HIGHLIGHTS

Mitochondrial quality control and its emerging role in the pathogenesis of diabetic kidney disease

The impact of hypoxia-inducible factors in the pathogenesis of kidney diseases: a link through cell metabolism

Serum and urine metabolomic biomarkers for predicting prognosis in patients with immunoglobulin A nephropathy

Electronic alert outpatient protocol improves the quality of care for the risk of postcontrast acute kidney injury following computed tomography

A questionnaire survey on the diagnosis and treatment of Fabry nephropathy in clinical practice
Aims and Scope

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

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

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

The journal is currently indexed in Science Citation Index Expanded (SCIE), Scopus, ScienceDirect, PubMed, PubMed Central (PMC), Directory of Open Access Journals (DOAJ), DOI/Crossref, Google Scholar, KoMCI, KoreaMed, ScienceCentral, CAS, Current Content Clinical Medicine and Essential Science Indicators.

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

Open Access

Every peer-reviewed research article in this journal is freely available via our website (https://www.krcp-ksn.org). Articles published in KRCP are distributed under the terms of the Creative Commons Attribution Non-Commercial and No Derivatives License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits unrestricted non-commercial use, distribution of the material without any modifications, and reproduction in any medium, provided the original works properly cited. ANY USE of the open access version of this Journal in whole or in part must include the customary bibliographic citation, including author and publisher attribution, date, article title, *Kidney Research and Clinical Practice* (*Kidney Res Clin Pract*), and the URL https://www.krcp-ksn.org and MUST include a copy of the copyright notice. If an original work is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For any commercial use of material from the open access version of the journal, permission MUST be obtained from KRCP. If necessary, please contact the Editorial Board through our editorial office (registry@ksn.or.kr). Proprietary rights notice for KRCP online were available at: https://www.krcp-ksn.org/authors/permission.php.

Publisher The Korean Society of Nephrology
Editor-in-chief Tae-Hyun Yoo, MD, PhD

Editorial office
The Korean Society of Nephrology
#301, (Miseung Bldg.) 23, Apgujeong-ro 30-gil, Gangnam-gu, Seoul 06022, Korea
Tel: +82-2-3486-8736  Fax: +82-2-3486-8737  E-mail: registry@ksn.or.kr

Publishing office
M2PI
#805, 26 Sangwon 1-gil, Seongdong-gu, Seoul 04779, Korea
Tel: +82-2-6966-4930  Fax: +82-2-6966-4945  E-mail: support@m2-pi.com

Published on September 30, 2023

# Table of Contents

## Editorials

539  **Metabolomics profiling: a potential tool for predicting immunoglobulin A nephropathy progression**  
*Dong Ki Kim; on behalf of KORNERSTONE investigators*

541  **Electronic alerts based on clinical decision support system for post-contrast acute kidney injury**  
*Hojin Jeon, Hye Ryoun Jang*

## Review Articles

546  **Mitochondrial quality control and its emerging role in the pathogenesis of diabetic kidney disease**  
*Jihyun Baek, Yu Ho Lee, Hye Yun Jeong, So-Young Lee*

561  **The impact of hypoxia-inducible factors in the pathogenesis of kidney diseases: a link through cell metabolism**  
*Orestes Foresto-Neto, Ana Ruth Paolinetti Alves da Silva, Marcella Cipelli, Fernanda Paula Roncon Santana-Novelli, Niels Olsen Saraiva Camara*

579  **Overview of aristolochic acid nephropathy: an update**  
*Qingqing Zhou, Lei Jiang, Tao Su, Gang Liu, Li Yang*

## Original Articles

591  **Serum and urine metabolomic biomarkers for predicting prognosis in patients with immunoglobulin A nephropathy**  
*You Hyun Jeon, Sujin Lee, Da Woon Kim, Suhyun Han, Sun Sik Bae, Eun Young Seong, Sang Heon Song*

606  **Electronic alert outpatient protocol improves the quality of care for the risk of postcontrast acute kidney injury following computed tomography**  
*Seokwoo Park, Jinyeong Yi, Yoon Jin Lee, Eun-Jeong Kwon, Giae Yun, Jong Cheol Jeong, Ho Jun Chin, Ki Young Na, Sejoong Kim*

617  **Baseline characteristics of the Korean genetic cohort of inherited cystic kidney disease**  
*Jeong Min Cho, Hayne Cho Park, Jin Woo Lee, Hyunjin Ryu, Yang Chul Kim, Curie Ahn, Kyu-Beck Lee, Yeong Hoon Kim, Seungyeup Han, Yaerim Kim, Eun Hui Bae, Hee Gyung Kang, Eujin Park, Kyungjo Jeong, Seoon Kang, Jungmin Choi, Kook-Hwan Oh, Yun Kyu Oh*

628  **A questionnaire survey on the diagnosis and treatment of Fabry nephropathy in clinical practice**  
*Soo Jeong Choi, Su Hyun Kim, Min Sung Lee, Samel Park, Eunjung Cho, Seung Seok Han, Eun Sil Koh, Byung Ha Chung, Kyung Hwan Jeong, Eun Hui Bae, Eun Young Lee, Young Joo Kwon*
639  Acute kidney injury in hospitalized adults with chronic kidney disease: comparing cROCK, KDIGO, and combined criteria
   Ling Sun, Rui-Xue Hua, Yu Wu, Lu-Xi Zou

649  COVID-19 incidence and outcomes among patients with kidney replacement therapy
   Siribha Changsirikulchai, Pornpen Sangthawan, Jirayut Janma, Songyas Rajborirug, Thammasin Ingviya

Images in Practice
660  Unilateral renal displacement in an autosomal dominant polycystic kidney disease patient
   Jin Kim, Seong Kwon Ma, Soo Wan Kim, Eun Hui Bae

Correspondence
662  Acute pancreatitis after trimethoprim/sulfamethoxazole exposure during desensitization for kidney transplantation
   Hanbi Lee, Hyung Duk Kim, Chul Woo Yang, Sook Young Lee, Hwa Young Lee, Byung Ha Chung

The image on the front cover: Park et al reported the electronic alert for post-contrast acute kidney injury. Schematic illustration for the electronic alert protocol for post-contrast acute kidney injury. Please see the text for more details (pp. 606–16).
Metabolomics profiling: a potential tool for predicting immunoglobulin A nephropathy progression

Dong Ki Kim¹,²; on behalf of KORNERSTONE* investigators

¹Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea
²Kidney Research Institute, Seoul National University, Seoul, Republic of Korea

Immunoglobulin A nephropathy (IgAN) is the most prevalent primary glomerulonephritis and one of the leading causes of end-stage kidney disease globally [1,2]. Identifying reliable biomarkers of disease progression is crucial for timely intervention and improved patient outcomes in IgAN. Despite the variable prognosis, there are currently no definitive biomarkers that accurately predict the outcomes of the disease. In recent years, rapid advancements in high-throughput omics technologies have empowered researchers to extract vast quantities of data from limited samples, such as blood, urine, or kidney tissue. These technologies have emerged as powerful tools for biomarker discovery in glomerular diseases, including IgAN [3].

Metabolomic analysis has shown promise in the field of nephrology, as it allows for a comprehensive assessment of various factors influencing complicated pathophysiology of kidney diseases. Metabolites can provide a real-time snapshot of systemic and local kidney metabolic status, thereby offering potential insights into disease progression and therapeutic responses. The human metabolome is the end product of numerous influences, including genetic variability, environmental factors, internal biochemical processes, and their complex interactions [4]. Consequently, the multifaceted pathophysiological mechanisms of IgAN, characterized by multiple pathologic hits, are likely to result in related shifts in the concentrations of specific metabolites. Thus, metabolites could serve as pathognomonic biomarkers, capable of linking the dots between the sequential pathological hits of IgAN [5].

¹H-NMR spectroscopy offers a unique advantage in metabolomics analysis. It provides quantitative results, offering absolute concentration values for metabolites. This technique detects a broad range of metabolites, and its excellent reproducibility makes it suitable for high-throughput analysis. It can detect a wide spectrum of metabolites, including small organic molecules and metabolites of lipids, amino acids, and carbohydrates, offering a comprehensive view of the metabolic shifts in disease. Also, the non-destructive nature of ¹H-NMR allows potential re-analysis of samples, making it particularly useful for lon-
gitudinal studies to monitor disease progression and treatment response. Furthermore, the presence of high-abundance metabolites might overshadow the detection of less abundant, yet potentially significant ones [6]. In terms of 1H-NMR spectroscopy, although it provides broad metabolite coverage, its sensitivity is lower compared to other techniques like mass spectrometry, requiring high-quality samples and careful data interpretation. Moreover, variability in urine metabolite concentrations due to diet, lifestyle, volume status, medications, and other factors necessitates rigorous study design and data normalization strategies.

Jeon et al. [7] investigated the metabolomic profiles in the serum and urine of patients with IgAN to identify potential biomarkers for disease progression. The study included 20 IgAN patients, of whom a proportion exhibited disease progression, with higher urine protein/creatinine ratios than the non-progressor group. Through 1H-NMR spectroscopy, distinct clusters were observed between the control and IgAN groups, as well as between progressors and non-progressors. Pathway enrichment analysis identified several altered metabolic pathways associated with IgAN progression, including glycerolipid metabolism, aminoacyl-tRNA biosynthesis, valine, leucine, isoleucine biosynthesis, and glycine, serine, and threonine metabolism. Predictive models incorporating these identified metabolites and proteinuria showed improved prognostic power for IgAN progression. In the serum, the combination of glycerol, threonine, and proteinuria had an area under the curve (AUC) of 0.923 for disease progression, while in urine, the combination of leucine, valine, and proteinuria had an AUC of 0.912. These results highlight the potential of metabolomic profiling in predicting IgAN progression and in revealing altered metabolic pathways in IgAN patients. Further studies involving a larger patient cohort are warranted to validate these findings and explore the mechanistic links between the altered metabolic pathways and IgAN progression.

Conflicts of interest
The author has no conflicts of interest to declare.

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

ORCID
Dong Ki Kim, https://orcid.org/0000-0002-5195-7852

References
Clinical decision support systems (CDSS) are computer-based programs designed to improve patient care by providing patient-specific information and clinical knowledge to healthcare professionals [1]. These systems were developed to improve clinical decision-making by integrating relevant data and offering tailored recommendations based on individual patient characteristics. CDSS are already widely used in hospital settings in forms of alerting physicians and other healthcare professionals regarding medication interactions, potential diagnoses, and treatment alternatives. These systems can aid healthcare professionals in enhancing patient safety and improving clinical outcomes by offering timely and relevant data to minimize medical errors. A randomized trial demonstrated the effectiveness of electronic alert systems in reducing drug interactions [2]. CDSS can also assist with disease management, clinical protocols, and guideline adherence. For example, the system for an asthma attack, entitled ‘ACAFE (Asthma Clinical Assessment Form and Electronic) decision support’, improved asthma plan provision, documentation of asthma severity, and other important clinical parameters [3].

Park et al. [4] performed a retrospective cohort study to demonstrate the impact of CDSS in patients at risk of acute kidney injury (AKI) after contrast-enhanced computed tomography (CECT). They compared two time periods (before and after) according to the initiation of a new electronic alert system as CDSS. The alert system was activated when CECT was prescribed for patients undergoing kidney replacement therapy or with a baseline estimated glomerular filtration rate (eGFR) of < 45 mL/min/1.73m². Although the incidence and major outcomes of AKI were similar between the two groups, the frequency of kidney function monitoring by nephrologists significantly increased from 29.4% to 66.7% and the volume of prophylactic fluid was smaller in the alert group compared to the historical control group.

Extensive use of intravenous contrast media for CECT or radiologic interventions has been associated with an increased risk of AKI. The Contrast Media Safety Committee (CMSC) of the European Society of Urogenital Radiology (ESUR) defined ‘post-contrast acute kidney injury (PCA-KI)’ as a sudden deterioration in kidney function within 48 hours after intravascular administration of iodine-based contrast media [5]. The pathogenesis of PCAKI involves two distinct pathways: direct nephrotoxic effects of con-
Contrast media and indirect disturbances in kidney blood flow (Fig. 1) [6]. Direct effects are caused by contrast media-induced adverse changes in tubular flow and physiology, including increased viscosity, loss of polarity in tubular cells, and the subsequent apoptosis and necrosis in tubular cells, leading to tubular obstruction and injury. Indirect effects arise from elevated blood viscosity, which disrupts normal blood flow patterns and causes endothelial dysfunction [7,8]. These effects are accompanied by increased endothelin levels, activation of the renin-angiotensin system, and reduced levels of nitric oxide and prostaglandin I2, resulting in vasoconstriction of renal arterioles. These indirect effects can further lead to microvascular thrombosis and prolonged ischemia in the renal medulla. Ultimately, the overall harmful effects caused by contrast media deteriorate kidney function. In a multicenter cohort study including 288 hospitalized patients diagnosed with PCAKI, the incidence of persistent kidney dysfunction was 46.9% and the all-cause mortality rate for 1 year was as high as 13.5% [9]. The risk factors of PCAKI included age, preexisting kidney dysfunction, proteinuria, hypertension, and diabetes mellitus [10,11]. CMSC recommended eGFR measurements within 7 days before contrast media exposure for patients with acute illness or acute deterioration of preexisting chronic disease and for inpatients [11]. PCAKI following CECT is associated with all-cause mortality and subsequent chronic kidney disease (CKD) after severe or persistent PCAKI may impede the optimal evaluation and treatment of primary illnesses [12]. Therefore, systemized strategies for early detection and prevention of PCAKI are critical for avoiding unfavorable clinical outcomes and progression of CKD especially in patients receiving repeated CECT due to underlying diseases such as cancer. Since intravenous fluid therapy, specifically intravascular volume expansion, has been reported as a preventive measure for PCAKI, the ESUR recommended preventive fluid therapy to reduce the risk of PCAKI in all patients with risk factors [11,13,14]. The recommended intravenous fluid regimens for preventing PCAKI include the administration of either 3 mL/kg/hr of bicarbonate 1.4% (or 154 mmoL/L solution)

---

**Figure 1.** Pathophysiology of post-contrast acute kidney injury.
for 1 hour before contrast media (in the case of intra-arterial administration, followed by 1mL/kg/hr of bicarbonate 1.4% for 4 to 6 hours after contrast media) or 1mL/kg/hr of 0.9% saline for 3 to 4 hours before and 4 to 6 hours after contrast media infusion [11].

It is noteworthy that Park et al’s study [4] did not find a significant difference in the incidence of PCAKI despite of fluid volume difference between the groups. The volume of administered fluid in the alert group (750 mL of isotonic fluid) was lower than that in the control group (1,000 mL of isotonic fluid). Administering large volume of fluids can cause intrarenal venous congestion and compartment syndrome because kidneys are encapsulated organs [15]. Thus, careful monitoring of volume status and administration of the optimal volume of prophylactic fluid are imperative to prevent PCAKI. In an outpatient clinic-based management for PCAKI, the shortening of fluid infusion time is preferred. Reduction of fluid volume by the alert system in Park’s study [4] may contribute to the establishment of an optimized fluid protocol for patients undergoing CECT in outpatient clinics.

Although there were no statistically significant differences in the incidence of PCAKI, the risk of hospitalization, and kidney replacement therapy following the implementation of an electronic alerts system, the frequency of consultation and follow-ups with nephrologists were increased by the alert system in Park’s study [4]. There were several studies to determine the efficacy of CDSS for AKI. In a randomized study with 1,201 patients assigned to the AKI alert group and 1,192 patients assigned to the control group, the primary outcomes including relative maximum changes in serum creatinine, dialysis, and mortality within 7 days were comparable between the groups [16]. Despite insufficient data demonstrating the effectiveness of an AKI alert system on overall kidney outcome and mortality, early detection of AKI and timely consultation with nephrologists by an alert system are anticipated to contribute to the earlier management of AKI and the improvement of patient overall outcomes including mortality [17]. The effectiveness of CDSS in reducing the occurrence of overlooked AKI cases

Figure 2. A schematic representation of hospital-based interactions within a knowledge-based clinical decision support system.
was reported by showing that early nephrology consultation was linked to improved clinical outcomes in hospitalized patients [18]. Consequently, the implementation of an electronic AKI alert system holds promising potential to improve patient outcomes.

In a previous study with AKI patients, alerts without CDSS education were associated with a significantly increased risk of mortality [19]. In a randomized controlled study [20], the efficacy of an intervention comprising of education on AKI, PCAKI prevention approaches, safe target volumes of contrast, and feedback was investigated for the prevention of AKI following coronary angiography or percutaneous coronary intervention. In this study, the intervention groups showed a lower likelihood of developing PCAKI compared to the control group [20], supporting the importance of comprehensive CDSS education to physicians and implementation of adequate management strategies for AKI. The presence of well-educated physicians who are proficient in CDSS utilization holds the potential to improve the prognosis of PCAKI and overall patient outcomes.

In Park et al.’s study [4], the electronic alert system for outpatient protocol based on CDSS effectively reduced the volume of preventive fluid therapy without an increase in the incidence of PCAKI and facilitated active consultations with nephrologists after CECT. Therefore, CDSS-based electronic alert system seem to have the potential to prevent PCAKI or mitigate the severity of AKI in patients with risk factors. To advance the effectiveness of CDSS for PCAKI prevention, education on PCAKI and the implementation of an electronic alert system are critically required (Fig. 2). Although there is some evidence supporting the efficacy of CDSS in improving patient care, further prospective studies are required to refine the design of these systems and enhance the optimal utilization of a CDSS-based electronic alert system in clinical practice.

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.

References

10. Davenport MS, Khalatbari S, Dillman JR, Cohan RH, Caolli EM, Ellis JH. Contrast material-induced nephrotoxicity and intravenous low-osmolality iodinated contrast material. Radiology


Gottlieb ER, Mendu M. Clinical decision support to prevent acute kidney injury after cardiac catheterization: moving beyond process to improving clinical outcomes. JAMA 2022;328:831–832.
**Mitochondrial quality control and its emerging role in the pathogenesis of diabetic kidney disease**

Jihyun Baek¹²*, Yu Ho Lee¹*, Hye Yun Jeong¹†, So-Young Lee¹†

¹ Division of Nephrology, Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Republic of Korea
² Department of Biomedical Science, College of Life Science, CHA University, Pocheon, Republic of Korea

Most eukaryotic cells have mitochondrial networks that can change in shape, distribution, and size depending on cellular metabolic demands and environments. Mitochondrial quality control is critical for various mitochondrial functions including energy production, redox homeostasis, intracellular calcium handling, cell differentiation, proliferation, and cell death. Quality control mechanisms within mitochondria consist of antioxidant defenses, protein quality control, DNA damage repair systems, mitochondrial fusion and fission, mitophagy, and mitochondrial biogenesis. Defects in mitochondrial quality control and disruption of mitochondrial homeostasis are common characteristics of various kidney cell types under hyperglycemic conditions. Such defects contribute to diabetes-induced pathologies in renal tubular cells, podocytes, endothelial cells, and immune cells. In this review, we focus on the roles of mitochondrial quality control in diabetic kidney disease pathogenesis and discuss current research evidence and future directions.

**Keywords:** Diabetic nephropathies, Endothelial cells, Mitochondria, Quality control, Podocytes

**Introduction**

Mitochondria are double membrane-bound intracellular organelles essential for energy production in most eukaryotic cells [1]. Besides adenosine triphosphate (ATP) production, mitochondria also participate in various cellular processes such as redox homeostasis, intracellular calcium handling, cell differentiation, proliferation, and death [2]. A healthy and well-functioning mitochondrial population, achieved by so-called mitochondrial quality control, is therefore vital for cellular health [3]. Biogenesis, proteostasis, and mitophagy are critical components of mitochondrial quality control, and failure of mitochondrial quality control failure has been implicated in several human diseases [2,4].

Diabetes mellitus alters available energy substrates and results in excessive oxidative stress, followed by increased levels of inflammatory and profibrotic cytokines and cell...
Mitochondrial quality control mechanisms comprise molecular and organelle quality control mechanisms that interact to maintain a healthy mitochondrial population [2,4]. Molecular-level mechanisms are composed of antioxidant defenses, protein quality control, and a DNA damage repair system (Fig. 1). Organelle-level mechanisms consist of mitochondrial fusion, fission, mitophagy, and mitochondrial biogenesis (Fig. 2).

Mitochondria are major sources of reactive oxygen species (ROS) due to oxidative phosphorylation, which is the metabolic process that results in ATP synthesis. In this context, mitochondria contain are enriched in antioxidants for redox homeostasis. When the balance between mitochondrial ROS production and the antioxidant system is disrupted, an oxidative stress situation develops with deleterious effects on organisms [10]. Mitochondria function as ROS amplifiers due to mitochondria-to-mitochondria and mitochondria-to-different ROS sources crosstalk with positive feedback [11]. Excessive ROS can lead to impairment of the electron transport chain (ETC) and low ATP production as a result of reduced cytochrome c oxidase activity [10]. In addition, defects in the ETC across the mitochondrial inner membrane can induce the loss of mitochondrial membrane potential and leakage of proapoptotic proteins into the cytosol [12]. Accumulation and decompartmentalization of mitochondrial ROS can result in oxidation of proteins, lipids, and DNA, leading to cellular dysfunction and disruption of crucial cellular signaling pathways [2,10].

The mitochondrial protein quality control system consists of two parts: the ubiquitin-proteasome system and chaperones [2,13]. Phosphatase and tensin homolog-induced putative kinase 1 (PINK1)-parkin signaling also contributes to mitochondrial protein quality control by inducing the formation of mitochondrial-derived vesicles or mitophagy [14]. Mitochondrial unfolded protein response (mtUPR) refers to the upregulation of mitochondrial proteases and chaperone gene transcription. This response is activated when the amount of unfolded or misfolded mitochondrial proteins overwhelms the capacity of the mitochondrial protein quality control system. Therefore, any defects in the mitochondrial protein quality control system are associated with failure of the mtUPR, and result in mitochondrial dysfunction and cell death [15].

Mitochondrial genome encodes 13 polypeptides essential for oxidative phosphorylation and ATP synthesis; failure to repair damaged mitochondrial DNA can therefore result in insufficient ATP production and jeopardize cell survival [16]. Mitochondrial DNA has a 10- to 20-fold higher rate of mutagenesis than nuclear DNA because of its susceptibility to oxidative stress [17]. Absence of histone proteins, proximity to ROS production sites, and specific DNA replication through asymmetric patterns are possible explanations for the vulnerability of mitochondrial DNA to mutagenesis [2]. Mitochondrial DNA is repaired in the same manner as nuclear DNA [18].

Mitochondria maintain their abundance and functions through constant fusion and fission of the mitochondrial network [2]. Mitochondrial fusion allows the exchange of metabolites and DNA between mitochondria for their health, especially under metabolic and environmental stress [2,19]. In contrast, mitochondrial fission separates damaged and dysfunctional mitochondria from the mitochondrial network as a defense mechanism essential for mitochondrial regeneration, redistribution, and proliferation [2,20]. These dynamic mitochondrial morphological changes are controlled by expression of associated modulators and their posttranslational modification [2,3,19,20]. Mitochondrial fusion results in elongation of the mitochondrial network, which is associated with higher ATP production and maintenance of cell viability, whereas mitochondrial fission leads to short mitochondria, mitochondrial depolarization, and ROS overproduction with low ATP synthesis [3].

Mitophagy can selectively remove defective or surplus mitochondria from the mitochondrial network [21]. Several pathways are involved in labeling mitochondria and trans-
Figure 1. Molecular-level mechanisms of mitochondrial quality control. (A) Under normal physiological conditions, superoxide (O2-) is produced by mitochondrial ETC complexes, particularly complexes I and III, during ATP production. Subsequently, superoxide is transformed by antioxidant enzymes into less-damaging reactive oxygen species such as hydrogen peroxide (H2O2). Superoxide dismutase 2 (SOD2) catalyzes the conversion of superoxide into oxygen and hydrogen peroxide. Subsequently, GPx and Prx further reduce hydrogen peroxide to water (H2O). (B) The mitochondrial protein quality control system consists of two parts: the ubiquitin-proteosome system and chaperones. Heat-shock protein (HSP) 70 and HSP60 chaperone systems are localized to the matrix. They can aid in mitochondrial protein transport, folding, and clearance. Damaged or mistargeted mitochondrial proteins are eliminated by several proteases, including Lon protease in the matrix, ATP-dependent proteases in the inner mitochondrial membrane, and high-temperature requirement protein A2 in the intermembrane spaces. (C) Mitochondrial DNA integrity is maintained in the same manner as that of nuclear DNA, namely base excision repair, mismatch repair, homologous recombination, and non-homologous end-joining.

Mitochondrial biogenesis is a complex process in which mitochondria increase in size and number to meet cellular energy demands [2]. Both mitochondrial DNA and nuclear DNA encode mitochondrial proteins. Therefore, elaborate coordination of cytoplasmic and mitochondrial protein synthesis is necessary [23]. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) plays an important role in mitochondrial biogenesis by regulating the expression of genes involved in mitochondrial biogenesis and function.
in directing this process [2,23]. PGC1α activity is controlled at both transcriptional and posttranslational levels [24].

**Roles of mitochondrial quality control in diabetic kidney disease**

After the heart, the kidneys, which function to remove waste products from blood, regulate fluid and electrolyte balance, and maintain blood pressure, contain the second largest number of mitochondria [3,23]. More than 20 types of specialized cells in mammalian kidney fulfill these important roles in the human body [25]. The nephron is the microscopic, functional unit of the kidney. Normally about 1 to 1.5 million nephrons are found in one kidney [25]. A
nephron comprises a renal corpuscle (where urine formation begins by ultrafiltration) and a renal tubule responsible for absorption and excretion of various substances. In the renal corpuscle, fenestrated endothelium, glomerular basement membrane, and the foot process of podocytes comprise the filtration barrier and confer the nephron with charge and size-permselectivity. Renal tubules constitute approximately 90% of renal cortical cells and link the glomerulus to a collecting system. Defects in the filtration barrier and loss of nephrons result in proteinuria and a decreased glomerular filtration rate [26]. DKD is associated with a spectrum of characteristic morphologic changes of the glomeruli, tubules, interstitium, and vasculature [1].

