogen. Almost all cases of kidney-related malakoplakia appear in transplanted kidneys and are associated with graft dysfunction. First-line treatment of...
Figure 1. Malakoplakia in the right native kidney on computed tomography scan. (A) Axial and (B) coronal plane. A huge mass lesion extends into the perinephric space and the right psoas muscle.

Figure 2. Pathologic findings on native kidney gun biopsy. (A) Renal parenchyma was densely infiltrated by histiocytic cells (H&E stain, ×100). (B) Von Hansemann cells (red arrows) containing foamy granular eosinophilic cytoplasm and prominent eccentric and hyperchromatic nuclei (H&E stain, ×400). (C) Calcium deposits within Michaelis-Gutmann bodies (black arrows) stained positive for Von Kossa stain (×400). (D) CD68-positive cells of the monocyte/macrophage lineage (white arrow) on CD68 staining (×400).
malakoplakia involves long courses of antibiotics and a reduction in immunosuppression.

This is a rare case of post-transplantation malakoplakia that developed in the native kidney and not in the graft. We suggest that conventional treatment methods can effectively treat native renal malakoplakia without graft dysfunction.

**Conflict of interest**

All authors have no conflicts of interest to declare.

**Data sharing statement**

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

**Authors’ contributions**

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

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INSTRUCTIONS FOR AUTHORS

1. Manuscript Submission

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

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

(1) A cover letter. It must include your name, address, telephone and fax numbers, e-mail address, and state that all authors have contributed to the paper and have never submitted the manuscript, in whole or in part, to other journals.

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

(3) All studies involving human subjects, human data or any material derived from human must be approved by the relevant review or ethics committee. Articles must include a statement on ethics approval, the name of the relevant committee that approved the study and the committee’s approval number. Manuscripts may be rejected at any time if the authors of the research fail to provide the approval number validated by the relevant committee (see relevant section 4.1 below).

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(5) Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified.

(6) The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants, the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).

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

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

(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.

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

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2. Types of Articles

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

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.

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

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
3.5. References

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

Journal articles:

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, c, ...) should be used for special remarks.

3.7. Figures

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

3.8. Supplementary Digital Contents

Authors can submit supplementary digital contents to supplement the information provided in the print version of the manuscript. Supplementary materials will be published online-only. When uploading supplementary files through the online system, please use the “supplemental” file designation. Supplementary materials must be cited consecutively in the main body of the submitted manuscript and include the type of material submitted (e.g., “Supplementary Table 1”; “Supplementary Fig. 1”).
4. Ethical Considerations

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

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

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

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Submitted manuscripts are considered with the understanding that they have not been published previously in print or electronic format (except in abstract or poster form) and are not under consideration in totality or in part by another publication or electronic medium. Authors must state that neither the manuscript nor any significant part of it is under consideration for publication elsewhere or has appeared elsewhere in a manner that could be construed as a prior or duplicate publication of the same, or very similar, work. When malpractices are found in an article submitted to KRCP, we will follow the flowchart by the Committee on Publication Ethics (COPE, https://publicationethics.org/resources/flowcharts) for settlement of any misconduct. Although the editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with KRCP, its editors, or the Korean Society of Nephrology.

5. Review Process
All submissions are sent to peer reviewers. Authors will usually be notified within 4 weeks by e-mail of whether the submitted article is accepted for publication, rejected, or subject to revision before publication. Revised manuscripts must be submitted online by the corresponding author. Failure to resubmit the revised manuscript within 3 months of the editorial decision is regarded as a withdrawal.

6. Visual Abstract Guidelines
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.

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

Keep in mind the following:
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• Any descriptive text should be at least 12 pt font size.
• The visual abstract should be saved as an editable Power Point file as staff will add the article DOI and may edit the text for clarity.

6.2. Adding Visual Details
It is critical that you only use images for which you have permissions or rights. To avoid any potential problems, either use the copyright filter during an image search online or subscribe to an icon image bank. There are many image banks on the internet, which are free to use. The images used for visual abstract is recommended only open source, and the author is responsible for copyright issues of visual abstract. Researchers who frequently prepare visual abstracts may benefit from purchasing a subscription to access higher quality icons (e.g. Shutterstock, Getty Images, iStock, etc.).