Renal tubular cells

Renal tubular cells are rich in mitochondria, which are required to generate ATP and power tasks such as active reabsorption of sodium, glucose, and other metabolites from urine [5]. Diabetic milieu can promote renal tubular cells to utilize more ATP than usual to increase active reuptake of urinary metabolites, particularly glucose reabsorption via the sodium-glucose cotransporter 2 (SGLT2). Due to the increased intracellular energy requirement, transport of fuel substrates such as free fatty acids to renal tubular cells is significantly increased in patients with diabetes [27]. Thus, any cellular event that decreases the fuel supply or increases oxygen demand (i.e., supply-demand imbalance) is an important pathophysiological contributor to diabetic tubulopathy in DKD [28]. Interestingly, the properties of mitochondria in proximal and distal tubules differ [29]. Studies using multiphoton live imaging of rat kidneys have shown that mitochondria in proximal tubules are more numerous but have lower membrane potentials and greater alterations in ROS generation upon ETC inhibition than those in distal tubules [29]. These properties may account for the clinical fragility of proximal tubules when mitochondrial homeostasis is disrupted. Fig. 3 shows how mitochondrial quality control in renal tubule cells is altered under high glucose conditions.

Excessive mitochondrial ROS can be induced by hyperglycemic stimuli, and these can contribute to diabetic tubulopathy [30]. An experimental study using rat renal proximal tubular cells demonstrated that high glucose media can induce the overproduction of mitochondrial superoxide, change the mitochondrial membrane potential, and decrease ATP generation with complex III dysfunction, culminating in tubular cell death [30]. It has been suggested that renal mitochondrial oxidative stress is exacerbated by reduced sirtuin 3 activity in Zucker diabetic fat rats (ZDFRs) [31]. Conversely, a CD38 (cluster of differentiation 38) inhibitor was shown to restore the intracellular NAD+/NADH ratio and sirtuin 3-mediated mitochondrial antioxidative enzyme activity in the kidneys of ZDFRs [32]. Sirtuin 3 functions as an antioxidant in mitochondria by activating isocitrate dehydrogenase 2 and superoxide dismutase 2 [32]. A recent study reported that hypoxia-inducible factor 1 (HIF1) is involved in mitochondrial redox homeostasis in diabetes [33]. Zheng et al. [33] showed that HIF1 activity was suppressed and that blood ROS levels increased in response to hypoxic exposure in patients with type 1 diabetes. They also demonstrated that hyperglycemia could diminish the HIF1 response in renal tubular cells under hypoxia and in kidneys from diabetic animals. Low HIF1 levels are associated with mitochondrial oxidative stress through increased mitochondrial respiration. In addition, it has been suggested that HIF1 can improve mitochondrial homeostasis via heme oxygenase 1 in renal tubules in diabetic environments [34].

Mitochondrial dynamics are profoundly altered in DKD. An increase in mitochondrial fragmentation has been shown to be an early phenomenon in renal tubules of experimental diabetes models [35]. Mitofusin (MFN) 1, a mitochondrial fusion-related protein, has been shown to be downregulated whereas dynamin-related protein 1 (Drp1), a pro-fission protein, has been shown to be upregulated in human renal proximal cells (HKC8) under high glucose conditions as well as in the kidneys of diabetic mice [36]. Several mechanisms have been suggested to mediate mitochondrial fragmentation in diabetic tubular injury [37–39]. Liu et al. [37] demonstrated that hyperglycemia can inhibit AMP-activated protein kinase (AMPK) phosphorylation and induce an increase in SP1 (specificity protein 1) followed by PGAM5 (phosphoglycerate mutase family member 5) upregulation, resulting in Drp1-dependent mitochondrial fission in diabetic renal tubular injury. Zhang et al. [38] demonstrated that stromal cell-derived factor-1a (SDF-1α)/CXCR chemokine receptor 4 (CXCR4)/signal transducer and activator of transcription 3 (STAT3) signaling is associated with mitochondrial dysfunction in
Under high glucose conditions, the activity of AMPK, the major energy-sensing enzyme, is reduced and phosphorylation of PGC1α, the master regulator of mitochondrial gene expression, is suppressed, resulting in impaired mitochondrial biogenesis and low mitochondrial mass. A decrease in SIRT3 activity after CD38 upregulation is associated with impaired antioxidant capacity and excessive ROS production in mitochondria. Reduced HIF1 expression is also associated with mitochondrial ROS overproduction under high glucose conditions. Overexpression of MIOX can enhance the effects of high glucose by inhibiting the PINK1/parkin pathway. TXNIP can inhibit autophagy/mitophagy flux via mTOR signaling in renal tubular cells under high glucose conditions. The IncRNA NEAT1 inhibits mitophagy via the miR150-5p-Drp1 axis. Upregulation of TIPE1 in tubular epithelial cells disrupts PHB2-mediated mitophagy. PACS2 downregulation interferes with MAM integrity and suppresses mitophagy progression. Upregulation of PGAM5 can promote Drp1-dependent mitochondrial fission. Activation of SDF-1α/CXCR4/STAT3 signaling by hyperglycemic conditions increases mitochondrial fission through OPA1 inhibition, p66Shc mediates hyperglycemia-induced mitochondrial fragmentation and apoptosis signaling.

**AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; CD38, cluster of differentiation 38; CXCR4, CXC chemokine receptor 4; Drp1, dynamin-related protein 1; ETC, electron transport chain; ERR, estrogen-related receptor; Glu, glucose; HIF1, hypoxia-inducible factor 1; IncRNA NEAT1, long noncoding RNA nuclear paraspeckle assembly transcript 1; MAM, mitochondrial-associated endoplasmic reticulum membrane; MFN1, mitofusin protein 1; MIOX, myo-inositol oxygenase; miR150, microRNA 150; mTOR, mammalian target of rapamycin; NRF1, nuclear respiratory factor 1; NRF2, nuclear factor erythroid 2-related factor 2; OPA1, optic atrophy 1; P, phosphorylation; PACS2, phosphofurin acidic cluster sorting protein 2; Parkin, E3 ubiquitin-protein ligase parkin; PGAM5, phosphoglycerate mutase family member 5; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; PHB2, prohibitin 2; PINK1, phosphatase and tensin homolog-induced putative kinase 1; ROS, reactive oxygen species; SDF-1α, stromal cell-derived factor-1α; SGLT2, sodium glucose cotransporter 2; SIRT3, sirtuin 3; STAT3, signal transducer and activator of transcription 3; TFAM, mitochondrial transcription factor A; TIPE, tumor necrosis factor alpha-induced protein 8-like 1; TXNIP, thioredoxin interacting protein.**
Mitophagy plays a protective role against cell damage. Inappropriate mitophagy is associated with hyperglycemia-induced cytotoxicity [1,21] while reduction of mitophagy is associated with accumulation of intracellular mitochondrial ROS. Overexpression of myo-inositol oxygenase (MIOX), an enzyme that inhibits PINK1-parkin-mediated mitophagy, increased mitochondrial ROS production in high glucose-treated renal tubular cells [7]. In contrast, mitochondrial-targeting antioxidant mitoquinone mesylate was shown to restore mitophagy activity and reduce tubular cell death through restoration of nuclear factor erythroid 2-related factor 2 (NRF2) and PINK1 activity in an experimental DKD model [40]. Studies on the mechanism of mitophagy regulation in diabetic tubulopathy are ongoing. Thioredoxin interacting protein has been reported to inhibit autophagy/mitophagy flux via mammalian target of rapamycin signaling in renal tubular cells under high glucose conditions [41]. Overexpression of optineurin, a coordinator protein linking damaged mitochondria and autophagy, was shown to promote mitophagy and relieve cellular senescence in renal tubular cells of diabetic mice [42]. It has been suggested that an increase in the long non-coding RNA (lncRNA) NEAT1 (nuclear paraspeckle assembly transcript 1) can inhibit mitophagy via the miR150-5p-Drp1 axis in high glucose-exposed HK2 human proximal tubular cells [43]. Recent studies have shown that upregulation of TIPE1 (tumor necrosis factor alpha-induced protein 8-like 1) in tubular epithelial cells can interfere with PHB2 (prohibitin 2)-mediated mitophagy and exacerbate diabetic tubulopathy [44]. Melatonin, a pineal hormone involved in regulation of circadian rhythms, can ameliorate diabetic tubulopathy via the AMPK-PINK1-mitophagy pathway in HK2 cells and streptozotocin (STZ)-induced diabetic mice [45].

Mitochondria-associated endoplasmic reticulum (ER) membranes (MAMs) play a critical role in mitochondrial quality control during ER stress [46]. MAMs are a region of interaction between the ER and mitochondria. They have diverse functions such as nutrient and hormone signaling, regulation of calcium homeostasis, autophagy, and apoptosis [46]. Li et al. [46] demonstrated that phosphofurin acidic cluster sorting protein 2 (PACS-2) expression is decreased in renal tubules of patients with DKD and that PACS-2 overexpression in HK2 cells can restore MAM integrity and promote the formation of mitophagosomes.

Analytical studies of human and animal samples have demonstrated inefficient mitochondrial bioenergetics and reduced functional mitochondrial mass in DKD [6,47,48]. PGC1α, a key regulator of mitochondrial biogenesis, is highly expressed in renal proximal tubules where mitochondria are abundant. Notably, failure of PGC1α to be upregulated under hyperglycemic circumstances can aggravate diabetic tubulopathy [49]. Therefore, enhancing PGC1α activity could be a potential therapeutic strategy for treating DKD. Reduction of PGC1α expression is accompanied by aberrant mitochondrial dynamics, excessive ROS production, and tubular cell death, whereas administration of the pharmacological PGC1α activators 5-aminomidazole-4-carboxamide-1-ß-D-ribofuranoside (AICAR) or metformin can restore mitochondrial homeostasis and reduce apoptosis. Beneficial effects of AICAR and metformin have been confirmed in STZ-induced diabetic mice; AICAR and metformin increase renal PGC1α expression, improve mitochondrial fragmentation and ROS production, and improve hyperglycemia-related tubular damage and renal fibrosis [47]. In addition, chloroquine and amodiaquine (traditionally used to treat malaria) can effectively abrogate downregulation of AMPK and PGC1α phosphorylation due to hyperglycemia, restore mitochondrial homeostasis, and alleviate albuminuria and renal histopathological changes in the kidneys of diabetic mice [48,50].

Interestingly, the PGC1α-induced increase in mitochondrial biogenesis affects mitochondrial ROS generation, dynamics, and autophagic elimination of mitochondria, suggesting complex, synergistic interactions among various mechanisms for adequate quality control of mitochondria in diabetic tubulopathy.
When the mitochondrial fission regulator Drp1 is deleted present before the clinical manifestations of DKD pathognomonic feature of diabetic podocytopathy and is podocytopathy plays an important role in the pathophysiology of diabetic formation induced by the NLRP3 inflammasome pathway containing 3 (NLRP3) inflammasome \[\ldots\]. Overproduction of ROS from mitochondrial and non-mitochondrial sources can oxidize cardiolipin, a mitochondrial membrane-specific phospholipid, which is an initiator for the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Activation of the NLRP3 inflammasome can lead to caspase 1-dependent release of proinflammatory cytokines. Persistent inflammation induced by the NLRP3 inflammasome pathway plays an important role in the pathophysiology of diabetic podocytopathy.

Unopposed mitochondrial fission in podocytes is a pathognomonic feature of diabetic podocytopathy and is present before the clinical manifestations of DKD. When the mitochondrial fission regulator Drp1 is deleted in podocytes, albuminuria is decreased and major pathologic features of DKD are improved in mice. Wang et al. found that Rho-associated coiled-coil containing protein kinase 1 (ROCK1) contributes to Drp1 recruitment to mitochondrial membrane by phosphorylation of Drp1 and aggravates mitochondrial fission and ROS production in podocytes under high glucose conditions. Podocyte-specific deletion of ROCK1 can inhibit apoptosis and mitochondrial ROS production. MFN2, a fusion-related protein, is located at ER membranes and regulates the dynamics of MAMs. Cao et al. demonstrated that MFN2 overexpression attenuated the high glucose effect in an animal model. They also confirmed that MFN2-protein kinase RNA-like ER kinase (PERK)-regulated MAMs and had antiapoptotic effects in podocytes under hyperglycemic conditions.

A few studies have investigated the role of mitophagy in diabetic podocytopathy. Mitophagy is an important defense mechanism during cellular stress, and inappropriate mitophagy can exacerbate podocyte injury. Forkhead transcription factor O1 (FoxO1) is inactivated by the PI3K/Akt (phosphatidylinositol-3-kinase/Akt) pathway under high glucose conditions. FoxO1 upregulation can increase PINK1/parkin-dependent mitophagy to restore podocyte damage in STZ-induced diabetic type 1 diabetic mice. Progranulin is an autocrine growth hormone involved in development, inflammation, cell proliferation, and protein homeostasis. Recombinant human progranulin treatment was shown to facilitate mitophagy and mitochondrial biogenesis in the podocytes of diabetic mice through progranulin-sirtuin1-PGC1a/FoxO1 signaling.

Mitochondrial biogenesis is suppressed in podocytes under high glucose conditions. Reduced expression of PGC1α and mitochondrial dysfunction have been observed in the podocytes of DKD animal models. Analysis of kidney biopsies from patients with DKD revealed a reduction in PGC1α expression in micro-dissected glomeruli. As a positive control for PGC1α, podocyte-specific sirtuin1 knockdown of sirtuin1 can increase susceptibility to diabetic nephropathy in a murine model. In contrast, podocyte-specific sirtuin1 overexpression can ameliorate podocyte loss and DKD progression. IncRNA taurine upregulated gene 1 (TUG1) is a recently discovered PGC1α regulator. Podocyte-specific overexpression of TUG1 in podocytes.
Figure 4. Altered mitochondrial quality control in podocytes under high glucose conditions. In a diabetic state, the expression of PGC1α is usually suppressed and its downstream signaling networks are also altered. Downregulations of SIRT1 and TUG1 are associated with reduced expression of PGC1α. Reduced TGR5 mRNA transcription inhibits SIRT1, SIRT3, and NRF1, leading to reduced mitochondrial biosynthesis and overproduction of mitochondrial ROS. Upregulation of A3AR is associated with suppression of PGC1α. Nuclear translocation of TFEB can increase the expression of PGC1α and antioxidant enzymes. Hyperglycemia-induced mGPDH downregulation can inhibit PGC1α activity by modulating receptors for RAGE signaling. ROCK1 phosphorylates Drp1 and aggravates mitochondrial fragmentation. MFN2 is decreased in podocytes of diabetic kidney disease patients. MFN2 is localized in the endoplasmic reticulum membrane and can regulate MAM dynamics. As FoxO1 mediates PINK1 transcription, phosphorylated and inactivated FoxO1 under high glucose conditions can contribute to mitophagy reduction. The PPARγ-Klotho axis can help attenuate diabetic podocytopathies by inhibiting mitochondrial ROS overproduction and activation of NRF2 signaling. NOX4 can produce excess ROS under high glucose conditions and is associated with HIF1α signaling. Excessive ROS can activate the NLRP3 inflammasome to induce caspase 1-dependent release of proinflammatory and profibrotic cytokines.

A3AR, A3 adenosine receptor; Drp1, dynamin-related protein 1; ETC, electron transport chain; FoxO1, forkhead box protein 01; Glu, glucose; GLUT, glucose transporter; HIF1α, hypoxia-inducible factor 1α; Inc, long noncoding; MAM, mitochondrial-associated endoplasmic reticulum membrane; MFN2, mitofusin protein 2; mGPDH, mitochondrial glycerol-3-phosphate dehydrogenase; mRNA, messenger RNA; NLRP3, NLR family pyrin domain containing 3; NOX4, NADPH oxidase 4; NRF1, nuclear respiratory factor 1; NRF2, nuclear factor erythroid 2-related factor 2; P, phosphorylation; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; PINK1, phosphatase and tensin homolog-induced putative kinase protein 1; PPARγ, peroxisome proliferator-activated receptor γ; RAGE, receptor for advanced glycation end products; ROCK1, Rho-associated coiled-coil-containing protein kinase 1; ROS, reactive oxygen systems; SIRT, sirtuin; TFEB, transcription factor EB; TGR5, G protein-coupled receptor 5; TUG1, taurine upregulated 1.
diabetic mice restored PGC1α expression, leading to improved mitochondrial ATP production along with amelioration of diabetic glomerulopathy [9]. Proteomic analysis using glomeruli from individuals with or without DKD has revealed that levels of pyruvate kinase M2 (PKM2), a rate-limiting glycolytic enzyme, are elevated in diabetes patients without DKD [70]. Podocyte-specific PKM2 knockout mice with diabetes showed aggravated albuminuria with more severe pathologic changes in the glomeruli than wild-type diabetic mice. Conversely, treatment with a PKM2 activator was shown to induce mitochondrial biogenesis and reverse glomerular pathology in part by increasing glycolytic flux and PGC1α transcription [70]. Factors that affect mitochondrial biogenesis in diabetic podocytopathy by acting directly or indirectly on PGC1α such as TGR5 (G protein-coupled bile acid receptor), A3 adenosine receptor, transcription factor EB, and mitochondrial glyceral 3-phosphate dehydrogenase are continuously being discovered [71–74]. NRF2 is a downstream transcription factor of PGC1α. It is responsible for gene expression in the mitochondrial redox system and the induction of TFAM (mitochondrial transcription factor A) [2]. Wang et al. [75] showed that NRF2 activation attenuated high glucose-induced injury in mouse podocytes and that downregulation of NRF2 promoted more severe injury and increased ROS production in podocytes exposed to high glucose levels.

Evidence published to date indicates that mitochondrial quality control plays an important role in diabetic podocytopathy. There are several unanswered questions in this area. For example, the precise roles and interactions of each process involved in mitochondrial quality control and the detailed role of MAMs during the development and progression of diabetic podocytopathy require further exploration.

**Endothelial cells**

In DKD, glomerular endothelial cell injury is an early process that occurs before the onset of albuminuria. This event contributes to DKD by paracrine mediator release, leading to subsequent kidney injury [76]. Although the mechanism of glomerular endothelial cell-podocyte cross-talk regulation in DKD is obscure, a previous study showed that podocyte detachment and a decrease in endothelial cell fenestration play an important role in kidney injury in type 2 diabetes [77]. In addition, conditioned medium derived from endothelial nitric oxide synthase-deficient glomerular endothelial cells induced podocyte injury under high glucose conditions, suggesting the importance of communication between endothelial cells and podocytes in diabetes [78]. Qi et al. [79] investigated the role of glomerular endothelial mitochondrial dysfunction in DKD. They found that mitochondrial dysfunction and oxidized mitochondrial DNA in glomerular endothelial cells were related to high glucose-induced podocytopathy after comparing transcriptome profiles between DKD-resistant and susceptible mice strains [79]. They demonstrated that diabetes-induced endothelin-1 (Edn1)/Edn1 receptor type A (Ednra) signaling facilitated mitochondrial stress and injury in endothelial cells [79]. They also observed that mitochondrial DNA damage resulting from mitochondrial ROS was associated with glomerular endothelial Ednra expression and rapid DKD progression. Notably, an Ednra antagonist inhibited endothelial mitochondrial oxidative damage and reduced albuminuria and podocyte depletion in DKD-susceptible mice [79].

Hyperglycemia can lead to mitochondrial fission in glomerular endothelial cells coupled with mitochondrial ROS production and cell death [61]. ROCK1 contributes to Drp1 activation by phosphorylating Drp1 at serine 600 and triggers mitochondrial fragmentation, as it does in podocytes under high glucose conditions [61].

Although the content of mitochondria in endothelial cells is low, mitochondria play an important role in endothelial cell signaling in response to external stimuli. Therefore, studies on mitochondrial quality control of endothelial cells in diabetic nephropathy are necessary for new drug development.

**Immune cells**

Intrarenal inflammation is one of the most important contributing factors to the initiation and progression of DKD. Given that immune cell infiltration is frequently observed in biopsy-proven DKD, it is a major pathologic criterion used in pathologic classification of DKD [80]. Research studies have consistently demonstrated that the severity of interstitial inflammation is significantly associated with renal outcomes in patients with DKD [81–83]. Notably, kidney transcriptomic profiles of human diabetic kidneys
have revealed decreased expression levels of inflammation-associated genes in early diabetic kidneys compared to healthy controls, whereas expression levels of these genes are significantly increased in advanced diabetic kidneys, suggesting that the roles of inflammation in early and late DKD are different [84]. Deconvolution of RNA-sequencing data revealed significantly increased infiltration of virtually all immune cell types including macrophages, monocytes, B and T cells, and plasma cells, in advanced DKD compared to early DKD and control samples [84]. The significant increase in proinflammatory stimuli in patients with advanced DKD suggests that intrarenal inflammation plays a pivotal role in the progression of DKD. Consistent with these data, several studies have demonstrated that intrarenal macrophage infiltration is associated with albuminuria, the severity of histologic damage, and adverse renal outcomes [83,85].

Hyperglycemia can significantly affect mitochondrial homeostasis in immune cells and induce phenotypic changes. Several studies have shown that hyperglycemic stimuli can provoke mitochondrial dysfunction in circulating macrophages, which in turn can increase the proportion of proinflammatory M1 macrophages [86]. Hyperglycemia can induce the proinflammatory polarization of T cell compartments by increasing Th17 and Th1 subsets and decreasing regulatory T subsets [87]. Hyperglycemia can also induce mitochondrial inner-membrane hyperpolarization and increase the production of intracellular ROS and activation-induced interferon-γ [88]. Several studies have shown that B cells can also acquire proinflammatory phenotypes upon hyperglycemic stimulation. B cells have also been suggested to be involved in the pathogenesis of DKD, mainly by producing antibodies that can lead to the formation and deposition of immune complexes in the kidney [89]. Nevertheless, the role of immune cell mitochondria in DKD requires further investigation.

**Conclusions and future perspectives**

Defects in mitochondrial quality control and mitochondrial dysfunction are common characteristics of damaged kidney cells under hyperglycemic conditions [4,90]. Studies using diabetic rats have shown that abnormal changes in mitochondria precede the onset of albuminuria and histopathology [35]. Thus, disruption of mitochondrial homeostasis has been postulated to be a primary initiator of DKD and a potential target for developing new drugs. However, most studies that have investigated mitochondrial homeostasis-targeting drugs for DKD are in preclinical trials, with the exception of bardoxolone methyl [91]. Bardoxolone methyl, an activator of the NRF2 pathway, can inhibit mitochondrial ROS generation and nuclear factor kappa B signaling and is currently being investigated in a phase 3 clinical trial for DKD patients (NCT03550443).

Interestingly, although newly introduced antidiabetic drugs with reno-protective effects were not initially developed to target mitochondria, subsequent studies have shown that they improve mitochondrial health and dynamics [36,37,92]. *In vivo* and *in vitro* experiments have revealed that SGLT2 inhibitors can reduce mitochondrial fission and facilitate mitophagy in DKD [36,37]. Similarly, analysis of urine metabolites in patients with type 2 diabetes mellitus treated with atrasentan (an endothelin A receptor antagonist) suggested that this drug can prevent renal mitochondrial dysfunction under hyperglycemic conditions [92]. Therefore, the ability of a drug to affect mitochondrial dynamics and function may be used to determine its potential therapeutic effectiveness in DKD.

Due to the increasing prevalence of diabetes worldwide and the adverse effects of DKD on mortality, it is critical to gain a comprehensive understanding of the pathogenesis of DKD. Although many studies have investigated mitochondrial quality control, much remains to be clarified. Mitochondrial quality control deserves further investigation as a potential novel therapeutic target for DKD.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korean government (MSIT) (No. 2022R1A2C2006713, 2022R1F1A1073067, and 2021R1G1A1014115).

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: JB, SYL
Funding acquisition: HYJ, SYL
Visualization: JB, YHL
Writing—original draft: JB, YHL
Writing—review & editing: HYJ, SYL
All authors read and approved the final manuscript.

**ORCID**

Jihyun Baek, https://orcid.org/0000-0002-8328-9289
Yu Ho Lee, https://orcid.org/0000-0001-5231-0551
Hye Yun Jeong, https://orcid.org/0000-0002-5613-7079
So-Young Lee, https://orcid.org/0000-0003-1877-2124

**References**


www.krcp-ksn.org 557
ASL, Chertow GM, Luyckx VA, Marsden PA, Skorecki K, Taal MW

27. Gerich JE. Role of the kidney in normal glucose homeostasis and
in the hyperglycaemia of diabetes mellitus: therapeutic implications.

28. Østergaard JA, Cooper ME, Jandeleit-Dahm KA. Targeting oxidative
stress and anti-oxidant defence in diabetic kidney disease. J
Nephrol 2020;33:917–929.

29. Hall AM, Unwin RJ, Parker N, Duchen MR. Multiphoton imaging
reveals differences in mitochondrial function between nephron

30. Munusamy S, MacMillan-Crow LA. Mitochondrial superoxide
plays a crucial role in the development of mitochondrial dys-

Renal mitochondrial oxidative stress is enhanced by the redu-

32. Ogura Y, Kitada M, Xu J, Monno I, Koya D. CD38 inhibition by
apigenin ameliorates mitochondrial oxidative stress through restoration of the intracellular NAD+/NADH ratio and Sirt3

33. Zheng X, Narayanan S, Xu C, et al. Repression of hypoxia-induc-
able factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes. Elife 2022;11:e70714.


35. Coughlan MT, Nguyen TV, Penfold SA, et al. Mapping time-


40. Xiao L, Xu X, Zhang F, et al. The mitochondria-targeted anti-
oxidant MitoQ ameliorated tubular injury mediated by mito-


42. Chen K, Dai H, Yuan J, et al. Optineurin-mediated mitophagy protects renal tubular epithelial cells against accelerated senes-

43. Yang DY, Zhou X, Liu ZW, Xu XQ, Liu C. LncRNA NEAT1 acceler-
ates renal tubular epithelial cell damage by modulating mito-

44. Liu L, Bai F, Song H, et al. Upregulation of TIE1 in tubular ep-


46. Li C, Li L, Yang M, et al. PACS-2 ameliorates tubular injury by fa-
cilitating endoplasmic reticulum-mitochondria contact and mi-

47. Lee SY, Kang JM, Kim DJ, et al. PGC1α activators mitigate dia-


49. Tran MT, Zsengeller ZK, Berg AH, et al. PGC1α drives NAD bio-
synthesis linking oxidative metabolism to renal protection. Na-
ture 2016;531:528–532.


51. Kravets I, Mallipattu SK. The role of podocytes and podocyte-as-
ociated biomarkers in diagnosis and treatment of diabetic kid-


53. Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-induced


81. An Y, Xu F, Le W, et al. Renal histologic changes and the outcome...


The impact of hypoxia-inducible factors in the pathogenesis of kidney diseases: a link through cell metabolism

Orestes Foresto-Neto¹,²,* Ana Ruth Paolinetti Alves da Silva², Marcella Cipelli¹, Fernanda Paula Roncon Santana-Novelli⁷, Niels Olsen Saraiva Camara¹,²,*

¹Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
²Division of Nephrology, Department of Medicine, Federal University of São Paulo, São Paulo, Brazil

Keywords: Immune system, Lung injury, Metabolism, Microbiota

Background

The number of patients with end-stage renal disease is increasing, largely due to the aging of the population and increased incidence of hypertension and type 2 diabetes [1,2]. Several hemodynamic [3], inflammatory [4,5], and metabolic [6,7] factors are related to progressive loss of renal function and replacement of the renal parenchyma by fibrotic tissue. Recent studies have demonstrated that changes in the metabolism of podocytes and tubular cells and/or immune cells in kidneys are involved in the pathogenesis and progression of kidney diseases [7–9].

Podocytes require a high energy supply to maintain their cellular functions [10]. Dysfunctions in energy metabolism may result in cell damage and glomerular diseases [9,11]. Dysregulation of podocyte mitochondrial dynamics and function is associated with development of focal segmental glomerulosclerosis (FSGS) [12]. After exposure to high glu-
Glomerular lesions can extend to the tubulointerstitial area with the passage of protein and pro-inflammatory material to the neighboring interstitium and consequent inflammation and fibrosis [15]. However, interstitial fibrosis can start and become chronic in the absence of glomerular lesions. This process involves the participation and interaction of several cell types, including fibroblasts and tubular epithelial cells [16,17]. Recent studies have shown that the fibrogenic process in the kidneys is also associated with alterations in the metabolism of tubular epithelial cells [18,19]. Inhibition of genes related to regulation of the oxidative metabolism of fatty acids in tubular epithelial cells results in ATP depletion, intracellular lipid deposition, cell death, and dedifferentiation, as are observed in renal fibrosis [18]. The “Warburg effect,” which is the cellular metabolic reprogramming that exchanges oxidative metabolism for activation of aerobic glycolysis [20], may also be involved in the damage to tubular epithelial cells in kidney diseases. However, whether such changes in energy metabolism occur in tubular epithelial cells and what metabolic pathways are altered in the process of renal fibrosis remain unclear.

Kidney-resident immune cells participate in the initiation and spread of kidney damage and respond to metabolic shifts by changing their phenotype and functions. Macrophages are plastic cells; their phenotype can polarize to pro-inflammatory or anti-inflammatory, which require different metabolic pathways. Pro-inflammatory macrophages (M1) rely mainly on glycolysis, whereas anti-inflammatory macrophages (M2) have high oxidative phosphorylation (OXPHOS) activity and fatty acid oxidation [7]. Under homeostatic conditions, kidney-resident immune cells exhibit OXPHOS as the predominant metabolic pathway. Affected kidneys are infiltrated by pro-inflammatory cells, and predominance of glycolytic activity is observed [21].

The maintenance of renal cell energy homeostasis is under the control of a series of pathways and molecules involved in modulation of cell metabolism, among which the hypoxia-inducible factor (HIF) pathway plays an important role [22].

**Hypoxia-inducible factor: mechanism and function**

HIFs are oxygen-sensitive transcription factors that act in metabolic reprogramming to provide cell adaptation under hypoxic conditions [23]. HIF proteins consist of two subunits, α and β, that form a functional complex. The α subunit is unstable, and three distinct HIF-α have been identified: HIF-1α, HIF-2α, and HIF-3α [24,25]. The β subunit is stable and always present in the protein complex. Once the functional complex is formed through dimerization of the two subunits, the complex translocates to the nucleus and activates target genes and cofactors [25]. Studies have shown that HIFs can regulate the expression of genes related to iron regulation, glycolysis, cell survival, erythropoiesis, apoptosis, and angiogenesis [26,27].

HIF-1α and HIF-2α are differently distributed in organs and cells. Both contain a dimerization domain in the N-terminus and a transactivation domain in the C-terminus and regulate common target genes such as facilitated glucose transporter 1 (GLUT1), vascular endothelial growth factor (VEGF), and adrenomedullin genes [25,28,29]. Both HIF-1α and HIF-2α regulate physiological and pathological angiogenesis [30,31] and also regulate specific target genes. HIF-1α is responsible for glycolytic and carbonic anhydrase-9 gene regulation, while HIF-2α regulates TGF-α and cyclin D1 genes [32,33]. In addition, Elvert et al. [34] observed that expression of the VEGF receptor 2 gene is induced by HIF-2α but not by HIF-1α. Less is known about the role of HIF-3α; however, it has been shown to negatively regulate the expression of genes up-regulated by HIF-1α and HIF-2α [35].

HIF activity is regulated by proteosomal-mediated degradation of the HIF-α subunit [36]. Under normoxic circumstances, prolyl hydroxylase domain (PHD) proteins hydroxylate HIF-α at prolyl residues. Once the HIF-α is hy-
hydroxylated, the von Hippel–Lindau (VHL) protein E3 ubiquitin ligase ubiquitinates HIF-α for subsequent degradation by the 26S proteasome system [25,37]. The role of PHDs is oxygen dependent; cellular PHD activity is mostly regulated by oxygen partial pressure, but it can also be modulated by reactive oxygen species (ROS), succinate, and nitric oxide (NO) [38,39]. HIF-α can be also acetylated by the arrestin-like acetyltransferase, which enhances the interaction between VHL and HIF-α and promotes its degradation [25]. Under hypoxic conditions, HIF-α is modulated by a small ubiquitin-like modifier (SUMO), which enhances the affinity between HIF-α and VHL and promotes PHD-independent degradation [40]. This occurs in the absence of a sentrin/SUMO-specific protease 1 (SENP1). In the presence of SENP1, HIF-α loses the SUMO modification in a process called deSUMOylation, preventing HIF-α degradation. Once HIF-α is stable, HIF-α and HIF-β dimerize, producing a functional transcriptional complex capable of activating hypoxia-response element genes [25,40].

Kidney oxygenation, hypoxia, and hypoxia-inducible factor

Physiological kidney oxygenation involves a balance of O₂ demand and supply. Renal O₂ consumption is mostly related to solute exchange in tubular cells and renal aerobic glycolysis. Most of the energy required for the kidneys is obtained through aerobic production of adenosine triphosphate (ATP). However, segments of the nephron present in the renal medulla can rely on anaerobic metabolism for energy production, since the vascular architecture promotes a physiologic hypoxic condition in the medullary area [25,41]. The parallel arrangement of peritubular capillaries located along the nephrons allows oxygen to diffuse from the descending branch of the vessel toward the ascending branch, which has lower oxygen tension. This countercurrent exchange of O₂ decreases O₂ availability in the medulla, even though the kidney receives a high amount of blood perfusion [42]. Due to these specific characteristics, the kidneys are extremely sensitive to stresses that cause hypoxia [43].

Many individual risk factors and environmental and behavioral causes might lead the kidneys to a pathological state of hypoxia. Anemia, hypertension, air pollution, hyperglycemia, smoking, atherosclerosis, and acute kidney injuries (AKIs) are some of the factors that reduce oxygen delivery, promoting renal hypoxia [44]. Once activated by hypoxia, the renal HIF pathway can regulate the gene expression of VEGF, glucose transporters, erythropoietin, and endothelin 1, improving angiogenesis, energy metabolism, and erythropoiesis. Therefore, HIF is crucial for cellular adaptation to hypoxia by improving survival and tissue oxygenation and preventing damaging effects [45–47]. This adaptation involves increase of glycolysis and reduction of cellular oxygen consumption [48]. In hypoxic conditions, HIF upregulates the expression of pyruvate dehydrogenase kinase 1 (PDK1) [49], which inhibits the mitochondrial enzyme pyruvate dehydrogenase, repressing the production of acetyl-CoA from pyruvate and decreasing the mitochondrial oxygen consumption in the tricarboxylic acid cycle. Pyruvate is then redirected to the glycolytic pathway [48]. Thus, the HIF pathway promotes a metabolic shift from OXPHOS to glycolysis, decreasing the cellular necessity of oxygen to produce ATP. Moreover, this metabolic shift also protects the cells by reducing ROS formation [50]. Metabolic reprogramming could lead to a cellular bioenergetic crisis once glycolysis produces less ATP than OXPHOS [48]. However, HIF activation leads to an increase in glucose uptake through upregulation of glucose transporters GLUT1 and GLUT3 [48,51] and increases the expression of enzymes involved in the glycolytic pathway [50,51]. In addition, HIF downregulates the medium-chain acyl-CoA dehydrogenase and the long-chain acyl-CoA dehydrogenase enzymes [52] and inhibits carnitine palmitoyltransferase A 1 [53], decreasing the transport of fatty acid in the mitochondrial membrane and fatty acid oxidation and further reducing cellular oxygen consumption [48]. When hypoxia is brief and transitory, this mechanism of defense is often effective. However, when hypoxia is prolonged, the defense can be insufficient and pathological cellular pathways are recruited, aggravating the hypoxia state and consuming more oxygen for their processes.

The regulatory activity of HIF-1α and its transcriptional response vary in different renal cell types [54,55]. The energy metabolism of proximal tubular epithelial cells relies mostly on oxidative mitochondrial metabolism. Although the capacity for glycolysis is limited in proximal tubular cells, during oxygen deprivation, the transcriptional activity of HIF-1α is activated and promotes the expression of enzymes involved in glycolytic anaerobic metabolism,
which can provide small amounts of ATP to renal cells [54]. In podocytes, hypoxia-induced HIF activation increases the expression of VEGF, which promotes vascular development [56] and improves mitochondrial metabolism of other glomerular cells such as endothelial cells in a paracrine manner [57]. Due to the key role of cell metabolism in the maintenance of cell viability and integrity, disturbance of oxygen homeostasis and HIF activity can impact the pathogenesis and/or progression of several pathological conditions, including kidney diseases [25,58].

**Hypoxia-inducible factors in kidney diseases**

In addition to regulating cell metabolism in kidneys [25,59,60], HIF-1α plays a role in the process of renal injury in different conditions [61–63]. Several lines of evidence indicate that HIF-1α acts as a profibrotic effector in kidney diseases. Activation of HIF-1α in tubular epithelial cells by hypoxia was shown to promote epithelial-mesenchymal transition in vitro and was associated with tubulointerstitial lesion in mice that underwent unilateral ureteral obstruction (UUO) and patients with chronic kidney disease (CKD) [64]. Deletion of VHL in tubular cells resulted in HIF-1α stabilization in the renal cortex and increased fibrosis in mice that underwent 5/6 nephrectomy. Pharmacological blockade of HIF-1α with YC-1 [65] or deficiency of HIF-2α in renal interstitial cells [66] ameliorated renal interstitial fibrosis in UUO mice. Inhibition of renal HIF-1α by short hairpin RNA attenuated glomerular damage and tubulointerstitial fibrosis induced by angiotensin II infusion [67] or chronic renal ischemia [62] in rats. Glomerular type I collagen accumulation was reduced by HIF-1α knockout in the NEP25 model of FSGS [68]. Selective deletion of HIF-1α in proximal tubular epithelial cells ameliorated renal fibrosis in an aristolochic acid mouse model of kidney disease [69].

Another study showed that global stabilization of Hif-1α and Hif-2α through genetic inactivation of VHL attenuated the renal inflammatory status of UUO mice [70]. Pretreatment with FG-4487, a PHD inhibitor, induced accumulation of HIF-1α and HIF-2α in tubular cells and reduced kidney injury and apoptosis in rats with AKI induced by renal ischemia/reperfusion injury (IRI) [71]. In vitro, treatment with enarodustat (a pan-PHD inhibitor) or small interfering RNA (siRNA) knockdown of PHD2 reduced the levels of ROS and increased the viability of renal proximal tubule cells with ischemia by blockade of OXPHOS or oxygen-glucose deprivation. These protective effects of PHD inhibition were attributed to the HIF-1α–induced expression of enzymes involved in glycogen synthesis, with enhanced glycolysis and delayed ATP depletion [72]. In cisplatin-treated mice, pretreatment with roxadustat (FG-4592), a novel PHD inhibitor, enhanced HIF-1α in tubular cells, improved renal function, and reduced markers of renal inflammation/injury [73]. Roxadustat reduced renal crystal deposition and ameliorated renal dysfunction and tubulointerstitial damage in a model of CKD by adenine overload [74]. However, roxadustat had no effect on renal fibrosis or macrophage infiltration in UUO mice [75]. Oral administration of roxadustat corrected anemia and reduced serum hepcidin level, which was increased by inflammation [76], in dialysis and nondialysis patients with CKD [77–79]. Together, these findings suggest that the role of HIF in kidney damage can vary according to the pathological context and the mechanism applied to modulate its activation. Furthermore, therapeutic approaches may interfere with the activation of HIF in cells other than renal cells, such as immune cells.

**Physiological hypoxia-inducible factor activity in immune cells**

Hypoxia plays a key role in the bone marrow, providing a specific niche for hematopoietic stem cells, maintaining the self-renewal capacity of these cells, and sustaining their survival [80]. Recent research has demonstrated the importance of HIF expression by immune system components in response to physiological hypoxia or inflammation. Evidence shows that activation of HIFs in innate and adaptive immune cells plays a central context-specific manner role in controlling their functions and cell metabolism (Fig. 1) [48]. In neutrophils, for example, HIF-1α expression is associated with a metabolic shift to a glycolytic profile, associated with increased phagocytic capacity and formation of neutrophil extracellular traps (NETs), in addition to promoting survival [81]. Human and mouse neutrophils express HIF-2α, which is up-regulated by lipopolysaccharide (LPS). HIF-2α–depleted inflammatory neutrophils derived from murine bronchoalveolar lavage fluid from LPS-challenged mice expressed lower level of the antioxidant enzyme catalase than wild-type neutrophils and underwent
Figure 1. Hypoxia-inducible factors (HIFs) and immune cells. (A) HIFs regulate immune cells by interfering with their metabolic pathways and function/phenotype. In neutrophils, HIF-1α activation favors glycolysis, increasing the cellular phagocytic capacity, surveillance, and release of neutrophil extracellular traps (NET). In natural killer (NK) cells, HIF-1α is associated with the balance between antibacterial function and tissue repair. HIF-1α increases the migration capacity of both eosinophils and dendritic cells (DCs). In pro-inflammatory macrophage (M1), HIF-1α is fundamental for the activation of glycolysis and pentose phosphate pathways (PPP) and increases the phagocytic capacity and nitric oxide synthase 2 (NOS2) expression, while suppressing oxidative phosphorylation (OXPHOS). HIF-2α is associated with higher tissue repair capacity through arginase-1 expression and lower glycolysis in anti-inflammatory macrophage (M2), which rely on OXPHOS to meet their energy demands. (B) HIF pathways balance the expression of transcriptional factors and effector functions in adaptive immunity. HIF-1α downregulates forkhead box P3 (Foxp3) and upregulates the retinoid orphan receptor gamma t (RORγt) in CD4+ T cells, interfering with their immune functions. During Th1 differentiation, HIF-1α increases the expression of interferon gamma (IFN-γ). In CD8+ T cells, HIF is associated with increased release of granzyme B and perforin and enhanced glycolytic activity. In B cells, HIF induces glycolysis, antibody production, and proliferation and inhibits apoptosis.

In dendritic cells, activation of HIF-1α has been associated with the capacity to migrate and to activate T cells [83]. Recently, HIF-1α expression was also observed in natural killer cells in the skin and was shown to play an important role in the balance between tissue repair and antibacterial defense [84]. Although its participation in metabolism has not been investigated, the expression of HIF-1α was associated with a greater migratory capacity in eosinophils [85]. In macrophages, activation of the glycolytic pathway through expression of HIF-1α is associated with a pro-inflammatory profile (M1), increasing the phagocytic capacity and favoring the production of cytokines essential for clearance of viruses and bacteria [86, 87]. Tannahill et al. [88] demonstrated that LPS-induced HIF-1α stabilization leads to a metabolic shift toward glycolysis and the pentose phosphate pathway (PPP) in macrophages by inducing the expression of genes essential for glycolysis, which results in accumulation of intermediates of the Krebs cycle such as fumarate, malate, and succinate. Increasing succinate in macrophages leads to inhibition of PHDs through generation of ROS by mitochondrial reverse electron transport from complex I or even by competition for the binding site of its cosubstrate, alpha-ketoglutarate [89, 90]. This inhibi-
tion finally results in accumulation of HIF-1α, which induces a pro-inflammatory phenotype in macrophages, increasing the production of inducible NO synthase, which drives NO synthesis [86,88,91]. Notably, suppression of HIF-2α increases the levels of NO and efferocytosis in macrophages [91,92]. This demonstrates that the HIF-α isoforms regulate NO production and phagocytic capacity in macrophages in an antagonistic way (Fig. 1A).

HIF-1α also participates in several processes essential for the development and functioning of T and B cells and generation of antibodies upon an antigenic challenge (Fig. 1B) [93,94]. Cho et al. [93] demonstrated that HIF signaling increases the glycolytic metabolism of germinal center B cells, affecting their antibody production, proliferation capacity, and apoptosis. In line with this finding, another group observed that the deletion of VHL leads to a decrease in antigen-specific terminal center B cells, impairing the generation of high-affinity antibodies [94]. Activation and differentiation into subtypes of CD4 T cells and the cytotoxic capacity of CD8 T cells have also been associated with induction of the HIF-1α-dependent glycolytic pathway [81]. HIF-1α promotes the differentiation of Th17 cells through transcriptional activation of the retinoid orphan receptor gamma t (RORγt), the main transcription factor of Th17 cells. HIF-1α binds to p300/RORγt, and this protein complex binds to the interleukin 17 (IL-17) promoter, enhancing IL-17 gene expression and contributing to Th17 function [95]. In addition, signal transducer and activator of transcription 3 (STAT3), which is activated by IL-21, IL-6, and IL-23, can physically interact with the promoter of HIF-1α and increase its expression [96,97]. Concomitantly, HIF-1α can direct the major transcriptional factor of regulatory T cells (Tregs), forkhead box P3 (Foxp3), to proteasomal degradation [95]. Shehade et al. [98] showed that T cell differentiation under hypoxic conditions had a higher number of IL-17-producing cells, whereas the Foxp3+ Treg frequency was significantly decreased. In Th1 differentiation, HIF-1α can act as an enhancer of the glycolytic pathway and interferon gamma (IFN-γ) expression through retroactive activation of STAT3 [81,98]. The higher amount of HIF-1α in Th1 cells leads to an increase in lactate dehydrogenase A, which increases acetyl-CoA accumulation in the cells, promoting histone acetylation and trancription of IFN-γ [99]. HIF signaling is also important for CD8+ T cell function, promoting the expression of crucial transcription, effector, and costimulatory-inhibitory molecules and favoring the clearance of viruses and tumors. Deletion of VHL and increase in HIF-1α and HIF-2α result in higher effector function in CD8+ T cells during chronic infection, culminating in hyperinflammation [100]. These examples demonstrate the complexity behind the roles of HIF-1α in different immune cells, which also depend on the surrounding cellular environment.

Considering that HIF can alter the metabolism of immune cells, interfering with their inflammatory activity, it is conceivable that its activation acts as a regulatory link between events that impairs kidney function/cell metabolism (e.g., hypoxia, LPS, ROS) and the inflammation that is established and progresses in kidney diseases.

**Role of hypoxia-inducible factor in immunity in acute kidney injury**

The pathophysiology of renal IRI is accompanied by intense inflammation, and researchers have increasingly demonstrated a relationship between HIF-1α activation and immune cells during this process. Increased transcriptional activity of HIF-1α in renal tubular cells has been closely associated with macrophage-dependent inflammation [101]. The nuclear factor kappa B (NF-κB) pathway is highlighted for its central role in promoting inflammation and is activated by inflammatory cytokines, leading to their retroactive production. Li et al. [101] demonstrated that NF-κB binds the HIF-1α promoter, leading to increased HIF-1α expression and consequent protection against tubular injury during AKI.

Using the cisplatin model of AKI and other models of acute and chronic hypoxic kidney injury, Yamaguchi et al. [102] found that the inflammation-related transcription factor CCAAT/enhancer binding protein δ (CEBPδ) is a regulator of HIF-1 in the kidney, binding directly to the HIF-1α promoter and potentiating its transcription. In tubular epithelial cells, CEBPδ was rapidly induced by inflammatory cytokines produced by macrophages, such as IL-1β, through NF-κB activation, which increases HIF-1α expression during hypoxia and is essential for non-hypoxic induction of HIF-1α.

Folic acid, also known as vitamin B9, is necessary for one-carbon transfer reactions and nucleic acid synthesis; however, it causes toxicity and AKI when administered in
high doses [103]. Interestingly, pretreating human monocyte THP-1 cells with folic acid decreased the nuclear accumulation of HIF-1α protein and reduced the expression of IL-1β and tumor necrosis factor alpha (TNF-α), while it increased the level of IL-10. In addition, KC7F2 (an HIF-1α inhibitor) reduced the levels of these hypoxia-induced cytokines, whereas dimethyloxalylglycine (DMOG, a PHD inhibitor) induced their over-expression [104]. Gentamicin nephrotoxicity is also a common cause of drug-induced AKI. Dose-dependent elevation of renal HIF-1α messenger RNA level and increased tubulointerstitial infiltration of ED1+ macrophages have been reported in rats with gentamicin-induced acute injury [105,106]. Treatment with cobalt activates HIF-1α and reduces macrophage infiltration in kidneys exposed to gentamicin [106].

The AKI to CKD transition and development of fibrosis in mice treated with low-dose cisplatin was accompanied by a significant increase in the total macrophage population, with a higher amount of M2 macrophages expressing arginase 1 [107], which is induced by HIF-2α and suppresses NO production [91]. A recent study investigated the role of macrophages in response to repeated low doses of cisplatin-induced fibrosis using liposome-encapsulated clodronate to deplete macrophages in mice. The authors showed that renal depletion of F4/80high and M2 macrophages with decrease in arginase 1 expression attenuates the development of renal fibrosis, suggesting a pathogenic role for kidney-resident M2 macrophages in the progression of fibrosis [108].

**Chronic kidney disease-related complications and hypoxia-inducible factor activation**

After AKI, an adaptive repair process can restore the integrity of the renal tubules. In contrast, incomplete repair with undifferentiated and atrophic tubules and persistent inflammation can result in renal fibrosis and progression to CKD.

Hypoxia is an early event in the development and progression of experimental diabetic kidney disease, in which inflammation and macrophage polarization play a key role [109]. TGF-β-activated kinase 1 (TAK1) binding protein and TGF-β-activated (TAK1) binding protein form a complex (TAB1/TAK1) that can activate the NF-κB signaling pathway in bone marrow-derived macrophages, activating HIF-1α, increasing glycolytic metabolism, and promoting polarization of these cells toward the M1 pro-inflammatory phenotype [110]. In contrast, myeloid cell-specific activation of HIF suppressed inflammation in UUO mice, whereas specific inactivation of HIF in these cells enhanced inflammation. Furthermore, prolonged exposure to hypoxia suppressed the expression of multiple inflammatory molecules in non-injured kidneys [70]. Thus, hypoxia and/or HIF activation in myeloid cells seem to attenuate the renal inflammation associated with UUO. Consistent with these findings, we observed that exposure to chronic hypoxia reduced renal infiltration by CD68+ macrophages and attenuated renal oxidative stress, innate immunity activation, and injury in rats with 5/6 nephrectomy, while it did not promote renal injury or inflammation in sham-operated rats [111]. HIF-1 and HIF-2 exert different effects on macrophage function in vitro; HIF-1 promotes polarization to the M1 phenotype, while HIF-2 activation induces the M2 anti-inflammatory phenotype [70].

Immunoglobulin A nephropathy (IgAN) is one of the most common types of primary glomerulonephritis. Infiltration of CD68+ and CD206+ macrophages is increased in the kidneys of patients with IgAN [112,113], and the presence of CD68+ macrophages in the tubulointerstitial area was associated with increased renal activation of NF-κB [114], which binds the HIF-1α promoter and enhances its transcription [101]. Indeed, the expression of HIF-1α has been detected in biopsy material from patients with IgAN. Notably, higher HIF-1α expression was associated with lower serum creatinine level, low interstitial fibrosis score, and low glomerular sclerosis in early CKD. However, once fibrosis progresses in the later stages of CKD, lower HIF-1α expression is detected [115]. Thus, renal expression of HIF-1α may be beneficial in early CKD, when active tissue damage is ongoing.

Cardiovascular complications are the leading cause of death in CKD patients [109]. While HIF-1α induces expression of VEGF, endothelin-1, and matrix metalloproteinases in endothelial cells to facilitate angiogenesis, it induces the proliferation of vascular smooth muscle cells in atheroma by upregulation of CD98 and macrophage migration inhibitory factor. HIF-1α also modulates the function of macrophages derived from diseased foam cells, making the cells more inflammatory and apoptotic [116]. Notably, activation of HIF in immune cells plays different roles in different
contexts of CKD, especially in macrophages, promoting chronic inflammation or slowing disease progression. The HIF-mediated inflammatory response resulting from CKD directly affects the kidneys but can reach the circulation and affect other organs.

**Lung-kidney crosstalk in diseases: a role for hypoxia-inducible factor?**

Lungs and kidneys are related in structure and function. Both organs contain an epithelial barrier that regulates the amounts of fluid and solutes that move across two distinct compartments [117–119]. Evidence indicates a close relationship between kidneys and lungs in several diseases [120,121]. Damaged kidneys can interfere with pulmonary disorders by altering the acid-base or fluid balance or through the production of inflammatory mediators [119]. Lungs are highly susceptible to circulating mediators such as TNF-α, IL-1, IL-6, IL-8, NO, and caspase-3 due to their extensive capillary network [118].

Although the lungs are among the most oxygenated organs in the human body [122], HIFs appear to play a critical role in lung function [123]. HIF-1α is involved in lung vascular development via upregulation of angiogenic factors [124,125]. HIF-2α participates in the formation of alveoli and production of surfactant [126]. HIF-3α knockout mice showed impaired lung remodeling at the late embryonic stage and right ventricular enlargement in the adult stage [127].