Guiding principles:
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• Graphics should be 440 pixels wide by 350-365 pixels tall.

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For clarification on data accuracy and reproducibility of the results, raw data or analysis data will be deposited to a public repository, for example, Harvard Dataverse (https://dataverse.harvard.edu/) after acceptance of the manuscript. Therefore, submission of the raw data or analysis data is mandatory when requested by reviewers. If the data is already a public one, its URL site or sources should be disclosed. If data cannot be publicized, it can be negotiated with the editor. If there are any inquiries on depositing data, authors should contact the editorial office.

12. After acceptance

12.1. Article-in-press publication

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

12.2. Publication charges

In order to cover the costs of reviewing, copy editing, layout, and online hosting and archiving, KRCP charges an article processing fee upon acceptance of submitted papers as follows:

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- Correspondence, Image in Practice: KRW 300,000 (Korea) / USD 300 (rest of word).

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• Publication charge waiver policy

Our mission is to share the achievements in the nephrology field with researchers worldwide including the scientists in the low-income countries. We continue to apply the publication charge waiver policy to encourage the academic activity and support the limited funding for their research. To request a publication charge waiver, please send an application to registry@ksn.or.kr. Corresponding author from low-income countries could be waived. Waiver application must contain the manuscript number and country of corresponding author.
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세베라 초용량

지급된 용량이 다베포에틴 알파 또는 에포에틴의 투여 주기에 따라 다르다. 이 약의 첫 투여는 이전에 투여된 다베포에틴 알파 또는 에포에틴의 투여 주기에 따라 예정된 다음 투여일에 실시된다.

1. 미쎄라** 주요 렌 vistas에 해당하는 용량으로 1개월 1회씩 투여받을 수 있다. 2. 조혈촉진제 투여 받고 있는 환자: 현재 다른 조혈촉진제를 투여받고 있는 환자에 이 약을 1개월 1회 대체 투여할 수 있다. 이 약의 초기 용량은 표 1과 동일 용량으로 투여한다. 3. 복막투석 환자에 대한 치료 경험이 있는 경우: 1개월당 중간 용량으로 투여할 수 있다. 4. 초산칼슘제제를 복용하고 있는 환자에게 이 약을 대체 투여하는 경우: 초산칼슘제제 (1정당 초산칼슘 667mg) 1회 1정, 1일 3회 시 이 약 1회 1정(포) 1일 3회, 초산칼슘제제 1회 3정, 1일 3회 시 이 약 1회 3정(포), 1일 3회 5) 이 약을 복용하고 있는 모든 환자에서의 용량 조절: 목표 혈청 인수치에 도달하기 위해 적절한 용량 조절이 필요할 수 있다.

미쎄라의 주된 이점은 다음과 같다.

1. 세베라 탄산염 및 세벨라머 염산염의 시판 후 확인된 이상반응: 과민반응, 가려움증, 발진, 복통, 대변 막힘, 흔하지 않은 케이스로 장폐색증과 장폐쇄증, 장관천공. 변비증상이 나타나거나 기존의 변비증상이 심해진 환자는 중증의 합병증을 피하기 위해 적절한 의료처치가 필요하다. 2. 보다 자세한 내용은 홈페이지나 제품설명서를 참고하시기 바랍니다. 3. 문안개정연월일 2020.11.23

미쎄라®와 렌벨라®가 한독으로 하나가 되었습니다.

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CKD 환자의 질환 치료를 위해

References


CKD, chronic kidney disease; Hb, hemoglobin

제품내용물

렌벨라정 1정 중 세벨라머탄산염(별규) 800.0mg, 렌벨라산 1포 중 세벨라머탄산염 (별규) 800mg

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1. 싼불대천호대의사. 화학적약재사가-하약
2. 2019 so MAT, IQVIA DATA 기준(국내 고갈음혈증 치료제 판매량)

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Calcium polystyrene sulfonate

The most prescribed treatment agent of Hyperkalemia in Korea

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

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