HIFs also play an important role in pulmonary diseases [128,129]. Acute lung injury (ALI) is an inflammatory disease characterized by pulmonary edema attributed to increased permeability in endothelial cells and infiltration of protein-rich fluid into the alveoli, which reduces the efficacy of air exchange and can result in hypoxemia and alveolar hypoxia. Sepsis, renal IRI, severe traumatic injury, and cigarette smoking are some of the major risk factors for ALI. Although there is no evidence that hypoxia is a direct cause of ALI, studies suggest that hypoxia can contribute to the pathogenesis of the disease [129]. Experimental acute exposure to hypoxia resulted in an ALI-like scenario in rats, with increased infiltration of immune cells and vascular leakage in lungs, suggesting that lung injury is perpetuated by alveolar hypoxia [130,131]. Indeed, alveolar hypoxia in ALI can trigger an inflammatory response with immune cell infiltration, especially macrophages, and increased production of inflammatory molecules, such as intercellular adhesion molecule-1, TNF-α, macrophage inflammatory protein-1β, and monocyte chemoattractant protein-1 (MCP-1), aggravating the injury to the lungs [122,131]. Furthermore, the lungs are among the organs that express the highest levels of HIF-2α in hypoxia [132], and this HIF-2α upregulation increases the activation of nuclear factor of activated T cells c2 and the proliferation of pulmonary fibroblasts [133]. In contrast, other studies have reported that hyperoxygenation increased mortality in experimental models of ALI, whereas hypoxia reduced inflammation in the lungs [134]. Augmentation of HIF-1α downregulated the expression of toll-like receptor 4 and TNF-α in macrophages and decreased inflammatory impairment in ALI rats [135]. In cultured pulmonary alveolar type II cells, TNF-α induced the upregulation of HIF-1α and inhibited vasodilator-stimulated phosphoprotein expression, which plays an important role in the impairment of the alveolar-capillary barrier in ALI [136].

HIF-1α also plays a metabolic role in ALI. Administration of DMOG protected alveolar epithelial cells from neutrophil-LPS–induced ATP decline and cell death in vitro, whereas knockdown of HIF-1α with siRNA or inhibition of glycolysis using media containing 2-deoxy-D-glucose abolished the protective effect of DMOG, suggesting that HIF-1α activation protects alveolar epithelial cells in ALI by enhancing their glycolytic activity. In addition, treatment with DMOG protected the alveolar epithelial barrier, improved arterial oxygenation, and prevented lung ATP decline in mice with LPS-induced lung injury [137]. Woods et al. [138] demonstrated that tissue-resident alveolar macrophages can adapt to hypoxia through HIF-1α activation in ALI. Hypoxia or treatment with FG-4592 stabilized HIF-1α in resident alveolar macrophages, increasing their glycolytic function and survival, and ameliorated lung injury in mice, suggesting that therapies inducing HIF-1α in macrophages may be beneficial in ALI [138]. Repeated injuries in the pulmonary epithelium can cause abnormal wound healing, with recruitment of immune cells and activation of fibroblasts for extracellular matrix protein production, such as fibronectin and collagen, and result in pulmonary remodeling/fibrosis, which hinders air exchange and leads to systemic hypoxemia [128]. Inhibition of the HIF-1α/PDK1 axis in lung fibroblasts attenuated bleomycin-induced pulmo-
nary fibrosis in mice [139]. Activation of HIF-1α by hypoxia polarized activated macrophages to a fibrotic phenotype through increasing adenosine A2B receptor expression and production of profibrotic mediators [120]. These findings suggest an important role of HIF-1α as an amplifier of pulmonary fibrosis.

Patients with chronic obstructive pulmonary disease (COPD) present long-term respiratory symptoms and airflow limitation caused by remodeling of lung structure, leading to hypoxic conditions. HIF-1α has been implicated in the increase of deoxycytidine kinase, which is responsible for accumulation of deoxyATP and apoptosis in COPD [140]. HIF-1α also promotes mucus hypersecretion in COPD by increasing the expression of mucin 5AC, a major component of airway mucus, in airway epithelial cells [141]. CKD has been shown to affect the long-term survival of COPD patients [142]. Gjerde et al. [143] reported that systemic inflammatory markers are associated with a higher risk of renal failure in COPD patients.

Patients with AKI are twice as susceptible to respiratory failure as those without AKI and can progress to ALI [144]. Mice who underwent ALI after induction of AKI had increased lung neutrophilia in bronchoalveolar lavage fluid and elevated MCP-1 levels in kidneys, serum, and lungs compared with ALI mice without AKI [145]. Moreover, after kidney ischemia, lungs present an inflammatory profile, with increased concentrations of pro-inflammatory cytokines and chemokines, upregulation of caspase-dependent apoptosis, and increased macrophage-mediated pulmonary vascular permeability [146]. Pulmonary stabilization of HIF-1α had a protective effect in mice exposed to LPS by inducing the expression of the antioxidant enzyme heme oxygenase-1 (HO-1) [147]. Notably, hemin-induced HO-1 production reduces the systemic inflammation and improves the renal outcomes after IRI. In addition, treatment with hemin ameliorated lung inflammation in AKI mice [148]. These findings suggest the existence of lung-kidney crosstalk in different pathological scenarios and that HIF-induced antioxidant enzymes as well as HIF-1α target genes play a role in this process (Fig. 2).

The gut-kidney axis: a role for hypoxia-inducible factor?

Oxygen concentrations along the intestinal tract and large intestine are lower than those in other organs such as the lung, liver, and heart [149]. The partial pressure of oxygen level is low in the gut due to the anatomical juxtaposition of the outermost mucosal surface with the oxygen-depleted lumen and a countercurrent oxygen exchange system in the intestinal villi [149,150]. In addition, the gut contains a diverse and dense microbial population that includes aerobic facultative and anaerobic bacteria necessary for breakdown of food nutrients and regulation of intestinal and systemic immune responses [151,152].

HIFs are crucial for O2 regulation in the gut. In hypoxic conditions, HIFs promote the expression of factors responsible for adaptation of the gut to hypoxia, such as genes involved in erythropoiesis, angiogenesis, and metabolism [39]. In addition to the protective barrier in the gut composed of mucus, intercellular tight junctions, and adherens junctions in the external (luminal) and internal (vascular) compartments, HIF-1α was also shown to participate in protection of the intestinal epithelia during intestinal hypoxia [153,154]. Moreover, HIF-1α and HIF-2α have been shown to modulate intestinal epithelial barrier integrity, function, and homeostasis in colitis [155,156].

HIFs play critical roles in inflammatory response in the intestinal tissue. Acute intestinal inflammation involves the accumulation of neutrophils and can progress to chronic inflammation. Recruitment of neutrophils reduces O2 level in the environment sufficiently to activate HIF-1 in the gut of acute colitis model mice [157]. Intestinal tissue from patients with active ulcerative colitis showed increased positive staining for HIF-1α, whereas tissue from patients with Crohn disease showed intense expression of HIF-2α [158]. HIF-1α and HIF-2α were shown to upregulate the expression of creatine kinases in intestinal epithelial cells, which promotes rapid ATP generation via the phosphocreatine-creatine kinase system, improving cellular bioenergetics and reducing the damage to the intestinal epithelium in colitis [156]. Inhibition of PHD with FG-4497 resulted in stabilization of HIF-1α and reduction of intestinal inflammation in murine colitis [159]. Deficiency of PHD1 ameliorated colitis in mice by reducing apoptosis in the inflamed colon and enhancing epithelial barrier function [160]. Wild-type mice treated with AKB-4924 (a PHD inhibitor) showed reduced serum levels of endotoxin, IL-1β, IL-6, and TNF-α and preservation of the intestinal barrier during colitis, whereas mice with epithelial-specific HIF-1α
Hypoxia-inducible factors (HIFs) in the lung-kidney and gut-kidney axes.

Increased systemic inflammation is associated with a higher risk of renal failure in patients with chronic obstructive pulmonary disease. Presence of kidney disease increases neutrophilia in bronchoalveolar lavage fluid and the levels of monocyte chemoattractant protein-1 (MCP-1), cytokines, and apoptosis in the lungs. HIF activation induces the expression of the antioxidant enzyme heme oxygenase 1, which can reduce the injury to the damaged lungs and kidneys. Renal damage also results in accumulation of uremic toxins, which promote intestinal dysbiosis and inflammation. Gut dysbiosis and lesions on the intestinal epithelium increase the passage of inflammatory molecules from the gut to circulation, reaching the kidneys, where they promote inflammation and dysfunction. A balanced gut microbiota produces short-chain fatty acids (SCFAs), which are increased by HIF activation and exert positive effects on the intestinal mucosa and immunity and reduce kidney inflammation. Therefore, kidneys, gut, and lungs receive a large blood supply, resulting in high exposure to circulating immune cells and molecules. HIFs can interfere with cell metabolism and the expression of cytokines by immune and epithelial cells, increasing the possibility that HIFs play a role in the lung-kidney and gut-kidney axes. However, it remains unclear whether HIF activation is beneficial or acts as a link between the diseases.

GFR, glomerular filtration rate.

deficiency had no protection against colitis in the presence of AKB-4924 treatment, suggesting that HIF-1α is essential for the modulation of gut inflammatory diseases [161].

The roles of the gut microbiota in the healthy gut and in intestinal diseases have been extensively investigated. A regulated host-microbiota interaction is essential for physiologic homeostasis and regulation of the immune system [151,162]. Dysbiosis is the imbalance of the gut microbiota with changes in the host metabolic capacity and inflammatory responses that can result in damage to different organs. Lifestyle and environmental factors, use of antibiotics, and a diet poor in fibers and high in sugar and fat are associated with dysbiosis [151,163]. Alterations in the gut microbiota composition with a predominance of pathogenic bacteria lead to the synthesis of harmful molecules that damage the gut and are released to the circulation [151,164,165]. Dysbiosis and lesions on the intestinal epithelium also increase the passage of bacteria and other inflammatory players from the gut to the circulation and therefore to other extraintestinal sites such as the kidneys,
where they promote inflammation (Fig. 2). Loss of renal function results in accumulation of uremic toxins, which promotes gut dysbiosis. This gut-kidney crosstalk also involves immune cells and cytokines that are regulated by bacterial metabolites [151]. A balanced gut microbiota produces short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate through anaerobic fermentation of dietary fibers or through metabolism of amino acids [166]. SCFAs play an important role in intestinal homeostasis and exert positive effects on the intestinal mucosa and immune response as well as reduce kidney inflammation [151,166]. Butyrate is one of the main SCFAs that act in gut homeostasis [166]. In the healthy gut, butyrate concentration can exceed 30 mM and is a significant energy source for colonic epithelial cell metabolism. Thus, changes in microbiota can result in abnormal colonocyte function [167]. In colonocytes, absorbed butyrate can be converted to acetyl-CoA through β-oxidation in mitochondria, contributing to the consumption of oxygen and activation of HIF-1α and transcription of its target genes [168]. Zhou et al. [169] demonstrated that intestinal epithelial-specific deletion of HIF-1α changed the composition of the gut microbiota and decreased butyrate production, increasing the susceptibility of mice to induced colitis. In addition, butyrate can upregulate the expression of tight junction proteins in the intestinal epithelium in an HIF-1α-induced manner, which reduces intestinal inflammation and protects the barrier function of the gastrointestinal tract [170,171]. Germ-free and antibiotic-treated mice had reduced colonic butyrate content and weaker HIF activation, both of which were restored by butyrate supplementation [172]. Koury et al. [173] showed that the prolonged increase in HIF-1α after experimental gut IRI is mediated by contact of bacterial products within the gut lumen with the stressed intestinal mucosa. These findings suggest a role of the HIF-1α pathway on the protective effect of SCFAs produced by the gut microbiota in intestinal inflammatory diseases.

Evidence indicates that SCFAs produced by the intestinal microbiota also exert a renoprotective effect in AKI. Acetate, propionate, and butyrate improved renal function and reduced inflammation in experimental IRI-induced AKI mice. Furthermore, treatment with SCFAs reduced activation of NF-κB signaling, production of ROS, and translocation of HIF-1α to the nucleus in tubular epithelial cells stimulated with an inflammatory cocktail [174]. Gut dysbiosis and the consequent release of pro-inflammatory cytokines and chemokines by the intestinal epithelium are also involved in the pathogenesis and progression of CKD [162,164,175,176]. This raises the possibility that therapeutic interventions aimed at interfering with renal HIF-1α in kidney diseases also interfere with intestinal HIF activation and microbiota. Kidneys and gut receive a large blood supply, increasing their exposure to HIF-related molecules released by other damaged organs into the bloodstream.

**Conclusions**

The role of hypoxia and HIF in kidney diseases has become a topic of interest to nephrologists. However, whether HIF activation is beneficial or participates in the pathogenesis and progression of kidney diseases remains unclear. HIF-1α stabilization has been shown to promote epithelial-mesenchymal transition in vitro and act as a pro-fibrotic effector in experimental CKD. In contrast, other studies have reported that HIF-1α activation ameliorates renal inflammation, apoptosis, and injury. HIF also regulates the metabolism of immune cells and can modulate immune responses in kidney diseases. Furthermore, evidence suggests HIF-1α activation as a mechanism linking lung and gut damage to worsening kidney disease. Further studies on the role of HIF in the kidney-lung crosstalk and in the kidney-gut axis are needed. Current research supports HIF-1α and its transcriptional activity as important therapeutic targets to prevent human kidney diseases. However, variations in the HIF response to different pathological context need to be considered when targeting the HIF pathway.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This work was supported by the São Paulo Research Foundation (FAPESP, grant No. 2017/05264-7, 2019/02893-9, 2021/06748-3, 2022/03740-4, and 2022/01226-1), the National Council for Scientific and Technological Development (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.
Acknowledgments

The figures were created with BioRender.com.

Data sharing statement

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

Authors’ contributions

Conceptualization: OFN, NOSC
Writing–original draft: All authors
Writing–review & editing: OFN, NOSC
All authors read and approved the final manuscript.

ORCID

Orestes Foresto-Neto, https://orcid.org/0000-0001-7548-4327
Ana Ruth Paolinetti Alves da Silva, https://orcid.org/0000-0001-8023-2749
Marcella Cipelli, https://orcid.org/0000-0001-9185-3938
Fernanda Paula Roncon Santana-Novelli, https://orcid.org/0000-0001-5837-4310
Niels Olsen Saraiva Camara, https://orcid.org/0000-0001-5436-1248

References


36. Huang LE, Gu J, Schau M, Bunn HF. Regulation of hypoxia-inducible factor-1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc Natl Acad Sci USA* 1998;95:7987–7992.


49. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-medi-


Philip K, Mills TW, Davies J, et al. HIF1A up-regulates the ADORA2B receptor on alternatively activated macrophages and contributes to pulmonary fibrosis. *FASEB J* 2017;31:4745–4758.


Overview of aristolochic acid nephropathy: an update

Qingqing Zhou¹², Lei Jiang¹²³, Tao Su¹²³, Gang Liu¹²³*, Li Yang¹²³*

¹Division of Renal, Department of Medicine, Peking University First Hospital, Beijing, China
²Institute of Nephrology, Peking University, Beijing, China
³Research Units of Diagnosis and Treatment of Immune-mediated Kidney Diseases, Chinese Academy of Medical Sciences, Beijing, China

Aristolochic acid nephropathy (AAN) is a rapidly progressive renal interstitial fibrosis caused by medical or environmental exposure to aristolochic acid (AA). Since the outbreak of AAN in Belgium was reported nearly 30 years ago, the safety of herbal remedies has drawn considerable attention, and AAN has become a global public health problem. Breakthroughs have been made to better understand the disease, including the toxicity of AAs, the possible mechanisms of AAN, the disease patterns, and the pathological features; however, some critical problems remain unresolved. Because of the insidious onset of the disease, the incidence of AAN and the prevalence of exposure to AAs are unknown and might be largely underestimated. During the past decades, AA-containing herbs have been strictly administrated in many regions and the occurrence of AAN has declined sharply, yet cases of AAN are still sporadically reported. Despite the progress in the understanding of the disease’s pathogenesis, there is no effective treatment for delaying or reversing the renal deterioration caused by AAN. Therefore, the risk of exposure to AAs should be taken seriously by public health workers and clinicians. In this review, we updated the latest data on AAN, summarized the advances throughout these years, and put forward some challenges for future research.

Keywords: Aristolochic acids, Aristolochic acid nephropathy, Herbal therapy, Interstitial nephropathy, Renal tubular dysfunction

Introduction

In 1964, a Chinese doctor named Songhan Wu first described two patients with acute renal failure after excessive intake of Aristolochia manshuriensis, yet the nephrotoxicity of Aristolochia was not taken seriously at that time [1]. In the early 1990s, a group of Belgian scholars reported that a cohort of young female patients suffered from rapidly progressive interstitial renal fibrosis [2]. The onset of the epidemic was attributed to continuous consumption of the same slimming pills containing Chinese herbs; therefore, this new type of kidney disease was initially termed “Chinese herbs nephropathy” [2]. Very soon after, researchers revealed that roots of Stephania tetrandra (Pin Yin: Han Fang Ji), an original component in the slimming regimen, were mistakenly replaced with...
roots of an aristolochic acid (AA)-containing herb called *Aristolochia fangchi* (Pin Yin: Guang Fang Ji), because they both belong to the “Fang Ji” family in traditional Chinese medicine and share similar names in Pin Yin [3,4]. Further studies then identified AA as the causative agent, leading to the renaming of this renal tubulointerstitial disease as “aristolochic acid nephropathy” (AAN) [5]. Furthermore, AA exposure has been proven to be associated with a high incidence of urothelial carcinoma [6,7]. Since its discovery, cases related to AA intoxication have been reported all over the world, and AA exposure has received considerable attention [8]. Due to its hidden onset, the incidence of AAN could be largely underestimated, especially in Asia [8]. As traditional medicines are extremely popular in Asia, the wide application of *Aristolochia*, as well as the frequent substitution of botanical products by AA-containing herbs, increased the potential risk of AAN [8–10]. Despite the banning of *Aristolochia* in many countries, botanical remedies containing AA are still accessible and sporadic cases are occasionally reported, reminding clinicians not to overlook the harm of AA exposure [11].

**Epidemiology of aristolochic acid nephropathy**

The outbreak of AAN in Belgium first reported in the early 1990s included nine female patients [2], but the number of patients involved rose to more than a hundred in 1998 [12]. Cases with similar or different phenotypes related to AA nephrotoxicity were reported thereafter in Europe [13–16], the United States [17], China [18,19], Japan [20,21], Korea [22], Australia [23], and Bangladesh [24], illustrating that AA-containing herbs were widely used for treating an assortment of diseases. Due to its insidious onset, low awareness, as well as lack of strict diagnostic criteria, the prevalence of AAN remains largely unknown and is probably underestimated, especially in Asia [8]. Thousands of cases have been reported in China in the past decades among patients previously diagnosed with chronic tubulointerstitial nephritis of unknown origin [25]. At our center in Beijing, 300 patients were diagnosed with AAN between 1997 and 2006 [18]. As for Korea and Japan, the number of persons affected by AAN appears to be relatively lower than in China [21,22]. Although no cases of AAN have been reported in India, the high proportion of patients with chronic interstitial nephritis among the chronic kidney disease (CKD) population might be associated with dietary and environmental AA exposure [26,27]. In the Balkan region, so-called Balkan endemic nephropathy is regarded as an endemic type of AAN with similar clinical and pathological features; it occurs after the chronic ingestion of food made from flour contaminated by the seeds of *Aristolochia clematitis* [28].

When it comes to risk factors, the cumulative dose of AA ingestion has been proven to be correlated with progressive renal dysfunction [25]. According to a survey conducted in China, regular use of nephrotoxic medications (analgesics or AA-containing pills) increased the risk of renal impairment (odds ratio [OR], 2.19) [29], and a cumulative dose of over 0.5-g aristolochic acid I (AA-I) intake was tightly associated with a higher CKD incidence (OR, 5.625) [30]. In recent years, the number of patients with newly diagnosed AAN has reduced sharply because of the warning and strict supervision of AA-containing herbs in many countries, yet sporadic cases are reported occasionally, reminding clinicians to take AA exposure seriously.

**Aristolochic acid: the culprit**

**Herbs containing aristolochic acids**

AAs are found in the plants of the genus *Aristolochia* and *Asarum* belonging to the *Aristolochiaceae* family, which are widely distributed worldwide [31]. Herbal remedies of *Aristolochia* can date back to more than 2,500 years ago in Europe, and at least 1,500 years ago in China [32]. Throughout the long history, AA-containing medications have been utilized to treat various diseases and indications, including eczema, headaches, colds, chronic pain, infections, inflammatory diseases, snake bites, as well as obstetrical and gynecological diseases [31,33–35]. At least seven species of *Aristolochia*, as well as four species of *Asarum*, are used medicinally (Table 1) [36,37]. In the clinical practice of traditional Chinese medicine, dozens of AA-containing herbs have been reported, including Ma Dou Ling (*Aristolochiae Fructus*), Guan Mu Tong (*A. manshuriensis Caulis*), Qing Mu Xiang (*Aristolochiae Rhizoma*), Tian Xian Teng (*A. fangchi Radix*), Guang Fang Ji (*A. fangchi* Radix), Tian Xian Teng (*Aristolochiae Herba*), Xi Xin (*Asari Radix et Rhizoma*), etc. [31,38]. A number of Chinese patent medicines have been tested with the content of AA, some of which were associated with AAN in previously reported cases [31].
Metabolism of aristolochic acids

AA is a generic term for a family of structurally related nitrophenanthrene carboxylic acids [39]. Among all those compounds, AA-I and aristolochic acid II (AA-II) are the most common components extracted from the Aristolochia species (the structures of AA-I and AA-II are shown in Fig. 1) [40]. In human cells, AA-I and AA-II are mainly reduced to aristolactams (ALs), including AL-I, AL-Ia, AL-II, etc. (Fig. 1) [41]. During the metabolic process, AA-I and AA-II are activated to reactive cyclic nitrenium ions with delocalized charge, which then preferentially react with purines in the DNA to form AL-DNA adducts (predominantly dA-AAI and dG-AAI) [39,40,42]. The AL-DNA adducts, mainly located in the renal cortex and urothelium, might give rise to mutations in the TP53 tumor-suppressor gene, leading to urothelial malignancies [40].

The pharmacodynamics of AAs has been studied in animal models. After oral administration or intravenous injection, AAs are promptly absorbed in blood circulation and bind to plasma proteins, and are then distributed throughout the body [43,44]. AAs are first concentrated in the liver, and they are then transferred to the kidneys. The AA-albumin binding components are not filtrated through the glomerulus but flow further through the peritubular

Table 1. Common aristolochic acid-containing herbs in medicinal use

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aristolochia</td>
<td>Aristolochia clematitis L. (known as birthwort, located in Europe)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia serpentaria L. (virginia snakerooot, North America)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia indica L. (Indian birthwort, Asia)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia debilis Sieb. et Zucc. (China)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia bracteolahata Lam. (Africa)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia acuminata Lam (India)</td>
</tr>
<tr>
<td></td>
<td>Aristolochia trilobata L. (Central/South America, Caribbean)</td>
</tr>
<tr>
<td>Asarum</td>
<td>Asarum heteropoides f. mandshuricum (Maxim.) Kitag (China)</td>
</tr>
<tr>
<td></td>
<td>Asarum sieboldii Miq. (China)</td>
</tr>
<tr>
<td></td>
<td>Asarum europaeum L. (Europe)</td>
</tr>
<tr>
<td></td>
<td>Asarum canadense L. (North America)</td>
</tr>
</tbody>
</table>

Aristolochic acid I (AA-I): R=OCH₃
Aristolochic acid II (AA-II): R=H

Figure 1. Metabolism of aristolochic acids in human cells.
capillaries, where AAs are transferred to the nearby proximal tubular epithelial cells (PTECs) via organic anion transporters (OATs) [44–46]. A study demonstrated that the distribution ratio of AA in the liver went down several days later, yet it remained high in the kidneys even after 40 days, implying its accumulation and slow elimination in the kidneys [46]. In humans, AA and its metabolites could be detected in the plasma of approximately half of AAN patients even after over 18 months of AA withdrawal [18]. Taken together, the organ-specific accumulation and the slow elimination of AAs shed light on possible mechanisms of how AAs persistently do harm to the kidneys and ultimately result in chronic AAN.

**Toxicity of aristolochic acids**

Although AA-I is regarded to be responsible for AA-related diseases in previous studies, other members of the AA family, as well as their intermediates in the process of metabolism, also show nephrotoxicity. A few *in vitro* and *in vivo* studies demonstrated that compounds like AL-I, 7-methoxy-AL-IV, AL-Iva, etc., also do harm to PTECs [47–50]. More interestingly, some of them even show much stronger nephrotoxicity than AA-I in several studies [47,49,50]. Structurally, the nitro and methoxy groups play crucial roles in AA-mediated intoxication [51,52]. These findings indicate that some nephrotoxic constituents of the AA family, besides AA-I, might contribute to the pathogenesis of AAN in AA-containing herbs or other species, which remind researchers that more attention and stricter supervision of such compounds are required in the future for the sake of herbal safety.

**Clinical manifestations and pathology**

**Clinical patterns of aristolochic acid nephropathy**

The initial presentation of AAN turns out to be silent, and renal dysfunction is often discovered by routine blood tests [8]. Nonspecific symptoms including nausea, fatigue, poor appetite, and edema occur in some AAN cases [18,22]. According to a previous study conducted by our center including 300 patients diagnosed as AAN between 1997 and 2006 with 2 to 156 months of follow-up, three clinical subtypes were defined [18]: chronic AAN, acute AAN, and renal tubular dysfunction (Table 2).

### Chronic aristolochic acid nephropathy

Over 90% of patients were reported to suffer from chronic AAN with decreased estimated glomerular filtration rate (eGFR) at different degrees, and most of them developed rapid progression to end-stage renal disease (ESRD) (me-
Acute aristolochic acid nephropathy

Approximately 5% of patients were shown to present with nonoliguric acute kidney injury and developed acute or subacute renal failure, which is often caused by continuous or excessive use of Chinese medicine decoctions containing AA in a short period of time.

Renal tubular dysfunction aristolochic acid nephropathy

Less than 3% of patients with intermittent and lowest cumulative AA-I intake showed varying degrees of renal tubular dysfunction or Fanconi syndrome.

In addition, hypertension, elevated serum creatinine, as well as anemia presenting earlier and more severe than anticipated from the progression of renal failure, are usually seen in physical examinations and laboratory tests [53]. Urinalysis is unremarkable in most cases. Mild proteinuria and glycosuria can be detected in some patients [8, 54]. Tubular-derived proteinuria is confirmed by the increased level of five kinds of low molecular weight proteins in urinalysis, including β2-microglobulin, α1-microglobulin, cystatin C, retinal-binding protein, and Clara cell protein [55]. Furthermore, levels of urinary neutral endopeptidase, a 94-kDa ectoenzyme of proximal tubule brush border, decrease significantly in patients with moderate renal failure and are almost undetectable in those with ESRD, indicating the loss of integrity of proximal tubules [56]. Taken together, all these findings imply that proximal tubules are the main target of AA-containing herbs.

Pathology of aristolochic acid nephropathy

Macroscopically, the kidneys of patients with chronic AAN are detected as shrunken and asymmetric, with the thinning of renal parenchyma on ultrasound testing [57]. Microscopically, the renal pathology of AAN often has certain characteristics. The immunopathological examination of renal tissue biopsy is usually negative. In patients taking excessive drugs containing AA, light microscopic examination shows severe injury of tubular epithelial cells similar to acute tubular necrosis, including severe cell degeneration and necrosis or disintegration with naked tubular basement membrane [57, 58]. The lesions appear to be diffuse or multifocal and are characterized by the lack of regeneration of tubular epithelial cells [58]. As for those with long-term intermittent AA consumption, the main pathological feature is extensive paucicellular interstitial fibrosis accompanied by apparent atrophy of proximal tubules, which starts predominantly from the superficial cortex and then progresses to the deep cortex, whereas little infiltration of interstitial inflammatory cells can be observed [2, 57, 59]. The glomeruli remain relatively spared. Furthermore, loss of peritubular capillaries and ischemic shrinkage of glomeruli are detected in some cases [58]. Apart from the lesions mentioned above, the swelling of organelles in the interstitial microvascular endothelial cells as well as the stratification or even rupture of the basement membrane are shown on electron microscopic examination.

Association with urothelial malignancies

Approximately 30% to 40% of AAN patients are accompanied by urinary translational cell carcinoma (TCC), which might be detected before or after the diagnosis of AAN, after over 10-year withdrawal of AA-containing herbs, or even after kidney transplantation [60–62]. Tumors can be observed multifocally throughout the whole urinary system, including the renal pelvis, ureter, and bladder with a high recurrence rate. Visible or invisible hematuria is regarded as the most common initial symptom, and flank pain occurs in about 20% of patients [63]. Abnormal urinary cytology, though not sensitive enough, indicates the existence of TCC [63]. Computed tomography (CT) urography or magnetic resonance urography, as well as invasive examinations, including cystoscopy, ureteroscopy, and simultaneous biopsy, assist with accurate diagnosis and tumor staging [63]. Furthermore, the long-term existence of AA-derived DNA adducts and the TP53 mutation spectra also serve as powerful biomarkers of AA exposure [40, 64].

Pathogenesis of aristolochic acid nephropathy

Breakthroughs on the pathogenesis of AAN have been made in the last few decades. Possible mechanisms of how AAs damage the kidney tissues are concluded as follows (Fig. 2).
Direct nephrotoxicity

AAs exert dose-dependent damage to renal tubules, leading to apoptosis and necrosis of PTECs via p53-mediated signaling [65].

Impairment of cell repair

Under normal circumstances, the tubular epithelial cells have strong capability for self-repair. After renal tubules are injured by nephrotoxic drugs, those spared or slightly injured tubular epithelial cells soon start their self-repair procedures by proliferating. However, renal biopsy shows lack of cell regeneration in patients with AAN, suggesting that AAs might somehow disrupt the self-repair process. Previous studies have demonstrated that exposure to AAs results in epithelial cell cycle arrest in the G2/M phase and reduced expression of epidermal growth factor, yet such inhibition cannot be reversed even after removal of extracellular AAs [66,67].

Chronic hypoxia and ischemic injury

Pathologically, a severe loss of peritubular capillaries (PTCs), as well as disrupted PTC lumina, strongly suggest that the injury of vascular endothelial cells might participate in AAN pathophysiology [58]. Cytotoxicity of AAs, decreased expression of vascular endothelial growth factor, as well as imbalance between the vasoactive factors, are associated with upregulation of hypoxia-inducible factor alpha and reduction of vascular network, illustrating that chronic hypoxia and ischemic injury might partially give rise to interstitial fibrosis and defect of cellular proliferation [58,68–71].

Infiltration of inflammatory cells

Though some cases are characterized by little infiltration of interstitial inflammatory cells, recent studies show that the immune system participates in AAN progression [72]. Innate immune cells, such as monocytes/macrophages, as well as adaptive immune cells, including both T lym-
phocytes and B lymphocytes, are detected in the medullary rays and in the outer medullae [72–74]. Inhibition of immune cell infiltration or suppression of relative inflammatory signaling pathways dramatically attenuates renal fibrosis [72,73,75]. Besides, the fact that steroid therapy might slow down the deterioration of renal dysfunction in AAN patients provides evidence for this immune-related process [76,77].

**Activation of profibrotic signaling**

Overexpression of profibrotic factors and massive production of extracellular matrix deposition are detected, suggesting that activation of profibrotic signaling plays an indispensable role in renal firosis [58,66]. After stimulation of AA, the tubular epithelial cells that were arrested in the G2/M phase tend to transfer to a profibrotic phenotype by excreting transforming growth factor beta 1 (TGF-β1) and connecting tissue growth factor, which further promote the activation of fibroblasts into myofibroblasts, and the production of collagen via TGF-β/Smad3-dependent and JNK/MAP kinase-dependent mechanisms [66,78,79].

**Diagnosis**

No universally accepted diagnostic criteria of AAN have been reached worldwide so far. In clinical practice, the diagnosis of AAN is usually based on the history of AA-containing medication intake, clinical manifestation of renal tubular injury or impaired renal function, and typical renal histopathology displaying hypocellular interstitial fibrosis [25]. Additionally, the detection of AA-derived DNA adducts in patients' renal/urinary tract tissues as well as AA and its metabolites in the blood/urinary samples support AA exposure [64]. Tubulointerstitial diseases caused by other reasons should be evaluated and differentiated from AAN in every suspected patient before a definite diagnosis is made. For patients with complex history of using several nephrotoxic medications (including AA-containing herbs, analgesics, antibiotics, etc.), different drug-induced nephropathies should be discriminated by the combination of clinical course and pathological manifestations.

**Management**

**Treatment and surveillance**

Unfortunately, there is no effective treatment for AAN. Several studies have demonstrated that steroid therapy delayed the progression of renal failure in some cases [76,77], supporting the hypothesis that immune factors could be involved in the progression of AAN [74]. Nevertheless, the long-term effect of steroid therapy on AAN and whether the benefits outweigh the side effects remain ambiguous. Renin-angiotensin system modulation by salt depletion and pharmacologic blockade with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin-receptor blockers are indispensable to managing CKD, yet no evidence supports that this strategy might improve renal function or slow down disease progression in AAN patients [80].

The principles of AAN management are similar to CKD patients of other causes, which include controlling blood pressure, treating complications, preventing infection, and time preparing for renal replacement therapy [25]. Because of the high incidence and recurrence rate of TCC in AAN patients, simultaneous bilateral nephroureterectomy has been recommended when performing renal transplantation surgery or at the start of dialysis [7,25,61]. Since the occurrence of TCC in bladder turns out to be much lower than that in renal pelvis and ureter, routine cystectomy seems unnecessary in most cases [7].

For further surveillance, routine urinary cytologic evaluation should be performed in all patients with AAN, yet more invasive methods are required due to its poor sensitivity. It has been suggested that yearly CT and ureteroscopy should be performed on patients who do not undergo the removal of bilateral native kidneys and ureters, whereas regular cystoscopy and bladder biopsy should be offered to patients every 6 months after nephroureterectomy [25]. If AA-derived DNA adducts are positive in the bladder specimens, which suggests a much higher risk of developing TCC in the bladder, cystectomy should be considered [25].

In recent years, advances have been made in understanding the pathogenesis of AAN, and new therapies and pharmaceutical targets have been explored in animal models. Drugs with the effect of inhibiting OATs can block the entry of AAs into PTECs in animal models, leading to prevention of cellular injury and AA accumulation [81]. A
recent study in rat model indicated that chymase-induced ACE-independent angiotensin II formation participates in kidney injury in AAN, and chymase inhibitor (with or without ACEI) significantly mitigates the progression of AAN, which might be a potential therapeutic target in the future [82]. In addition, the anti-renal fibrosis effects of some traditional Chinese formulas (such as Dahuang Fuzi Decoction [83], Fuzheng Huayu Recipe [84], Kangxianling [85], etc.) have been proven and might provide a viable approach for treating AAN as well as CKD induced by other causes [86]. Furthermore, the anti-fibrotic and regenerative effects of mesenchymal stem cells and their extracellular vesicles have been attested in animal models of AAN [87,88].

**Prognosis**

The prognosis of AAN is worse than tubulointerstitial renal diseases caused by other reasons, with irreversible renal dysfunction in most cases and a much lower 2-year kidney survival rate of only 17% [54]. In the light of follow-up data in our center, cumulative dose of AA ingestion turns out to be the decisive factor for the progression and outcome of AAN patients [18]. A small proportion of cases develop rapidly progressive renal failure to ESRD within a year. Some patients presenting with acute AAN and those with tubular dysfunction AAN might have a partial recovery after cessation of AA-containing medication and timely treatment. However, most patients undergo chronic deterioration of renal function and have to turn to renal replacement therapy after years of progression.

**Prevention**

Due to the nephrotoxicity and carcinogenicity of AAs, prevention of exposure to AAs has become a global public health priority. Regulatory authorities of many countries have sent out warnings against AA-containing remedies, and products with AAs have been banned and restricted in the drug markets. In the United States, the Food and Drug Administration issued an alert about the danger of AAs in 2001 [25]. In Europe, the enforcement of the 2004 European Directive on Traditional Herbal Medicinal Products in 2011 has imposed a ban on AA-containing remedies [25]. The Korea Food and Drug Administration has forbidden the use of AA-containing ingredients since 2005 in Korea [22]. Taiwan and Hong Kong laid embargoes on such herbal medications in 2003 and 2004, respectively. Here in mainland China, the medicinal standards for several species of Aristolochia, including Guan Mu Tong (A. manshuriensis Caulis), Guang Fang Ji (A. fangchi Radix), and Qing Mu Xiang (Aristolochiae Radix), were canceled from the “Chinese Pharmacopoeia” in 2004 [89,90], followed by the cancellation of Ma Dou Ling (Aristolochiae Fructus) and Tian Xian Teng (Aristolochiae Herba) in the latest version released in 2020 [91]. The National Medical Products Administration of China has stressed that the nephrotoxicity of AA-related patent medications must be marked clearly on the labels, and those AA-containing patent drugs and preparations are accessible only under the strict instructions given by licensed professional practitioners of traditional Chinese medicine [90]. Apart from official regulations, public education on the importance of rational use of herbal remedies is quite necessary for improving awareness of drug safety.

It is encouraging that the occurrence of AAN has decreased significantly, illustrating that those measures are quite effective in preventing exposure to AAs. Nevertheless, cases with newly-onset AAN are still occasionally detected nowadays, reminding clinicians not to let down their guard on AAN. Because of the poor prognosis and rapid progression of the AAN, it is important for doctors to identify the disease early and perform timely interventions.

**Conclusion and perspectives**

Since the first report of AA-related nephropathy, a great number of breakthroughs in understanding the pathogenesis of AAN have been made throughout these years. However, there still exist considerable challenges that require further investigation. The lack of universally accepted diagnostic criteria makes it harder for early and accurate diagnoses. Though specific biomarkers like AL-DNA adducts have been found, noninvasive biomarkers for AAN and exposure to AAs should be developed. Furthermore, potential therapeutic targets and remedies for reversing or delaying disease progression should be explored to improve the outcomes of patients with AAN. Lastly, strict regulation on AA-containing medications is required in the future to diminish this preventable disease. As such, there is still a long way to go to defeat AAN completely.
Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 82130021), the Beijing Young Scientist Program (No. BJJWZYJH01201910001006), the CAMS Innovation Fund for Medical Sciences (No. 2019-12M-5-046, 2020-JKCS-009), and the PKU-Baidu Fund (No. 2020BD026, 2020BD044).

Data sharing statement

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

Authors’ contributions

Conceptualization: QZ, GL, LY
Project administration, Supervision, Validation: GL, LY
Visualization: QZ, LJ
Writing–original draft: QZ
Writing–review & editing: LJ, TS, GL, LY
All authors read and approved the final manuscript.

ORCID

Qingqing Zhou, https://orcid.org/0000-0003-3664-5778
Lei Jiang, https://orcid.org/0000-0002-8177-4037
Tao Su, https://orcid.org/0000-0002-6857-8146
Gang Liu, https://orcid.org/0000-0003-1981-2634
Li Yang, https://orcid.org/0000-0002-2528-5087

References

19. Yang CS, Lin CH, Chang SH, Hsu HC. Rapidly progressive fibros-


46. Su T, Qu L, Zhang CL, Cai SQ, Li XM. [Studies on pharmacody-
64. Siborová M, Arlt VM, Schmeiser HH. DNA adducts formed by aristolochic acid are unique biomarkers of exposure and explain the initiation phase of upper urothelial cancer. Int J Mol Sci 2017;18:2144.


Background: Immunoglobulin A nephropathy (IgAN) is the most prevalent form of glomerulonephritis worldwide. Prediction of disease progression in IgAN can help to provide individualized treatment based on accurate risk stratification.

Methods: We performed proton nuclear magnetic resonance-based metabolomics analyses of serum and urine samples from healthy controls, non-progressor (NP), and progressor (P) groups to identify metabolic profiles of IgAN disease progression. Metabolites that were significantly different between the NP and P groups were selected for pathway analysis. Subsequently, we analyzed multivariate area under the receiver operating characteristic (ROC) curves to evaluate the predictive power of metabolites associated with IgAN progression.

Results: We observed several distinct metabolic fingerprints of the P group involving the following metabolic pathways: glycolipid metabolism; valine, leucine, and isoleucine biosynthesis; aminoacyl-transfer RNA biosynthesis; glycine, serine, and threonine metabolism; and glyoxylate and dicarboxylate metabolism. In multivariate ROC analyses, the combinations of serum glycerol, threonine, and proteinuria (area under the curve [AUC], 0.923; 95% confidence interval [CI], 0.667–1.000) and of urinary leucine, valine, and proteinuria (AUC, 0.912; 95% CI, 0.667–1.000) showed the highest discriminatory ability to predict IgAN disease progression.

Conclusion: This study identified serum and urine metabolites profiles that can aid in the identification of progressive IgAN and proposed perturbed metabolic pathways associated with the identified metabolites.

Keywords: Disease progression, IgA nephropathy, Metabolic networks and pathways, Metabolomics

Introduction

Immunoglobulin A nephropathy (IgAN) is the most common primary glomerular disease worldwide and progresses to end-stage kidney disease (ESKD) in approximately 30% of patients [1,2]. Oxford MEST-C classification [3] and International IgAN Prediction Tool [4] have been developed to assess the risk of disease progression in IgAN.

Received: July 7, 2022; Revised: November 9, 2022; Accepted: November 28, 2022

Correspondence: Sang Heon Song
Division of Nephrology, Department of Internal Medicine and Biomedical Research Institute, Pusan National University Hospital, 179 Gudeok-ro, Seo-gu, Busan 49241, Republic of Korea. E-mail: shsong0209@gmail.com
ORCID: https://orcid.org/0000-0002-8218-6974

You Hyun Jeon’s current affiliation: Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea

Copyright © 2023 by The Korean Society of Nephrology
© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial and No Derivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/) which permits unrestricted non-commercial use, distribution of the material without any modifications, and reproduction in any medium, provided the original works properly cited.
Kidney biopsy is mandatory for diagnosis and risk stratification in IgAN. However, there are concerns about the use of remotely performed biopsies in predicting patients’ dynamic clinical courses. Hence, efforts are needed to identify and validate readily available serum or urine biomarkers for prediction of IgAN disease progression with the goal of individualized treatment.

Generally, metabolites are the end products of cell reactions, and metabolomics has been used to obtain important information about the phenotypes of organisms and diseases [5,6]. Emerging high-throughput omics technologies have been applied in recent studies to gain insights into various kidney diseases and to identify diagnostic and predictive risk markers [7–9]. Untargeted metabolomic approaches have been used to discriminate metabolites associated with chronic kidney disease (CKD) progression [10,11], and a recent study identified 13 metabolites associated with progression of diabetic kidney disease (DKD) in the targeted analysis [12].

Several studies using omics techniques have also revealed that metabolites and proteins can serve as potential diagnostic biomarkers of IgAN [13–15]. A few studies have suggested the use of urinary metabolites and proteins identified through metabolomics and proteomics as biomarkers associated with outcomes of IgAN [16,17]. However, these studies had varied definitions and methods for selecting progressive disease groups. Therefore, further metabolomic investigations to clearly identify progressive disease and validate proposed biomarkers are needed.

We hypothesized that metabolites in serum and urine can serve as biomarkers of progressive IgAN as assessed by long-term changes in the estimated glomerular filtration rate (eGFR). Our ultimate goal is to identify biomarkers associated with kidney function deterioration to facilitate the development of minimally invasive risk-based treatment strategies.

**Methods**

**Study subjects**

We screened patients with primary IgAN diagnosed by biopsy at Pusan National University Hospital, between February 2007 and April 2016. Exclusion criteria were age of <20 years, follow-up period less than 5 years, other glomerular diseases identified on kidney biopsy, eGFR less than 30 mL/min/1.73 m², and unavailability of serum or urine samples. To determine the rate of disease progression, the annual eGFR slope was calculated using linear regression. Patients with an eGFR slope below the median and a final eGFR of 60 mL/min/1.73 m² or less were assigned to the progressor (P) group. Age- and sex-matched patients with an eGFR slope above the median were assigned to the non-progressor (NP) group. Subjects in the P group, NP group, and healthy controls were included in the final analysis (n = 10 for all three groups). Baseline clinical characteristics and laboratory results were also collected at the time of kidney biopsy. Healthy controls were living kidney transplantation donors who provided written informed consent and donated biospecimens to the Biobank. Serum and urine samples analyzed in our study had been collected earlier as biomarker research samples for a cohort study involving patients with glomerular kidney disease (No. 1610-003-003). Specimens from patients with IgA nephropathy who fasted after midnight were collected on the day of kidney biopsy. Similarly, samples from kidney donors as healthy controls were collected on the day of nephrectomy (Nx) surgery in the fasted state.

This study was approved by the Institutional Review Board (IRB) of Pusan National University Hospital (No. 2009-007-094). The IRB waived the requirement for informed consent because of the retrospective design of the study. Biospecimens and data used for the healthy control group were provided by the Biobank of Pusan National University Hospital, a member of the Korea Biobank Network.

**Sample preparation for proton nuclear magnetic resonance measurements**

Blood and urine samples from patients were collected before kidney biopsy and centrifuged for 15 minutes at 1,006 ×g and 4 °C. Serum and urine samples were immediately frozen and stored at ~80 °C. For proton nuclear magnetic resonance (^1H-NMR) analysis, frozen serum samples were fully thawed at room temperature. Serum was vortexed and then centrifuged at 12,000 ×g for 10 minutes at 4 °C. Sodium phosphate buffer solution (300 μL) in deuterium oxide (0.075-M NaH₂PO₄) containing 4-mM 3-trimethylsilyl propanic-2,2,3,3-d₄ acid sodium salt (TSP-d₄; Sigma-Aldrich)
as a chemical shift reference and sodium azide (NaN₃) as a preservative were added to 300 μL of supernatant from the serum samples. The resulting 600-μL solutions were adjusted to a pH of 7.4, mixed well, and transferred to 5 mm-NMR tubes for NMR measurement.

Urine samples were thawed and centrifuged using the same methods described above for the serum samples. Sixty microliters of potassium phosphate buffer solution in deuterium oxide at pH 7.4 (1.5-M KH₂PO₄) containing TSP-d₄ as a chemical shift reference and NaN₃ as a preservative were added to 540 μL of urine sample supernatant. The resulting 600-μL solutions were adjusted to a pH of 7.4, mixed well, and transferred to 5 mm-NMR tubes for NMR measurement.

Proton nuclear magnetic resonance spectral acquisition and processing analyses

\(^1\)H-NMR spectral acquisition of serum samples was performed using the Carré–Purcell–Meiboom–Gill (CPMG) pulse sequence with a relaxation delay time of 3 seconds, acquisition time of 3 seconds, and total time of 13 minutes 13 seconds using 128 scans to suppress water and macromolecule signals [18]. Signals were acquired using a 600-MHz Varian NMR spectrometer (Varian, Inc.) at 298 K. Phases and baselines of all \(^1\)H-NMR spectra were corrected manually, and chemical shifts were adjusted with the TSP signal to 0.00 parts per million (ppm) as an internal reference using VnmrJ software of Varian NMR spectrometer systems (Varian, Inc). All spectra with line broadenings of 0.3 Hz were binned into buckets of 0.003 ppm from 0.5 to 9.0 ppm after excluding the region of the water and urea signals (4.55 to 6.4 ppm) to minimize interference due to the variability of water suppression and urea signals, and spectra were normalized in the same manner as described for the serum samples.

Metabolic pathway analysis

Metabolic pathway analysis was performed using the pathway analysis module of MetaboAnalyst 5.0 (https://www.metaboanalyst.ca) for urine and serum metabolites with variable importance in projection (VIP) values of >1.0 in a comparison of the NP and P groups. Pathway analysis was conducted using as follows. Pathway enrichment analysis was performed using the “global test” to assess the statistical significance of the enrichment, and pathway topology was determined using “out-degree centrality” to evaluate the importance of the matched metabolites in identified metabolic pathways [19]. The Kyoto Encyclopedia of Genes and Genome (KEGG) database for Homo sapiens was used as the reference pathway library. Significantly disturbed metabolic pathways were defined as those with a p-value of <0.05 and an impact score of >0.1.

Statistical analysis

Multivariate pattern recognition analysis was performed using unsupervised principal component analysis (PCA) to identify outliers in the dataset; orthogonal partial least squares discriminant analysis (OPLS-DA) with unit variance scaling was the supervised method used to uncover discriminatory variances. Analyses were performed using SIMCA-P 12.0.1 software (Umetrics). Performances of the classification models were assessed using cumulative \(R^2\) and \(Q^2\) values indicating goodness of fit and prediction ability, respectively. Variables responsible for discrimination were identified using VIP in the model, and variables with VIP values of >1.0 were considered significant. To con-
firm the normality of the data sets, the Kolmogorov-Smirnov test was performed (p > 0.05). Significant differences in concentrations between NP and P groups were examined using the Mann-Whitney U test (p < 0.05). These analyses were performed using the IBM SPSS version 25 (IBM Corp.). Multivariate receiver operating characteristic (ROC) curve comparisons between the P and NP groups were performed using MetaboAnalyst 5.0. First, metabolites and clinical variables with high predictability were identified using univariate ROC analysis. We then assessed multicollinearity as measured by variance inflation factors (VIFs) and selected independent variables with VIF of <10. Thereafter, to enhance discriminatory accuracy, metabolites with area under the curve (AUC) above 0.7 were determined using multivariate ROC analysis.

**Results**

**Clinical characteristics of immunoglobulin A nephropathy patients**

Serum and urine samples from 20 IgAN patients were analyzed. Baseline characteristics of the patients are shown in Table 1. Urine protein/creatinine ratio (p = 0.027) was significantly higher in the P group, but there were no significant differences in age, sex, mean blood pressure, hematuria, World Health Organization (WHO) classification, or baseline kidney functions between the P and NP groups. Most patients were prescribed angiotensin II receptor blockers, and 10% of the NP group and 50% of the P group received immunosuppressive therapy with corticosteroid. During the median follow-up period of 6.2 years, the mean annual eGFR slope in the P group was \(-5.75 \pm 2.98 \text{ mL/min/1.73 m}^2/\text{year}\) (p < 0.001), and one patient (5%) developed ESKD. Fig. 1 illustrates the individual eGFR trajec-

### Table 1. Clinical and laboratory data of the study subjects at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (N = 20)</th>
<th>NP group (N = 10)</th>
<th>P group (N = 10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43.9 ± 10.9</td>
<td>46.8 ± 8.4</td>
<td>41.0 ± 12.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Male sex</td>
<td>16</td>
<td>9</td>
<td>7</td>
<td>0.26</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.24 ± 0.40</td>
<td>1.11 ± 0.30</td>
<td>1.37 ± 0.47</td>
<td>0.16</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>75.37 ± 27.93</td>
<td>79.24 ± 21.53</td>
<td>71.49 ± 33.91</td>
<td>0.55</td>
</tr>
<tr>
<td>eGFR slope (mL/min/1.73 m²)</td>
<td>-2.33 ± 4.09</td>
<td>1.08 ± 0.72</td>
<td>-5.75 ± 2.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio (mg/g)</td>
<td>686.6 (421.9–2,046.6)</td>
<td>522.1 (211.5–867.5)</td>
<td>949.0 (563.4–2,003.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hematuria (count/HPF)</td>
<td>0.66</td>
<td>0.66</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>11–29</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>100.0 (95.8–115.5)</td>
<td>102.5 (90.8–110.6)</td>
<td>100 (97.3–121.0)</td>
<td>0.65</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>0.61</td>
<td>0.61</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Use of ARB after kidney biopsy</td>
<td>19</td>
<td>9</td>
<td>10</td>
<td>0.31</td>
</tr>
<tr>
<td>Use of immunosuppressants after kidney biopsy</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>WHO classification</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, number only, or median (interquartile range). ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; HPF, high-power field; NP, non-progressor; P, progressor; WHO, World Health Organization.
Serum metabolic profiling

Serum samples from 10 healthy controls and 20 patients with IgAN were included in the metabolomics analysis. Multivariate analysis findings based on $^1$H-NMR spectroscopy of serum metabolites are shown in Fig. 2. We compared the P and NP groups after identifying metabolic patterns of IgAN that differed from the control group. Un-

Figure 1. eGFR trajectories of the study subjects. (A) The non-progressor (NP) group showed a decrease in kidney function with a mean eGFR slope of 1.08 mL/min/1.73 m$^2$/year. (B) The progressor (P) group showed preservation of kidney function with a mean eGFR slope of ~5.75 mL/min/1.73 m$^2$/year.

eGFR, estimated glomerular filtration rate.
Figure 2. Multivariate analysis of the metabolites of serum samples. (A) Principal component analysis (PCA) score plot of controls vs. patients with IgAN ($R^2_X = 0.515$, $Q^2 = 0.111$). (B) Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot of controls vs. patients with IgAN ($R^2_Y = 0.991$, $Q^2 = 0.770$). (C) PCA score plot of progressor (P) group vs. non-progressor (NP) group ($R^2_X = 0.263$, $Q^2 = 0.057$). (D) OPLS-DA score plot of P group vs. NP group ($R^2_Y = 0.948$, $Q^2 = 0.222$). The ellipse indicates the confidence region.

IgAN, immunoglobulin A nephropathy.

Supervised PCA showed differences in the distributions of serum metabolic profiles between the groups; however, there were some overlaps between clusters (Fig. 2A, C). To distinguish between the groups, OPLS-DA was performed. As shown in Fig. 2B and D, distinct clusters were observed between the groups (control vs. IgAN: $R^2_Y = 0.991$, $Q^2 = 0.770$; P vs. NP: $R^2_Y = 0.948$, $Q^2 = 0.222$). VIP scores, indicating the contributions of metabolites to the discriminant model, were obtained by comparison of the NP and P groups (Table 2), as well as the control and IgAN groups (Supplementary Table 1, available online). To identify potential biomarkers of disease progression, we evaluated common metabolites among variables with VIP score of $>1$ in each comparison group (control vs. IgAN and NP vs. P). Seventeen overlapping metabolites (3-hydroxybutyrate, 3-hydroxyisobutyrate, acetate, acetone, creatinine, formate, glucose, glutamine, glycerol, glycine, hypoxanthine, lysine, phenylalanine, proline, serine, threonine, and valine) were identified. Supplementary Table 2 (available online) provides the list of 43 metabolites identified in serum based on
Urine metabolic profiling

We performed metabolomics analyses of urine samples from healthy controls, NP, and P groups. Multivariate analysis based on 1H-NMR spectroscopy of urine samples was performed in the same manner as described for serum. PCA analyses revealed greater variability in clustering patterns in the IgAN group than in the control group (Fig. 3A). OPLS-DA was performed to identify differences between groups. Score plots from OPLS-DA showed discrimination between different groups (control vs. IgAN: R²Y = 0.971, Q² = 0.458; NP vs. P: R²Y = 0.937, Q² = 0.177) (Fig. 3B, D). The VIP scores of urinary metabolites that differed between the control and IgAN groups are provided in Supplementary Table 3 (available online). Table 3 lists the 20 urinary metabolites with VIP score of >1 obtained from OPLS-DA between the NP and P groups; among these, 18 metabolites were identified in both control vs. IgAN group and NP vs. P group comparisons. In addition, the urinary concentrations of 47 identified metabolites were compared between the P and NP groups using the Mann-Whitney U test (Supplementary Table 4, available online).

Pathway analysis of identified urinary metabolites

The 18 common urinary metabolites with VIP scores of >1.0 among comparison groups (control vs. IgAN and NP vs. P) were selected for pathway analysis. The topology of the pathway in Fig. 5 shows several altered metabolic pathways associated with IgAN progression. Each node represents a metabolic pathway; the darker is the color, the stronger is the impact. Labeled nodes (A–E in Fig. 4) indicate significantly perturbed metabolic pathways (all p < 0.05, impact > 0.1). In the P group, five metabolic pathways were altered compared to the NP group: glycerolipid metabolism; aminoacyl-tRNA biosynthesis; valine, leucine, and isoleucine biosynthesis; glycine, serine, and threonine metabolism; and glyoxylate and dicarboxylate metabolism. Matched serum metabolites and their impact on each pathway are listed in detail in Table 4.

Prediction of disease progression in immunoglobulin A nephropathy

Age, sex, baseline kidney function, proteinuria, and WHO classification were selected for univariate ROC analyses.

### Table 2. VIP values of serum OPLS-DA score plot between non-progressor and progressor groups

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Var ID (Primary, ppm)</th>
<th>VIP value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol*</td>
<td>3.571</td>
<td>2.548</td>
</tr>
<tr>
<td>Glycine*</td>
<td>3.562</td>
<td>2.399</td>
</tr>
<tr>
<td>Threonine*</td>
<td>3.586</td>
<td>2.251</td>
</tr>
<tr>
<td>Phenylalanine*</td>
<td>7.444</td>
<td>2.073</td>
</tr>
<tr>
<td>Proline*</td>
<td>3.341</td>
<td>1.964</td>
</tr>
<tr>
<td>Glucose*</td>
<td>3.526/3.766</td>
<td>1.874</td>
</tr>
<tr>
<td>Lysine*</td>
<td>1.438</td>
<td>1.838</td>
</tr>
<tr>
<td>3-Hydroxybutyrate*</td>
<td>1.207</td>
<td>1.626</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>1.126</td>
<td>1.618</td>
</tr>
<tr>
<td>3-Hydroxyisobutyrate*</td>
<td>1.078</td>
<td>1.547</td>
</tr>
<tr>
<td>Serine*</td>
<td>3.964</td>
<td>1.524</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>2.281</td>
<td>1.444</td>
</tr>
<tr>
<td>Acetone*</td>
<td>2.236</td>
<td>1.360</td>
</tr>
<tr>
<td>Formate*</td>
<td>8.458</td>
<td>1.354</td>
</tr>
<tr>
<td>Creatinine*</td>
<td>3.046/4.051</td>
<td>1.346</td>
</tr>
<tr>
<td>Acetate*</td>
<td>1.221</td>
<td>1.199</td>
</tr>
<tr>
<td>Valine*</td>
<td>2.269/3.610</td>
<td>1.196</td>
</tr>
<tr>
<td>Creatine</td>
<td>3.934</td>
<td>1.161</td>
</tr>
<tr>
<td>Hypoxanthine*</td>
<td>8.206</td>
<td>1.112</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>3.286</td>
<td>1.087</td>
</tr>
<tr>
<td>Glutamine*</td>
<td>2.455</td>
<td>1.067</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.069</td>
<td>1.031</td>
</tr>
</tbody>
</table>

OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection.

*It indicated commonly identified metabolites with those VIP score above 1 in comparison between control and immunoglobulin A nephropathy groups.
IgAN, immunoglobulin A nephropathy.

based on VIF values; among them, only proteinuria was higher than 0.7 in univariate analysis (Supplementary Table 5, available online). The AUC of six serum metabolites (glycerol, threonine, glycine, formate, valine, and acetone) and of urinary leucine and valine was also higher than 0.7. Although adding the identified metabolites to all clinical variables did not provide better predictive power, adding the serum or urinary metabolites to proteinuria improved predictive performance compared to proteinuria alone (Supplementary Table 6, available online). The multivariate prediction model including proteinuria and serum or urinary metabolites with an AUC greater than 0.8 showed the largest improvement in predictive performance (Supplementary Table 6, available online). The AUC for the combination of serum glycerol, threonine, and proteinuria was 0.923 (95% confidence interval [CI], 0.67–1.00) for disease progression of IgAN (Fig. 6B). Sensitivity and specificity were 70% and 90%, respectively. The ROC curve for the combination of urinary leucine, valine, and proteinuria had an AUC of 0.91 (95% CI, 0.67–1.00) with 80% sensitivity.

Figure 3. Multivariate analysis of the metabolites of urine samples. (A) Principal component analysis (PCA) score plot of controls vs. patients with IgAN ($R^2_X = 0.266$, $Q^2 = 0.068$). (B) Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot of controls vs. patients with IgAN ($R^2_Y = 0.971$, $Q^2 = 0.458$). (C) PCA score plot of progressor (P) group vs. non-progressor (NP) group ($R^2_X = 0.327$, $Q^2 = 0.025$). (D) OPLS-DA score plot of P group vs. NP group ($R^2_Y = 0.937$, $Q^2 = 0.177$). The ellipse indicates the confidence region.

Kidney Res Clin Pract 2023;42(5):591-605
and 70% specificity (Fig. 6C).

**Discussion**

In the current study, we identified unique serum and urinary metabolic profiles in the rapidly progressive IgAN group. We identified 22 serum and 20 urinary metabolites that differed between the control and IgAN groups. Among these, 17 serum and 18 urinary metabolites that also showed a significant difference between the NP and P groups were selected, and additional pathway analyses were performed to identify the metabolites associated with IgAN disease progression. Several metabolic pathways associated with IgAN disease progression were identified using integrated metabolomics analyses. The predictability of IgAN progression was most enhanced for the combination of proteinuria with serum glycerol and threonine or urinary leucine and valine.

Table 3. VIP scores of urinary OPLS-DA score plots between non-progressor and progressor groups

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Var ID (Primary, ppm)</th>
<th>VIP value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>0.942</td>
<td>2.697</td>
</tr>
<tr>
<td>Valine</td>
<td>0.978</td>
<td>2.319</td>
</tr>
<tr>
<td>Trigonelline</td>
<td>8.078</td>
<td>2.213</td>
</tr>
<tr>
<td>Formate</td>
<td>8.450</td>
<td>2.206</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.138</td>
<td>2.110</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.382</td>
<td>1.750</td>
</tr>
<tr>
<td>Pseudouridine</td>
<td>4.274</td>
<td>1.690</td>
</tr>
<tr>
<td>Mannitol</td>
<td>3.850</td>
<td>1.619</td>
</tr>
<tr>
<td>Pyroglutamate</td>
<td>2.406</td>
<td>1.518</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.890</td>
<td>1.490</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>7.642</td>
<td>1.372</td>
</tr>
<tr>
<td>Creatinine</td>
<td>3.034</td>
<td>1.354</td>
</tr>
<tr>
<td>3-Hydroxyisovaleric acid</td>
<td>2.354</td>
<td>1.335</td>
</tr>
<tr>
<td>Creatine phosphate</td>
<td>3.942</td>
<td>1.319</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>3.522</td>
<td>1.178</td>
</tr>
<tr>
<td>sn-Glycero-3-phosphocholine</td>
<td>3.218</td>
<td>1.166</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.114</td>
<td>1.153</td>
</tr>
<tr>
<td>N-Phenylacetylglycine</td>
<td>7.346</td>
<td>1.105</td>
</tr>
<tr>
<td>4-Hydroxyphenylacetate</td>
<td>7.158</td>
<td>1.022</td>
</tr>
<tr>
<td>Creatine</td>
<td>3.918</td>
<td>1.002</td>
</tr>
</tbody>
</table>

OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection.

*It indicates commonly identified metabolites with a VIP score above 1 in the comparison between control and immunoglobulin A nephropathy groups.

IgAN has a heterogeneous clinical presentation and natural course, making it difficult for physicians to predict disease progression. Kidney Disease: Improving Global Outcomes (KDIGO) guidelines proposed use of the International IgAN Prediction Tool for risk assessment in patients with primary IgAN [20]. Since this is based on histological and clinical data at the time of kidney biopsy, it is unclear whether dynamic changes during follow-up will be reflected in the initial biopsy. Indeed, in clinical practice, nephrologists are often faced with situations that require reassessment due to an abrupt increase in proteinuria or serum creatinine level. Histological changes may be followed by accumulation of metabolites from the disrupted pathways. Consequently, prognosis may be underestim-
Table 4. Metabolic pathways of serum metabolites associated with progression of IgAN

<table>
<thead>
<tr>
<th>Label</th>
<th>Pathway</th>
<th>Match status</th>
<th>Matched metabolites</th>
<th>p-value</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glycerolipid metabolism</td>
<td>1/16</td>
<td>Glycerol</td>
<td>0.001</td>
<td>0.100</td>
</tr>
<tr>
<td>B</td>
<td>Aminoacyl-tRNA biosynthesis</td>
<td>7/48</td>
<td>Phenylalanine</td>
<td>0.005</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glutamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lysine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Valine, leucine, and isoleucine biosynthesis</td>
<td>2/8</td>
<td>Threonine</td>
<td>0.006</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Glycine, serine, and threonine metabolism</td>
<td>2/33</td>
<td>Glycine</td>
<td>0.007</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Glyoxylate and dicarboxylate metabolism</td>
<td>4/32</td>
<td>Glycine</td>
<td>0.009</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Formate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glutamine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IgAN, immunoglobulin A nephropathy; tRNA, transfer RNA.

Figure 5. Pathway topology of urinary metabolites in disease progression of IgAN. The y-axis represents the -log (p) value of pathway enrichment analysis, and the x-axis represents the pathway impact value of topology analysis. (A) Valine, leucine, and isoleucine biosynthesis. (B) Aminoacyl-transfer RNA biosynthesis. IgAN, immunoglobulin A nephropathy.

Fortress have been made to identify diagnostic and prognostic markers of IgAN through metabolomics [13,14,16]. However, these studies involved diverse patient groups, and the follow-up periods were short. To predict disease progression, it is necessary to clearly distinguish between patients with and without progressive disease. Sui et al. [13] evaluated potential serum biomarkers by comparing 1H-NMR spectroscopy results between low-risk and high-risk IgAN patient groups. They identified IgAN-specific metabolic profiles in both groups in comparison with healthy controls; however, no metabolite differed significantly between the low- and high-risk groups. IgAN patients were classified solely based on histopathology according to the WHO diagnostic system for kidney biopsy [13]. In the present study, long-term clinical course was based on analyses of serum and urine samples at the time of diagnosis, and we were able to avoid the use of additional invasive methods. Furthermore, pathway analyses were performed to elucidate the functional pathways of the metabolic profiles identified using 1H-NMR spectroscopy.

Valine and leucine are branched-chain amino acids (BCAAs) whose serum concentrations have been shown to simultaneously drop as eGFR declines [25,26]. In CKD, metabolic acidosis is considered the main cause of amino acid degradation in skeletal muscles [27]. Previous animal
Table 5. Metabolic pathways of urinary metabolites associated with progression of IgAN

<table>
<thead>
<tr>
<th>Label</th>
<th>Pathway</th>
<th>Match status</th>
<th>Matched metabolites</th>
<th>p-value</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Valine, leucine, and isoleucine biosynthesis</td>
<td>2/8</td>
<td>Leucine</td>
<td>0.03</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Aminoacyl-tRNA biosynthesis</td>
<td>4/48</td>
<td>Glutamine</td>
<td>0.04</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leucine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tyrosine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IgAN, immunoglobulin A nephropathy; tRNA, transfer RNA.

Figure 6. Multivariate ROC curves for prediction of IgAN progression. (A) Using proteinuria alone (n = 20). (B) Using proteinuria and identified serum metabolites, glycerol and threonine (n = 20). (C) Using proteinuria and identified urinary metabolites, leucine and valine (n = 20).

AUC, area under the curve; ROC, receiver operating characteristic; IgAN, immunoglobulin A nephropathy.
experimental studies supporting this hypothesis have revealed 5/6 Nx-induced reductions in the levels of leucine and valine in rats that developed uremia and metabolic acidosis compared to those without metabolic acidosis [28]. Contrarily, another study on amino acid metabolomics reported that serum leucine and valine levels did not differ between patients with CKD stage 2–3 vs. 4–5, although serum hydrogen carbonate (HCO₃⁻) levels were different between the two groups [23]. Interestingly, we found significant group differences in BCAA metabolites even though kidney function was similar in the two groups at the time of diagnosis. This indicates that perturbed metabolic pathways (and not uremia) contribute to disease progression in IgAN. Furthermore, we observed increased urine levels of valine, which may reflect the decreased serum levels (Supplementary Tables 2, 4; available online). Duranton et al. [23] reported that the urinary excretion of valine is associated with proteinuria. Consistent with this, the P group in our study had more proteinuria than the NP group. Potential metabolic pathways involving leucine and valine include BCCA biosynthesis and aminoacyl-tRNA biosynthesis; therefore, impairment of these metabolic pathways may play a role in progression of IgAN associated with proteinuria. Moreover, as urine leucine and valine improved predictive performance in multivariate ROC analysis, they appear to have a prognostic role in disease progression of IgAN.

The results of our metabolomics study revealed significantly increased serum levels of formate alongside altered glyoxylate and dicarboxylate metabolism. Formate is mostly derived from the degradation of serine and the metabolic activities of the intestinal microbiome in humans [29]. In addition, urinary excretion of formate occurs via anion transporters in proximal tubular cells. A metabolomics study in rats showed that the plasma level of formate was significantly increased in a CKD rat model [30]. Those authors proposed that high levels of organic acids, including formate, are associated with metabolic acidosis in CKD rats. As mentioned above, kidney function was similar between groups in our study. Thus, formate may have a pathological role in abnormal metabolic pathways and not simply be produced in response to metabolic acidosis.

Glycine is a simple amino acid biosynthesized from serine. Several decades of in vivo experiments have demonstrated that glycine plays a protective role in the kidney [31–33]. A recent investigation of an Nx rat model revealed that altered glycine metabolism via the gut microbiota is associated with kidney function and hypertension [34]. The authors of that study reported higher serum levels of glycine-conjugated metabolites derived from the gut microbiome in CKD rats. In a metabolomics study involving humans, urinary glycine level was lower in CKD patients [35]. Park et al. [16] performed metabolomics analyses of urine samples from healthy control, patients with IgAN, membranous nephropathy, minimal change disease, and lupus nephritis to identify IgAN-specific biomarkers; they found that patients in the IgAN group had significantly higher urinary glycine level associated with a lower risk of reduced kidney function. However, in our study, there was no significant difference in the urinary concentration of glycine between the P and NP groups. Inconsistent results need to be interpreted in light of the major differences between two studies. These include IgAN patients who were younger and had higher levels of eGFR and proteinuria in the previous study than in our study. In addition, comparisons of the concentration of metabolites between the two studies are complicated because Park et al. [16] reported concentrations of urinary metabolites as creatinine-adjusted concentrations. In the current study, we found that the absolute concentration of serum glycine was significantly lower in the P group than in the NP group and associated with disease progression based on ROC analysis. Although it is unclear if excessive urinary glycine excretion leads to a lower serum concentration of glycine, significant differences in both serum and urinary glycine implicate altered metabolism of glycine in the disease progression of IgAN. Further mechanistic work is required to interrogate the biological role of glycine in IgAN nephropathy. Aminoacyl-tRNA biosynthesis pathway and glyoxylate and dicarboxylate metabolic pathways were identified as disturbed glycine-related metabolic pathways.

Threonine is an essential amino acid and a component of IgA. Previous studies have suggested that threonine is responsible for mucin synthesis and that it regulates the immune response by modulating inflammatory cytokines in animal intestines [36]. In a human study, threonine was identified as a metabolite associated with rapid deterioration of kidney function in CKD patients [11]. Since the only source of threonine is dietary intake, low levels of threonine represent high metabolic consumption of thre-
online in humans assuming a normal diet. In the current study, valine, leucine, and isoleucine biosynthesis; glycine, serine, and threonine metabolism; and aminoacyl-tRNA biosynthesis were identified as altered metabolic pathways associated with threonine. Further research is needed to determine whether the abnormal metabolism of threonine forms the basis of the pathogenesis of progressive IgAN or is a defense mechanism to maintain homeostasis due to progressive disease.

We identified perturbed glycerolipid metabolism as being associated with aggravation of IgAN. Glycerol is considered a nephrotoxic substance that induces oxidative stress and apoptosis in endothelial and tubular epithelial cells and is used for this purpose in acute kidney animal models [37,38]. Interestingly, serum glycerol level was significantly lower in the P group than in the NP group in our study. Compared to healthy controls, the IgAN group had a lower serum glycerol level; this implies that a low glycerol level is a consequence of an altered metabolic pathway rather than a contributing factor to disease progression in IgAN. A recent study using metabolomics and proteomics demonstrated that glycerolipid metabolism was altered in patients with DKD and that glycerol-3-galactoside showed a significant association with development of DKD [39].

In the current study, serum glycerol was significantly associated with disease progression in IgAN, despite having the same altered metabolic pathway as in a previous study on DKD. The addition of serum glycerol and threonine to proteinuria, which is commonly used as a clinical indicator of kidney function deterioration, showed strong predictive performance. Based on the results of multivariate ROC analysis, we suggest that serum glycerol and threonine be used as predictive indicators of IgAN progression.

The present study had several limitations that should be considered. First, we analyzed only a small number of patients. Therefore, unmeasured confounding factors, such as dietary patterns and previous medications, could have affected our findings. Our results should be validated by additional studies with larger number of patients and longitudinal biospecimens. However, our study has methodological utility as we stratified IgAN patients according to prognosis and observed distinctive metabolic fingerprints despite the small number of subjects included. Second, the International IgAN Prediction Tool could not be validated because our data did not include MEST histological score.

Therefore, we could not compare the predictive power of identified metabolites and International IgAN Prediction Tool. Furthermore, the relationships between pathologic findings and identified metabolites were not evaluated. Third, long-term sample storage was unavoidable because of the study design, and the effects of long-term storage on metabolic profiles have not been systematically investigated. Furthermore, because our study investigated only the Korean population, it is not appropriate to generalize the current results to other ethnic groups. Finally, this is the first step in identification of biomarkers of disease progression in IgAN. Untargeted metabolomics can reveal novel and unanticipated metabolic pathway perturbations. However, not all metabolites are indicative of disease progression because of the sensitivity of metabolomics. Further targeted metabolomic studies, external validation in independent cohorts, and mechanistic studies such as in vitro and animal studies are warranted to distinguish which metabolites are true biomarkers responsible for disease progression and to elucidate the mechanism(s) of disease progression.

Collectively, this untargeted metabolomics study identified serum and urinary metabolomics signatures of progressive IgAN that can help predict prognosis early in the clinical course of this disease. Furthermore, we identified impacted metabolic pathways involving the identified metabolites. Despite some of the limitations mentioned above, a strength of our study is the identification of metabolites related to progression of IgAN based on the clinical progression of 5 years or more. Furthermore, given that the metabolic signatures were established based on samples collected at the time of initial diagnosis, we were able to predict IgAN prognosis early in the clinical course. Additional prospective longitudinal and large-scale studies are required to verify that the biomarkers we identified are accurate predictors of IgAN disease progression.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This research was funded by the Biomedical Research Institute of Pusan National University Hospital (grant num-
Acknowledgments

The biospecimens and data used in this study were provided by the Biobank of Pusan National University Hospital, a member of the Korea Biobank Network.

Data sharing statement

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

Authors’ contributions

Conceptualization, Methodology: YHJ, SSB, MH, EYS, SHS
Formal analysis, Data curation: SL, SK
Funding acquisition: YHJ
Investigation: YHJ, DWK, MH, EYS
Supervision: SHS
Writing–original draft: YHJ, SL
Writing–review & editing: SK, SSB, SHS
All authors read and approved the final manuscript.

ORCID

You Hyun Jeon, https://orcid.org/0000-0001-7318-5753
Sujin Lee, https://orcid.org/0000-0001-8487-3981
Da Woon Kim, https://orcid.org/0000-0002-9471-5976
Suhyeun Kim, https://orcid.org/0000-0002-8320-2489
Sun Sik Bae, https://orcid.org/0000-0002-1027-6639
Miyeun Han, https://orcid.org/0000-0001-7304-2496
Eun Young Seong, https://orcid.org/0000-0002-6006-0051
Sang Heon Song, https://orcid.org/0000-0002-8218-6974

References

17. Rudnicki M, Siwy J, Wendt R, et al. Urine proteomics for predic-


Electronic alert outpatient protocol improves the quality of care for the risk of postcontrast acute kidney injury following computed tomography

Seokwoo Park¹²³, Jinyeong Yi¹, Yoon Jin Lee⁵, Eun-Jeong Kwon²³, Giae Yun²³, Jong Cheol Jeong²³, Ho Jun Chin²³, Ki Young Na²³, Sejoong Kim²³⁶

¹Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea
²Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
³Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
⁴Department of Health Science and Technology, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea
⁵Department of Radiology, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
⁶Center for Artificial Intelligence in Healthcare, Seoul National University Bundang Hospital, Seongnam, Republic of Korea

Background: Prevention and diagnosis of postcontrast acute kidney injury (AKI) after contrast-enhanced computed tomography is burdensome in outpatient department. We investigated whether an electronic alert system could improve prevention and diagnosis of postcontrast AKI.

Methods: In March 2018, we launched an electronic alert system that automatically identifies patients with a baseline estimated glomerular filtration rate of < 45 mL/min/1.73 m², provides a prescription of fluid regimen, and recommends a follow-up for serum creatinine measurement. Participants prescribed contrast-enhanced computed tomography at outpatient department before and after the launch of the system were categorized as historical and alert group, respectively. Propensity for the surveillance of postcontrast AKI was compared using logistic regression. Risks of AKI, admission, mortality, and renal replacement therapy were analyzed.

Results: The historical and alert groups included 289 and 309 participants, respectively. The alert group was more likely to be men and take diuretics. The most frequent volume of prophylactic fluid in historical and alert group was 1,000 and 750 mL, respectively. Follow-up for AKI was more common in the alert group (adjusted odds ratio, 6.00; p < 0.001). Among them, incidence of postcontrast AKI was not statistically different. The two groups did not differ in risks of admission, mortality, or renal replacement therapy.

Conclusion: The electronic alert system could assist in the detection of high-risk patients, prevention with reduced fluid volume, and proper diagnosis of postcontrast AKI, while limiting the prescribing clinicians’ burden. Whether the system can improve long-term outcomes remains unclear.

Keywords: Acute kidney injury, Automation, Contrast media, Electronic prescription, Quality of health care
Introduction

Broader use of intravenous contrast media during contrast-enhanced computed tomography (CECT) can increase the incidence of postcontrast acute kidney injury (PCAKI), particularly in patients with known risk factors. Not only is PCAKI after CECT associated with long-term mortality [1,2], but recurrent exposure to contrast may lead to more kidney failure in patients with diminished baseline renal function [3]. In populations where repeated CECT is an integral part of management, such as patients with cancer, appropriate measures to prevent and diagnose PCAKI are required to avoid adverse clinical outcomes, including the progression of chronic kidney disease (CKD), which prevents optimal evaluation and treatment of primary diseases [4].

Although the reported incidences of PCAKI vary widely depending on the specific study results, up to 36.5% is reported in moderate-to-severe CKD and diabetes [5–7]. In the absence of specific treatment, the European Society of Urogenital Radiology 2018 and the American College of Radiology 2018 guideline suggest that preventive hydration is a practical prevention strategy in high-risk patients with reduced estimated glomerular filtration rate (GFR) [8]. However, identification of patients indicated for prophylaxis is often difficult in outpatient settings at tertiary hospitals because attending clinicians ordering CECT, who may not be specialized in the pertinent field (i.e., nephrology or radiology), should be aware of the risk criteria and recommended regimen for intravenous volume administration. Absence of historical records of baseline renal function may also prohibit adequate assessment. Moreover, since the actual examination date of CECT is usually arranged on a different day from the date of order, time/place/personnel for intravenous fluid administration should be planned to match the schedule. Finally, the detection of PCAKI necessitates the measurement of serum creatinine (sCr) and follow-up visits to confirm the cases, which causes considerable inconvenience in outpatient clinics. These obstacles frequently cause insufficient hydration and missed PCAKI diagnosis [9].

With the advancement of artificial intelligence and information technology, clinical decision support systems are being actively developed and applied in various medical fields [10]. Automated alert systems incorporated into hospital information systems can help clinicians avoid inadvertent mistakes and promote multidisciplinary practices. In our hospital, we previously adopted an automated electronic alert system to detect in-hospital acute kidney injury (AKI) and proved that the system along with prompt intervention by nephrologists can help recover from AKI [11].

In this study, we leveraged an electronic alert system for the automatic identification of high-risk patients at the instance of CECT prescription in the outpatient department based on previous records of eGFRs. In addition, the protocolized order of the intravenous fluid replacement regimen was automatically provided to the prescribing clinician. We assessed the quality improvement of practices regarding PCAKI, namely, appropriate prevention, avoidance of underdiagnosis, and better clinical outcomes, after the launch of the system.

Methods

Ethics statement

The study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (No. B-1804-468-306). The requirement for informed consent was waived by the IRB.

Study design and population

This was a retrospective cohort study that compared the two time periods, namely before and after the launch of the electronic alert system on March 1, 2018. CECTs performed in the outpatient department from March 1, 2017, to February 28, 2020, were initially collected. Patients were then categorized into historical or alert cohorts based on the date of prescription. Because the study group categorization should depend on whether the system was working on the date of prescription, the historical and alert cohorts included the CECT prescribed from March 14, 2014, to February 28, 2018, and from March 1, 2018, to February 28, 2020, respectively. Exclusion criteria were as follows (Fig. 1): 1) second prescription or thereafter in cases with repeated prescription during the study period (only the first prescription was evaluated); 2) participants without available baseline sCr values within 6 months; 3) under renal...
replacement therapy (RRT); 4) baseline eGFR of more than 45 mL/min/1.73 m²; 5) heart diseases defined as ventricular hypertrophy, ischemia, or infarct at the last electrocardiography before the index prescription; and 6) previous history of partial or total nephrectomy. Baseline eGFR was calculated from the minimum sCr within 3 or 6 months (6 months, if sCr was unavailable within 3 months) from the date of computed tomography (CT) examination using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [12].

**Electronic alert system**

The alert system consisted of three main features: 1) identification of high-risk patients; 2) recommendation of fluid prescriptions; and 3) detection and follow-up management of PCAKI (Fig. 2).

**Identification**

When CECT is prescribed, the system opens a pop-up window automatically (Supplementary Fig. 1, available online).
if the patient is undergoing RRT or the baseline eGFR is less than 45 mL/min/1.73 m² [13]. This window alerts clinicians regarding the risk of PCAKI. The doctor should choose one of the four options provided to proceed: 1) consultation with the nephrologist; 2) prescription of a pre-set order for prophylactic intravenous fluid and follow-up measurement of sCr; 3) typing in the reason why prophylaxis should be skipped; and 4) canceling the order of CECT.

**Prescription**
Once the doctor decides to conduct prophylaxis, the order set consisting of following lines is automatically provided, which can be revised depending on the patient’s status and the doctor’s clinical decision: 1) normal saline of 250 mL intravenously or 30 minutes to 1 hour (before CECT); 2) normal saline of 500 mL intravenously for 1 to 2 hours (after CECT); 3) sCr, which is to be sampled within 2 to 7 days after CECT; 4) (optional) acetylcysteine 1,200 mg twice a day for 2 days, starting from the afternoon prior to the day when CECT is being scheduled.

**Postcontrast acute kidney injury detection and follow-up**
The patients are instructed to visit centers for blood sampling nearest to the residence, 2 to 7 days after CECT. However, we considered that sampling within 2 weeks of CECT was acceptable for the surveillance of PCAKI for patients’ convenience. The samples are delivered and analyzed in our hospital. The results of sCr determination and PCAKI diagnosis are transmitted to the patient via mobile phone. If the diagnosis of PCAKI is met, patients are further recommended to make another appointment with a nephrologist in our hospital, in addition to the doctor who prescribed CECT. PCAKI was defined as increase in sCr from baseline of more than 0.3 mg/dL or 50%, for this purpose [14].

**Data collection**
Baseline characteristics such as demographic data, department where the index prescription was made, comorbidities, medications, and baseline laboratory values were collected from electronic medical records. Baseline laboratory
values were the most recent measurements taken within 6 months from the date of CT examination. Comorbidities at the time of the index prescription were identified from all available electronic health records using diagnostic codes from the International Classification of Diseases.

**Outcomes**

The primary outcome was the frequency and odds ratio (OR) of follow-up measurement of sCr within 2 weeks, which we considered representative of surveillance for PCAKI. Secondary outcomes were the development and severity of PCAKI, admission within 6 months after CECT, mortality, and RRT. Mortality and RRT were monitored until the time of the event or June 30, 2020, whichever occurred first.

**Statistical analyses**

Baseline characteristics were compared using the chi-square test or Fisher exact test, as appropriate for categorical variables. Continuous variables were compared using the Mann-Whitney U test, as the variables showed a non-normal distribution, as they were tested using the Shapiro-Wilk test. Missing data, including blood urea nitrogen, hemoglobin, albumin, cholesterol, sodium, potassium, total CO₂, and spot urine protein-to-creatinine ratio were imputed by random forests algorithm, using missranger function in ‘missRanger’ package (version 2.1.3). At least five sets of imputed data were generated for multivariable regressions and the results were pooled using ‘mice’ package (version 3.14.0).

The efficacy of the alert protocol was assessed using logistic regression, where the follow-up measurement of sCr was an outcome variable. Univariable and multivariable regression models were constructed.

We determined the risks of PCAKI among the participants with sCr measurements depending on the implementation of the alert system, using logistic regression. Two different definitions of PCAKI were used: 1) at least 0.5 mg/dL or 25% increase in sCr [15]; and 2) at least 0.3 mg/dL or 50% increase in sCr [16]. Severe PCAKI was defined as an ≥50% increase in sCr from the baseline.

The long-term clinical outcomes of admission within 6 months after CECT, mortality, and RRT were investigated for all included participants. Cox regression analyses were performed for mortality and RRT since these were regarded as time-to-event variables.

Subgroup analyses were performed for baseline eGFR of 35 mL/min/1.73 m². The level of eGFR was chosen because the sample sizes of participants with baseline eGFR less than 30 mL/min/1.73 m² were only 37 and 42 for the historical and alert groups, respectively, which were too small to perform statistical testing.

Analyses were performed using the R software (version 4.1.2; R Foundation for Statistical Computing). Statistical significance was set at a two-sided p-value of <0.05.

**Results**

**Study flow**

Fig. 1 illustrates the flowchart of the study. Overall, 598 participants were included in this study. Among them, 291 had sCr values within 2 weeks after CECT, permitting the comparison of the incidence and severity of PCAKI.

**Baseline characteristics**

The baseline characteristics of the historical and alert groups are shown in Table 1. The median age was 76.0 years for both groups, and 32.9% of the participants had diabetes. Baseline eGFRs were 38.8 and 39.2 mL/min/1.73 m². Participants in the alert group were more likely to be male and using diuretics. Otherwise, the two groups showed no significant differences.

**Volume of administered fluid**

Among the four options on the alert window, when clinicians choose ‘Cancel the order’ option, we cannot identify such cases. For the remaining three options, the selection rates were 95.4%, 3.2%, and 1.4%, for ‘Prescribe protoco-lized prophylaxis regimen,’ ‘Proceed with the order after explaining specific reason,’ and ‘Consult with nephrology department, automatically,’ respectively. The volumes of prophylactic hydration are shown in Supplementary Table 1 (available online). Most participants in the alert group were administered 750 mL of isotonic fluid according to the default prescription of the pre-specified protocol, which is
Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Historical</th>
<th>Alert</th>
<th>p-value</th>
<th>No. of cases with missing value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>289</td>
<td>309</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>76.0 (71.0–81.0)</td>
<td>76.0 (70.0–80.0)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>168 (58.1)</td>
<td>209 (67.6)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Department</td>
<td></td>
<td></td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Internal medicine</td>
<td>133 (46.0)</td>
<td>149 (48.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urology</td>
<td>83 (28.7)</td>
<td>82 (26.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic surgery</td>
<td>28 (9.7)</td>
<td>29 (9.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General surgery</td>
<td>28 (9.7)</td>
<td>23 (7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>17 (5.9)</td>
<td>26 (8.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>55 (19.0)</td>
<td>45 (14.6)</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>97 (33.6)</td>
<td>100 (32.4)</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>7 (2.4)</td>
<td>2 (0.6)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>3 (1.0)</td>
<td>0 (0.0)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>9 (3.1)</td>
<td>22 (7.1)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>22 (7.6)</td>
<td>29 (9.4)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>27.1 (23.0–34.2)</td>
<td>27.5 (22.4–33.3)</td>
<td>0.72</td>
<td>21 (7.3) 77 (24.9)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.9 (9.9–12.3)</td>
<td>10.9 (9.9–12.5)</td>
<td>0.82</td>
<td>30 (10.4) 85 (27.5)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.8 (3.4–4.1)</td>
<td>3.7 (3.5–4.1)</td>
<td>0.97</td>
<td>27 (9.3) 79 (25.6)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>148.0 (130.0–168.1)</td>
<td>146.0 (127.0–172.0)</td>
<td>0.67</td>
<td>26 (9.0) 92 (29.8)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>139.6 (137.7–141.0)</td>
<td>139.8 (138.0–141.2)</td>
<td>0.17</td>
<td>46 (15.9) 99 (32.0)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.5 (4.3–4.8)</td>
<td>4.5 (4.3–4.8)</td>
<td>0.85</td>
<td>46 (15.9) 99 (32.0)</td>
</tr>
<tr>
<td>Total CO₂ (mmol/L)</td>
<td>22.4 (20.0–25.0)</td>
<td>23.0 (21.0–25.0)</td>
<td>0.44</td>
<td>95 (32.9) 176 (57.0)</td>
</tr>
<tr>
<td>Spot UPCR (g/g)</td>
<td>0.3 (0.2–0.8)</td>
<td>0.3 (0.2–1.0)</td>
<td>0.95</td>
<td>166 (57.4) 202 (65.4)</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.6 (1.4–1.8)</td>
<td>1.6 (1.5–1.9)</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Baseline eGFR (mL/min/1.73 m²)</td>
<td>38.8 (34.4–41.9)</td>
<td>39.2 (33.4–42.4)</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td></td>
<td></td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>65 (22.5)</td>
<td>78 (25.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–6</td>
<td>128 (44.3)</td>
<td>147 (47.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>96 (33.2)</td>
<td>84 (27.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

NSAID, nonsteroidal anti-inflammatory drugs; BUN, blood urea nitrogen; UPCR, urine protein-to-creatinine; eGFR, estimated glomerular filtration rate.

in the range of the recommended volume per body weight for most patients [17]. In the historical cohort, 1,000 mL of isotonic fluid infusion was the most frequent prescription. The mean infusion volume was significantly lower in the alert group than in the historical group (793 mL vs. 948 mL; p < 0.001 by two-tailed Student t test).

Follow-up measurement of serum creatinine

We investigated whether the alert system could prevent the underdiagnosis of PCAKI using follow-up sCr measurement within 2 weeks as a surrogate outcome. After the launch of the system, the frequency of follow-ups dramatically increased from 29.4% to 66.7%. In multivariable analysis, the alert system led to a significant increase in follow-up measurements after multivariable adjustment (OR, 6.00; 95% confidence interval [CI], 4.00–8.98; p < 0.001).

Clinical outcomes

Among the 291 participants with follow-up sCr values, we determined the incidence of PCAKI according to two different criteria, one by the PCAKI-specific consensus guideline and the other adopted from the KDIGO guideline.
for general forms of AKI (Table 2). Incidences ranged from 15.3% to 22.4% in the historical group and from 14.1% to 18.4% in the alert group. Protocolized prevention with less fluid volume did not show significant differences in the risk of PCAKI using both criteria, when analyzed by univariable (criteria 1: OR, 0.91; 95% CI, 0.45–1.85; p = 0.79; criteria 2: OR, 0.79; 95% CI, 0.42–1.46; p = 0.446) and multivariable (criteria 1: OR, 0.90; 95% CI, 0.40–2.03; p = 0.81; criteria 2: OR, 0.72; 95% CI, 0.35–1.56; p = 0.38) regressions. Similarly, the risk of severe AKI in multivariable analysis was not significantly different between the two groups (OR, 0.44; 95% CI, 0.13–1.48; p = 0.18).

We investigated whether timely diagnosis and management of PCAKI after CECT could improve long-term clinical outcomes. Our protocol encouraged patients with elevated sCr levels fulfilling the PCAKI criteria to visit the nephrology department, where aggravating factors of AKI can be corrected. Since the alert system assisted in more diagnosis of PCAKI than before by facilitating follow-up (Table 2, 3), we tested the association of the alert system with admission, mortality, and RRT (Table 4). We reasoned that more detection and appropriate management might result in better long-term outcomes. Numerically, proportion of patients experiencing the three outcomes were

---

**Table 2. Efficacy of alert system on follow-up measurements of serum creatinine in patients at risk for postcontrast acute kidney injury**

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>No. of event (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical (n = 289)</td>
<td>Alert (n = 309)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 2 wk</td>
<td>85 (29.4)</td>
<td>206 (66.7)</td>
<td>4.80 (3.39–6.79)</td>
<td>&lt;0.001</td>
<td>6.00 (4.00–8.98)</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio.

*Adjusted for all the baseline variables in Table 1 (i.e., age, sex, department, cardiovascular diseases, diabetes, lymphoma, multiple myeloma, diuretics, nonsteroidal anti-inflammatory drugs, blood urea nitrogen, hemoglobin, albumin, cholesterol, sodium, potassium, total CO₂, spot urine protein-to-creatinine ratio, baseline estimated glomerular filtration rate, and Charlson comorbidity index).

**Table 3. Risks of postcontrast AKI in subpopulation with follow-up sCr within 2 weeks after contrast-enhanced computed tomography**

<table>
<thead>
<tr>
<th>AKI criteria</th>
<th>No. of event (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical (n = 85)</td>
<td>Alert (n = 206)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKI criteria 1*</td>
<td>13 (15.3)</td>
<td>29 (14.1)</td>
<td>0.91 (0.45–1.85)</td>
<td>0.79</td>
<td>0.90 (0.40–2.03)</td>
</tr>
<tr>
<td>AKI criteria 2b</td>
<td>19 (22.4)</td>
<td>38 (18.4)</td>
<td>0.79 (0.42–1.46)</td>
<td>0.45</td>
<td>0.72 (0.35–1.50)</td>
</tr>
<tr>
<td>Severe AKId</td>
<td>9 (10.6)</td>
<td>11 (5.3)</td>
<td>0.48 (0.19–1.20)</td>
<td>0.12</td>
<td>0.44 (0.13–1.48)</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; CI, confidence interval; OR, odds ratio; sCr, serum creatinine.

*Increase in sCr from baseline within 2 weeks after computed tomography by either one of the following criteria: 1) 0.5 mg/dL or more, or 2) 25% or more. 
*Increase in sCr from baseline within 2 weeks after computed tomography by either one of the following criteria: 1) 0.3 mg/dL or more, or 2) 50% or more. 
*Increase in sCr from baseline within 2 weeks after computed tomography by the following criteria: 50% or more. 
*Adjusted for all the baseline variables in Table 1 (i.e., age, sex, department, cardiovascular diseases, diabetes, lymphoma, multiple myeloma, diuretics, nonsteroidal anti-inflammatory drugs, blood urea nitrogen, hemoglobin, albumin, cholesterol, sodium, potassium, total CO₂, spot urine protein-to-creatinine ratio, baseline estimated glomerular filtration rate, and Charlson comorbidity index).

**Table 4. Risks of long-term clinical outcomes before and after the application of alert system**

<table>
<thead>
<tr>
<th>Event</th>
<th>No. of event (%)</th>
<th>OR/HR (95% CI)</th>
<th>p-value</th>
<th>OR/HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical (n = 289)</td>
<td>Alert (n = 309)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission within 6 mo*</td>
<td>106 (36.7)</td>
<td>110 (35.6)</td>
<td>0.95 (0.68–1.33)</td>
<td>0.78</td>
<td>0.84 (0.55–1.28)</td>
</tr>
<tr>
<td>Mortality</td>
<td>22 (7.6)</td>
<td>13 (4.2)</td>
<td>0.84 (0.40–1.75)</td>
<td>0.63</td>
<td>1.08 (0.41–2.81)</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>14 (4.8)</td>
<td>6 (1.9)</td>
<td>0.66 (0.22–1.95)</td>
<td>0.43</td>
<td>0.17 (6.11e–84–4.92e4)</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio; OR, odds ratio.

*Admission for any causes within 6 months after computed tomography. ORs and 95% CIs were described for admission within 6 months. HRs and 95% CIs were described for mortality and renal replacement therapy.

*Adjusted for all the baseline variables in Table 1 (i.e., age, sex, department, cardiovascular diseases, diabetes, lymphoma, multiple myeloma, diuretics, nonsteroidal anti-inflammatory drugs, blood urea nitrogen, hemoglobin, albumin, cholesterol, sodium, potassium, total CO₂, spot urine protein-to-creatinine ratio, baseline estimated glomerular filtration rate, and Charlson comorbidity index).
reduced in the alert group by 1.1%, 3.4%, and 2.9%, respectively, albeit not statistically significant by two-tailed proportion test (data not shown). In univariable Cox regression analyses, the alert system tended to reduce the risk/hazards of admission (OR, 0.95; 95% CI, 0.68–1.33; p = 0.78), mortality (hazard ratio [HR], 0.84; 95% CI, 0.40–1.75; p = 0.63), and RRT (HR, 0.66; 95% CI, 0.22–1.95; p = 0.43), but the difference was not statistically significant. The results were similar to those of the multivariable analyses (Table 4).

Subgroup analyses

Additional analyses showed that the alert system was effective in supporting follow-up of sCr for both subgroups, with <35 or ≥35 mL/min/1.73 m² of baseline eGFR (Supplementary Table 2, available online). Regardless of the baseline eGFR, the alert system did not demonstrate a statistically significant benefit in admission, mortality, and RRT.

Discussion

In this study, we implemented a one-click electronic alert for PCAKI, which runs through the detection of at-risk patients, support for prophylaxis prescription, diagnosis, and referral to nephrologists. The system was developed with the intent of aiding clinicians prescribing CECT in the outpatient department and other personnel at a tertiary hospital. Automated alerts could significantly alter clinicians’ behavior, as more patients underwent surveillance of PCAKI. A more restricted volume of fluid infusion resulted in equivalent PCAKI incidence, and we observed a trend toward lower risks of long-term outcomes with the alert system, although this was not formally proven by statistical analyses.

Alerts supporting clinical decisions are increasingly adopted in-hospital information systems [10]. Several alerts aimed at early detection of AKI have been introduced in inpatient settings, with mixed results regarding their effectiveness on long-term endpoints [11,18,19]. One explanation for those alerts that could not improve the hard endpoints in previous studies was the absence of a universal treatment for AKI after it had already developed. Moreover, those alerts typically detect sCr elevation or a decrease in urine output from any cause, resulting in a heterogeneous study population. In contrast, the alert system in this study involves screening high-risk patients and preventing the development of PCAKI. Although the specific type or volume of intravenous fluid may be individualized, we could also provide attending clinicians with a common protocol for PCAKI prophylaxis based on clinical guidelines [8,20]. This could reduce alert fatigues, which many contemporary hospital information systems accompanying diverse electronic alerts are faced with. Another strength of our study was that, unlike previous studies, we targeted the outpatient population, which is usually difficult to follow. The result was that approximately two-thirds of patients underwent surveillance for PCAKI, which was more than double the follow-up rate from the historical group, suggesting acceptable user accommodation.

Reported incidences of PCAKI after CECT range widely from 0% to 21% depending on study designs, where outpatient data are usually from studies with small sample sizes [4,21]. In our study, the incidence of PCAKI was approximately 15% and did not demonstrate significant differences between the groups. The alert system helped providing the true rate of PCAKI in the outpatient setting, where many cases could be otherwise undetected due to omitted follow-up. Notably, severe PCAKI showed a greater tendency toward a reduced OR in the alert group without statistical significance. Since incidences could be measured only among patients with follow-up sCr, the limited sample size may have reduced the statistical power. Alternatively, selection bias could have been present, as patients in the alert group were more willing to participate in the follow-up sampling.

Importantly, we successfully restricted the volume of prophylactic fluid with equivalent AKI outcomes. In the outpatient clinic, the fluid infusion should be completed in a relatively short time, so reducing the volume of several hundred milliliters can be a great help by saving several hours and avoiding adverse effects due to rapid infusion. Volume overload easily complicates AKI, and excessive amount of intravenous fluid may cause deleterious effect [22]. It is suspected that previous AKI alerts that failed to improve patient outcomes provoked indiscriminate prescription of intravenous fluid upon detection of AKI causing harmful effects [18,19]. Thus, a reduction in the infusion volume minimizes the potential harm incurred by the alert protocol. Optimal volume and infusion rate to prevent PCAKI in the outpatient setting needs further investigation.
The alert system did not ultimately improve long-term clinical outcomes, including admission within 6 months, mortality, and RRT. First, this might be due to insufficient sample size because all results of the regression analyses showed a propensity to lower risk in the alert group, although this is merely a speculation. Because of the retrospective nature of the study, we could not determine the sample size in advance. Second, although we recommended appointments with nephrologist for patients diagnosed with PCAKI, the proportion of patients who visited the nephrology department was not satisfactory because it incurred excess medical expenses and time for the patients. Third, the association between CECT and adverse long-term outcomes may not be present. This issue has been explored in previous studies with inconclusive results. Experience of PCAKI or more frequent CECT was a significant risk factor for mortality and kidney failure in some studies [3,5], but others reported no difference in 30-day mortality or RRT following CECT [23,24]. Lastly, incomplete follow-up in one-third of the participants, still high even after considerable improvement, in the alert group may have weakened the efficacy of the intervention.

This study has several limitations. First, since the time periods when CECTs were performed in the two groups were different, the effects of secular differences in clinical practice could be present. However, the volume expansion protocol for outpatients was published in 2010 [25], and other preventive strategies, such as acetylcysteine and statins, have not been proven effective. Thus, the paradigms of prevention and treatment of PCAKI did not change considerably during the study period [26]. Second, the time window used for the diagnosis of PCAKI was 2 weeks after CECT, considering the convenience of patients who should visit clinics for sampling. Thus, some PCAKI cases that spontaneously improved within a few days could have been missed if sampling was performed later, although the overall impact of these recovery cases on the long-term prognosis might be rather weak. Moreover, causality between increases in sCr and contrast exposure cannot be proven because changes in sCr could occur for other reasons [27]. Third, since the study was carried out in a tertiary hospital, the results may not be generalizable without further research, preferably with a larger sample size. Lastly, specific treatment for PCAKI is limited, so the utility of the alert with respect to early detection may be attenuated. Previous studies have showed that early intervention by nephrologists is associated with better outcomes in AKI [28,29], and is a key determinant of prognostic benefit in in-hospital AKI alerts [11]. Thus, future research may provide evidence that the management of PCAKI by specialists, such as volume expansion or discontinuation of nephrotoxic drugs, can improve patient outcomes.

In conclusion, electronic alerts for PCAKI can be useful in automatic screening of high-risk patients, convenient prescription of protocolized fluid regimens, and follow-up of PCAKI at the outpatient department. Effective prevention of PCAKI is possible with a reduced fluid volume and desirable user acceptance. The clinical benefits of preventing PCAKI development and its long-term adverse outcomes need to be clarified.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

This research was supported by a grant of Patient-Centered Clinical Research Coordinating Center (PACEN) funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI19C0481, HC20C0054).

Data sharing statement

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

Authors’ contributions

Conceptualization: SK
Investigation, Data curation: SP, JY, YJL
Formal analysis: SP, JY, SK
Interpretation of data: SP, EJK, GY, JCJ, HJC, KYN, SK
Supervision: JCJ, HJC, KYN, SK
Writing–original draft: SP, SK
Writing–review & editing: SP, YJL, EJK, GY, JCJ, HJC, KYN, SK
All authors read and approved the final version of the manuscript.


Baseline characteristics of the Korean genetic cohort of inherited cystic kidney disease

Jeong Min Cho¹, Hayne Cho Park²–⁵, Jin Woo Lee¹, Hyunjin Ryu¹, Yong Chul Kim¹, Curie Ahn⁶, Kyu-Beck Lee⁶, Yeong Hoon Kim⁶, Seungyeup Han⁷, Yaerim Kim⁷, Eun Hui Bae⁸, Hee Gyung Kang⁹, Eujin Park¹⁰, Kyungjo Jeong¹¹, Seoon Kang¹¹, Jungmin Choi¹¹, Kook-Hwan Oh¹ and Yun Kyu Oh¹²

For further information on the authors’ affiliations, see Additional information.

Background: Identifying genetic mutations in individuals with inherited cystic kidney disease is necessary for precise treatment. We aimed to elucidate the genetic characteristics of cystic kidney disease in the Korean population.

Methods: We conducted a 3-year prospective, multicenter cohort study at eight hospitals from May 2019 to May 2022. Patients with more than three renal cysts were enrolled and classified into two categories, typical autosomal dominant polycystic kidney disease (ADPKD) and atypical PKD. We identified the clinical characteristics and performed a genetic analysis using a targeted gene panel.

Results: A total of 725 adult patients were included in the study, of which 560 (77.2%) were diagnosed with typical ADPKD and 165 (22.8%) had atypical PKD. Among the typical ADPKD cases, the Mayo imaging classification was as follows: 1A (55, 9.9%), 1B (149, 26.6%), 1C (198, 35.8%), 1D (90, 16.3%), and 1E (61, 11.0%). The atypical PKD cases were classified as bilateral cystic with bilateral atrophic (31, 37.3%), lopsided (27, 32.5%), unilateral (nine, 10.8%), segmental (eight, 9.6%), bilateral cystic with unilateral atrophic (seven, 8.4%), and asymmetric (one, 1.2%). Pathogenic variants were found in 64.3% of the patients using the ciliopathy-related targeted gene panel. The typical ADPKD group demonstrated a higher discovery rate (62.3%) than the atypical PKD group (41.8%).

Conclusion: We present a nationwide genetic cohort’s baseline clinical and genetic characteristics for Korean cystic kidney disease.

Keywords: Autosomal dominant polycystic kidney, Clinical epidemiology, Cystic kidney disease, Epidemiology, Polycystic kidney diseases

Introduction

Inherited cystic kidney disease is a heterogeneous group of diseases caused by mutations in genes involved in the cilium-centrosome complex, leading to cilium dysfunction and the development of kidney cysts of various sizes [1]. The disease spectrum of cystic kidney disease includes autosomal dominant polycystic kidney disease (ADPKD), nephronophthisis, autosomal recessive polycystic kidney disease, tuberous sclerosis complex, and autosomal dominant tubule-interstitial kidney disease [1]. Currently, more than 100 genes are known to be involved in kidney cystogenesis [2].

Among the various disease entities, ADPKD stands as the most prevalent inherited cystic kidney disease [3–5]. Clinical diagnosis of ADPKD typically relies on family history.
and kidney imaging [6,7], but accurately diagnosing patients with mild phenotypes or late-onset symptoms presents a significant challenge [8]. Genetic testing has greatly improved diagnostic accuracy [9], and recent research on genotype-phenotype correlations in ADPKD has yielded valuable insights into prognostic prediction [10–12].

However, despite ADPKD being caused by a single gene, it displays significant variability in both renal and extra-renal manifestations, which can be attributed to the presence of multiple variants within the disease-causing genes [13]. These challenges are further compounded by other gene-associated factors, such as the intricate nature of the PKD1 gene [14,15], the high allelic heterogeneity of both the PKD1 and PKD2 genes, and genotype-phenotype discrepancies. Consequently, achieving an accurate diagnosis of ADPKD remains a complex task [8]. In addition, there is a lack of research specifically focused on atypical ADPKD cases that do not have typical imaging features or family history [16]. Therefore, conducting a comprehensive genetic analysis linked to clinical data within a well-established cystic kidney disease cohort is essential. This approach will help identify specific genetic variants, establish associations between genotypes and phenotypes, and develop a precise diagnostic protocol that guides appropriate treatment planning.

We conducted a 3-year prospective, multicenter, nationwide cohort study of Korean cystic kidney disease to establish a comprehensive database of the disease and identify its genetic profiles. The primary focus of this study is to present the baseline clinical and genetic characteristics of the Korean cystic kidney disease cohort.

**Methods**

**Study design**

This 3-year prospective, multicenter study was designed to establish a cohort of Korean patients with cystic kidney disease. It aimed to develop an individualized genetic analysis protocol for each patient (Clinical Research Service: KCT0005580). The study design for the cohort was previously published [16].

**Study population**

Participants aged 18 years and older, presenting with three or more renal cysts in either or both kidneys, were registered between May 2019 and May 2021. The enrollment process took place in eight medical centers. Cases involving simple renal cysts or acquired cystic kidney disease resulting from kidney failure were excluded from the study. Patients who passed away or withdrew their consent were also excluded from the analysis. The participants were categorized into two groups: typical ADPKD and atypical polycystic kidney disease (PKD). Typical ADPKD cases were characterized by bilateral and diffuse distribution of cysts, resulting in the replacement of kidney tissue, and were identified based on the Pei-Ravine criteria. Patients displaying features not aligning with the typical radiological presentation were classified as having atypical PKD. In particular, within the atypical PKD group, further classification was performed, resulting in two distinct subclasses: 1) subclass 2A included cases with unilateral, segmental, asymmetric, or lopsided imaging findings, and 2) subclass 2B encompassed cases with bilateral cystic manifestation accompanied by either unilateral atrophy or bilateral kidney atrophy. Additionally, patients with typical radiological features but lacked a family history were classified as having clinically atypical PKD.

**Data collection**

Demographic data, including age, gender, height, and weight, were collected. The medical history of hypertension, diabetes mellitus, cardiovascular diseases, cerebrovascular accidents, malignancy, liver diseases, and complications associated with cystic kidney disease were obtained through patient interviews or by reviewing electronic medical records. The definitions of comorbidities and relevant drugs can be found in Supplementary Table 1, 2 (available online). Systolic and diastolic blood pressure measurements were taken, and laboratory analyses were conducted based on blood and urine sample test results. Kidney function was evaluated using the estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The stage of chronic kidney disease was defined according to the Kidney Disease Improving Global Outcome (KDI-
GO) serum creatinine classification. Non-enhanced kidney computed tomography (CT) scans were performed to classify patients as having typical or atypical cystic kidney disease. Genetic analysis was conducted using an 89-gene panel designed to detect pathogenic variants associated with ciliopathies, including polycystic kidneys and liver. The composition of the gene panel was described in our previous publication [16].

Assessment of volumetry and Mayo imaging classification

The assessment of well-established prognostic factors for ADPKD, including total kidney volume (TKV), height-adjusted TKV (htTKV), and Mayo imaging classification (MIC), was conducted [17,18]. TKV and total liver volume (TLV) were measured using both the ellipsoid and stereological volume equations [19]. For the stereological equation, kidney CT images were carefully screened to ensure complete coverage of both the kidneys and liver. The images were then reconstructed into 5-mm sections for axial images and 3-mm sections for coronal and sagittal sections. TKV and TLV were measured by a trained radiologist using the semiautomatic volumetry software ImageJ ver. 1.5a (https://imagej.nih.gov/ij/). Given the high correlation between the two volume measurement methods (Pearson correlation coefficient $r = 0.952$ [0.969–0.980], $p < 0.001$; unpublished data), stereologically calculated TKV and TLV values were analyzed. The htTKV was calculated by dividing the TKV by the patient’s height. Using MIC, patients with typical ADPKD were stratified into five subclasses (1A–1E) based on the yearly increase in htTKV estimated using baseline htTKV measurements and age-specific htTKV limits. The subclasses were defined as 1A for a yearly increase of $<1.5\%$, 1B for 1.5%–3%, 1C for 3%–4.5%, 1D for 4.5%–6%, and 1E for $>6\%$. In atypical PKD, patients were stratified into two subclasses: 2A (unilateral, asymmetric, segmental, lopsided) and 2B (bilateral cystic with unilateral atrophy, bilateral cystic with bilateral atrophy).

Variant prioritization

Variants were prioritized based on several criteria for predicted deleteriousness and rarity.

Class 1: Loss-of-Function (LoF) variants, including canonical splice site, frameshift insertion, frameshift deletion, and stop gain variants. Additionally, missense variants annotated as “(Likely) Pathogenic” for PKD-related phenotypes in ClinVar, polycystic kidney disease database, Rheinisch-Westfälische Technische Hochschule Aachen University’s Datenbank, or annotated as “DM” (Damaging) for PKD-related phenotypes in Human Gene Mutation Database (HGMD).

Class 2: LoF and missense variants predicted as “deleterious” by MetaSVM (Dmis). These variants are either annotated as “DM” in the HGMD for indirect relevance to PKD phenotypes, “DM?” for PKD-related phenotypes in HGMD or have a minor allele frequency (MAF) of $<2 \times 10^{-5}$ in both gnomAD and BRAVO when not reported in HGMD. Additionally, missense variants predicted as “tolerated” by MetaSVM (Tmis) are included if they are annotated as “DM” for indirect relevance to PKD phenotypes in HGMD, “DM?” for PKD-related phenotypes in HGMD while meeting additional deleteriousness criteria (CADD $\geq 20$ and REVEL $\geq 0.75$), or not reported in clinical databases in addition to any population database.

Class 3A: LoF and damaging missense (Dmis) variants with a MAF of $\leq 1 \times 10^{-3}$ ($1 \times 10^{-2}$ for homozygotes) in East Asian populations from gnomAD. Additionally, Tmis variants were annotated as “DM?” for PKD-related phenotypes in HGMD.

Class 3B: LoF or Dmis variants with a MAF of $\leq 1 \times 10^{-3}$ ($1 \times 10^{-2}$ for homozygotes) in East Asian populations from gnomAD and Tmis variants that do not meet the classification criteria for class 3A.

Class 4: LoF and missense variants annotated as “(Likely) benign” or “Likely neutral” for PKD-related phenotypes in clinical databases.

Statistical analyses

Statistical analyses were conducted using R version 4.2.1 (R Foundation for Statistical Computing). Continuous variables with a normal distribution were reported as mean ± standard deviation, while variables with a skewed distribution were reported as median (interquartile range [IQR]). Categorical variables were presented as frequencies. Baseline characteristics were compared between the typical ADPKD and atypical PKD groups using the independent t tests for continuous variables and the chi-square tests for categorical variables. A p-value of $<0.05$ was considered
**Results**

**Study population**

A total of 751 adults over 18 years old with three or more kidney cysts in either or both kidneys were included in the study conducted from May 2019 to May 2021. Twenty-six patients who died or withdrew their consent were excluded from the analysis. Ultimately, 725 individuals were enrolled in the study. Among them, 560 (77.2%) were classified as typical ADPKD cases, while 165 (22.8%) were classified as atypical PKD cases (Fig. 1).

**Demographic and clinical characteristics**

The mean age of the study population was 46.2 ± 14.0 years, with 48.4% being male. The average age at PKD diagnosis was 37.1 ± 13.1 years. Out of the study participants, 479 (66.7%) reported never smoking, 87 (12.1%) were current smokers, and 152 (21.2%) were former smokers. A history of drinking was reported by 338 participants (47.0%). The mean eGFR was 76.5 ± 32.9 mL/min/1.73 m², and the mean serum creatinine level was 1.3 ± 1.4 mg/dL. The median (IQR) urine protein/creatinine ratio was 0.11 g/g (0.09–0.31 g/g).

The mean ages of the typical ADPKD and atypical PKD groups were 45.3 ± 13.3 years and 48.9 ± 15.8 years, respectively, indicating a significantly younger age in the typical group (p = 0.003). The age at PKD diagnosis was also lower in the typical group (36.5 ± 12.4 years) compared to the atypical group (41.8 ± 14.6 years; p < 0.001). Blood urea nitrogen (BUN) levels were higher in the typical group (p = 0.03), while high-density lipoprotein (HDL) cholesterol was higher in the atypical group (p = 0.02). No significant differences were found in either serum creatinine or eGFR between the groups. Comorbidities, including diabetes mellitus, cerebrovascular disease, cardiovascular disease, malignancy, and liver disease, did not differ significantly between the groups. **Table 1** presents the demographic and clinical characteristics.

**Volumetry and Mayo imaging classification profiles**

Out of the total population, the median htTKV was 1,161.0 mL/m (IQR, 631.2–1,895.7 mL/m) and the median htTLV was 1,460.0 mL/m (IQR, 1,232.0–1,888.0 mL/m). The median htTKV of the typical ADPKD group (1,338.0 mL/m [IQR, 749.2–2,113.2 mL/m]) was significantly higher than that of the atypical group (651.5 mL/m [IQR, 408.0–1,013.8 mL/m]; p < 0.001). However, there was no significant difference in

---

**Figure 1. Study flowchart.**

ADPKD, autosomal dominant polycystic kidney disease; PKD, polycystic kidney disease.
Table 1. Characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Typical ADPKD group</th>
<th>Atypical PKD group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>725</td>
<td>560</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>351 (48.4)</td>
<td>267 (48.1)</td>
<td>84 (51.5)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.2 ± 14.0</td>
<td>45.3 ± 13.3</td>
<td>48.9 ± 15.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.3 ± 17.9</td>
<td>166.2 ± 9.5</td>
<td>165.3 ± 9.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.0 ± 17.5</td>
<td>67.4 ± 13.8</td>
<td>66.2 ± 13.5</td>
<td>0.34</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 ± 4.0</td>
<td>24.2 ± 3.7</td>
<td>24.1 ± 3.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>479 (66.7)</td>
<td>375 (67.7)</td>
<td>103 (63.2)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>87 (12.1)</td>
<td>64 (11.6)</td>
<td>23 (14.1)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>152 (21.2)</td>
<td>115 (20.8)</td>
<td>37 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td>338 (47.0)</td>
<td>257 (46.4)</td>
<td>81 (49.7)</td>
<td>0.51</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.3 ± 1.4</td>
<td>1.4 ± 1.3</td>
<td>1.35 ± 1.5</td>
<td>0.76</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>76.5 ± 32.9</td>
<td>75.6 ± 33.2</td>
<td>79.5 ± 32.0</td>
<td>0.19</td>
</tr>
<tr>
<td>CKD stage</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (90 ≤ eGFR)</td>
<td>304 (42.5)</td>
<td>232 (42.0)</td>
<td>2 (2.2)</td>
<td></td>
</tr>
<tr>
<td>2 (60 ≤ eGFR &lt; 90)</td>
<td>191 (26.7)</td>
<td>141 (25.5)</td>
<td>50 (45.3)</td>
<td></td>
</tr>
<tr>
<td>3a (45 ≤ eGFR &lt; 60)</td>
<td>74 (10.3)</td>
<td>61 (11.0)</td>
<td>12 (13.0)</td>
<td></td>
</tr>
<tr>
<td>3b (30 ≤ eGFR &lt; 45)</td>
<td>63 (8.8)</td>
<td>51 (9.2)</td>
<td>12 (13.0)</td>
<td></td>
</tr>
<tr>
<td>4 (15 ≤ eGFR &lt; 30)</td>
<td>52 (7.3)</td>
<td>43 (7.8)</td>
<td>9 (9.8)</td>
<td></td>
</tr>
<tr>
<td>5 (eGFR &lt; 15)</td>
<td>32 (4.5)</td>
<td>25 (4.5)</td>
<td>7 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.3 ± 1.7</td>
<td>13.3 ± 1.7</td>
<td>13.4 ± 1.9</td>
<td>0.37</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>20.1 ± 13.6</td>
<td>20.8 ± 14.0</td>
<td>18.2 ± 10.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.5 ± 1.6</td>
<td>5.6 ± 1.6</td>
<td>5.6 ± 1.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>174.7 ± 37.7</td>
<td>175.2 ± 39.6</td>
<td>176.1 ± 35.2</td>
<td>0.35</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>86.5 ± 46.7</td>
<td>102.2 ± 30.8</td>
<td>104.5 ± 31.3</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>44.8 ± 23.8</td>
<td>53.2 ± 14.3</td>
<td>54.4 ± 14.7</td>
<td>0.02</td>
</tr>
<tr>
<td>UPCR (g/g)</td>
<td>0.11 (0.07–0.23)</td>
<td>0.12 (0.07–0.34)</td>
<td>0.09 (0.06–0.22)</td>
<td>0.45</td>
</tr>
<tr>
<td>UACR (mg/g)</td>
<td>5.05 (1.4–15.1)</td>
<td>5.45 (1.6–13.4)</td>
<td>3.75 (1.0–13.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>35 (4.8)</td>
<td>23 (4.1)</td>
<td>12 (7.3)</td>
<td>0.15</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>77 (10.6)</td>
<td>62 (11.1)</td>
<td>15 (9.1)</td>
<td>0.44</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>32 (4.4)</td>
<td>23 (4.1)</td>
<td>9 (5.5)</td>
<td>0.18</td>
</tr>
<tr>
<td>Malignancy</td>
<td>24 (3.3)</td>
<td>19 (3.4)</td>
<td>5 (3.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Liver disease</td>
<td>59 (8.1)</td>
<td>38 (6.8)</td>
<td>21 (12.7)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age at diagnosis of PKD (yr)</td>
<td>37.1 ± 13.1</td>
<td>36.5 ± 12.4</td>
<td>41.8 ± 14.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HtTKV (mL/m)</td>
<td>1,161.0 (631.2–1,895.7)</td>
<td>1,338.0 (749.2–2,113.2)</td>
<td>651.5 (408.0–1,013.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HtTLV (mL/m)</td>
<td>1,460.0 (1,232.0–1,888.0)</td>
<td>886.0 (754.4–1,164.5)</td>
<td>791.7 (679.6–977.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>MIC subclass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>55 (9.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B</td>
<td>149 (26.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C</td>
<td>198 (35.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1D</td>
<td>90 (16.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1E</td>
<td>61 (11.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued to the next page)
the median htTLV between the typical ADPKD and atypical PKD groups (886.0 mL/m [IQR, 754.4–1,164.5 mL/m] vs. 791.7 mL/m [IQR, 679.6–977.2 mL/m]; p = 0.20). The distribution of the study population based on MIC is presented in Table 1. Among the participants in the typical ADPKD group (n = 553), they were categorized into different MIC classes based on htTKV and age to predict the change in eGFR over time: 1) 1A, 55 (9.9%), 2) 1B, 149 (26.9%), 3) 1C, 198 (35.8%), 4) 1D, 90 (16.3%), and 5) 1E, 61 (11.0%). In the atypical PKD group, 83 (50.3%) participants were classified into MIC class 2 based on radiological features. The most common subtype was bilateral cystic with bilateral atrophic type (31, 37.3%), followed by lopsided (27, 32.5%), unilateral (nine, 10.8%), segmental (eight, 9.6%), bilateral cystic with unilateral atrophic (seven, 8.4%), and asymmetric (one, 1.2%). Within the atypical PKD group, 119 participants (72.1%) did not have a known family history of PKD. Seven patients with typical ADPKD and 82 patients with atypical PKD were excluded from imaging classification due to unclear imaging tests or unmeasured total kidney volume.

Table 2. Renal and extrarenal manifestations of study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of participants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal manifestation</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>532 (73.4)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>187 (25.8)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>122 (16.8)</td>
</tr>
<tr>
<td>Kidney stone</td>
<td>68 (9.4)</td>
</tr>
<tr>
<td>Kidney pain</td>
<td>40 (5.5)</td>
</tr>
<tr>
<td>Cyst infection</td>
<td>21 (2.9)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>15 (2.1)</td>
</tr>
<tr>
<td>Cyst hemorrhage</td>
<td>12 (1.7)</td>
</tr>
<tr>
<td>Extrarenal manifestation</td>
<td></td>
</tr>
<tr>
<td>Liver cyst</td>
<td>461 (63.5)</td>
</tr>
<tr>
<td>Cerebral aneurysm</td>
<td>46 (6.3)</td>
</tr>
<tr>
<td>Hemia</td>
<td>8 (1.1)</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>76 (10.5)</td>
</tr>
<tr>
<td>Gout</td>
<td>35 (4.8)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4 (0.6)</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>4 (0.6)</td>
</tr>
</tbody>
</table>

Table 2 displays the renal and extrarenal manifestations observed in the study participants. Hypertension was the most prevalent kidney complication, observed in 73.4% of all participants. The mean age at diagnosis was 40.5 ± 11.6 years, and the mean systolic/diastolic blood pressure was 129.6 ± 14.0/80.3 ± 10.8 mmHg. The prevalence of hypertension was significantly higher in the typical ADPKD group (430, 76.8%) compared to the atypical group (102, 61.8%; p < 0.001). Proteinuria was the second most common kidney complication, observed in 187 participants (25.8%), followed by hematuria (122, 16.8%), kidney stones (68, 9.4%), kidney pain (40, 5.5%), cyst infections (21, 2.9%),...
urinary tract infections (15, 2.1%), and cyst hemorrhages (12, 1.7%). The most frequent extrarenal manifestation was liver cysts (461, 63.5%), followed by hyperuricemia (76, 10.5%), cerebral aneurysm (46, 6.3%), gout (35, 4.8%), hernia (eight, 1.1%), heart failure (four, 0.6%), and valvular heart disease (four, 0.6%).

Genetic characteristics

During the 3-year study period, a gene panel analysis was conducted on a total of 725 patients. The mutation detection rate in our cohort was 64.3% (466 out of 725). No variants were identified in 99 cases (13.7%), and variants of unknown significance were detected in 85 cases (11.7%). Damaging (DM) variants were found in 75 cases (10.3%). Fig. 2 presents the genetic profiles obtained from the gene panel analysis of the typical ADPKD and atypical PKD groups. Among clinically typical ADPKD patients (560 cases, 70.2%), the mutation detection rate was 62.3%. PKD1 was found to be the most common genotype (252, 45%), followed by PKD2 (67, 12.1%). The other genotypes responsible for the typical ADPKD phenotype were found to include COL4A5 (1.1%), TSC1 (0.5%), HNF1β (0.4%), AVP (0.4%), AHI1, ALG8, COL4A1, COL4A3, DYNC2H1, EYA1, HSPA6, LRP5, NEK8, PRKCSH, TSC2, and UMOD (all 0.2%). The clinically atypical PKD group (165 cases, 20.7%) presented a lower mutation detection rate (41.8%) based on a gene panel. In 45 cases (27.3%), no pathogenic variants were found based on a gene panel. PKD1 (36, 21.8%) and PKD2 (12, 7.3%) were the two most common genotypes, followed by HNF1β (2.4%), TSC1 (1.8%), GANAB (1.8%), COL4A3 (1.2%), COL4A5 (1.2%), UMOD (1.2%), TSC2, DYNC2H1, and PAX2 (all 0.6%). The patients with family histories of PKD but showing atypical imaging features comprised 45 cases (27.4%). Among those cases, the PKD2 genotype was predominant (15.9%) compared to those without family histories of PKD (4.2%).

Discussion

We conducted a prospective, multicenter, nationwide cohort study on Korean cystic kidney disease in order to identify the baseline characteristics and genetic profiles of this heterogeneous disease group. Over 3 years, we enrolled a total of 725 participants who had more than three kidney cysts, regardless of their clinical diagnosis, family history, kidney function, or phenotype. We collected and analyzed various clinicodemographic data and performed a primary genetic analysis using a targeted gene panel. Our analysis revealed significant differences in the age at PKD diagnosis, hTKV, and prevalence of hypertension between
patients with typical ADPKD and those with atypical PKD. Furthermore, our findings showed that the two most common genetic mutations among Korean cystic kidney disease patients were in the PKD1 and PKD2 genes.

Joint research consortia for inherited cystic kidney disease have been established in the United States, Canada, and Europe. Given that ADPKD is the most prevalent inherited kidney disease, multiple ADPKD cohorts have been formed worldwide, including the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) [20], the French Genkyst cohort [10], the Toronto Genetic Epidemiology Study of PKD (TGESP) [12], and the cohort for genotype-Phenotype correlation in ADPKD (HOPE-PKD) in Korea [21]. These cohorts have contributed to identifying various classification criteria and prognostic factors for ADPKD patients, such as the Mayo classification, TKV, and PKD1 or PKD2 genotype [17]. However, there is still a lack of comprehensive understanding regarding cystic kidney diseases. Specifically, limited data on predictive markers, diagnostic criteria, or classification guidelines for atypical PKD is available. Therefore, there is a need for a cystic kidney disease cohort that encompasses both clinical and genetic information for both typical ADPKD and atypical PKD.

Understanding the clinicodemographic factors associated with severity and renal outcomes in both typical ADPKD and atypical PKD is crucial, as these factors play a significant role. Previous studies have suggested that younger age at PKD diagnosis, higher TKV [17], elevated serum uric acid levels [22], decreased HDL levels [23], and increased proteinuria [24,25] are associated with adverse outcomes in ADPKD. Our current study identified significant differences in various factors between typical ADPKD and atypical PKD patients. Since atypical PKD is mainly classified based on radiologic patterns and its clinical characteristics are not fully understood [26], these findings may provide valuable insights for differential diagnosis, prognosis, and even potential endpoints in clinical trials involving patients with atypical PKD. However, further investigation is necessary to determine whether these factors are indeed associated with adverse outcomes in cystic kidney diseases.

Genetic profiling of cystic kidney disease can be a valuable tool for dissecting and classifying these heterogeneous disease entities. In our current study, we performed genetic analysis using a targeted gene panel to detect various genotypes in both typical ADPKD and atypical PKD groups. However, the overall mutation detection rate in our cohort was lower than that of other cohorts such as HALT/CRISP (92%) [11], TGESP (84.5%) [12], and Genkyst (89.9%) [10,27], which utilized molecular analysis techniques including Sanger sequencing, multiplex ligation-dependent probe amplification, and long-range polymerase chain reaction (Supplementary Table 3, available online). One possible explanation for this discrepancy could be the composition of our study population, as HALT/CRISP, TGESP, and Genkyst included patients with typical ADPKD but not those with atypical PKD. Furthermore, the complex structure of the PKD1 gene, responsible for nearly 85% of ADPKD cases, presents challenges in its detection using a targeted gene panel. The PKD1 gene consists of exons 1–33 with 97.7% identical six pseudogenes, which complicates sequencing [6,8,14]. Exon 1 of PKD1 has a guanine-cytosine nucleotide-rich content, so not all genomic regions are equally covered by next-generation sequencing [28]. The sensitivity of whole exome sequencing within exons 1–32 is only 7.14% [29]. While a targeted gene panel can be used as a potential screening method for cystic kidney disease patients, additional genetic analysis is necessary for patients who have been identified with no pathologic variants or variants of uncertain significance by the targeted gene panel.

Our study has several strengths. First, by including both typical ADPKD and atypical PKD groups in our cohort, we could identify differences in clinical and genetic characteristics between these two groups. This comprehensive approach adds to the understanding of these distinct conditions. Second, a noteworthy aspect of our cohort is the inclusion of approximately 21% of individuals with atypical PKD. Given the limited research on atypical PKD in the adult population, the substantial representation of atypical PKD cases in our study holds significant value. Third, we successfully detected genetic mutations in cystic kidney disease patients using a customized, targeted gene panel comprising 88 ciliopathy-related genes. In particular, for the challenging PKD1 gene, which is structurally complex and poses difficulties in mutation detection, we addressed technical issues by increasing read depth and coverage for PKD exon 1 (Supplementary Fig. 1, available online). Our study suggests that the targeted gene panel could be a potential screening method for cystic kidney disease patients,
considering that currently used genetic analytic methods such as Sanger sequencing and whole exome sequencing are time-consuming. Lastly, our study is a nationwide cohort that includes 725 patients, representing approximately 15% of the total cystic kidney disease population in the country. This large dataset provides sufficient statistical power for robust analyses. Moreover, given that most ADPKD cohorts primarily include Western populations, our cohort is optimized to reflect the characteristics of the Korean cystic kidney disease population. The clinical and genetic profiles identified in our cohort offer valuable insights for Korean patients with cystic kidney disease.

The current study has a limitation in terms of the relatively low mutation detection rate compared to other cohorts that specifically include typical ADPKD cases. This discrepancy may be attributed to the inclusion of a heterogeneous disease entity within our cohort. Therefore, further genetic investigations are warranted for individuals who were not genetically diagnosed through the targeted gene panel tests. Another limitation is the potential underestimation of comorbidities, renal complications, and extrarenal complications due to the reliance on self-reporting from patient interviews for clinical data. For instance, the prevalence of cerebral aneurysm or valvular heart disease may have been underestimated because not all patients underwent brain imaging tests or echocardiography. Additionally, certain renal complications, such as kidney pain, were reported based on subjective patient accounts, introducing potential bias in assessing these complications.

Our study provides valuable insights into the baseline clinical and genetic characteristics of the Korean cohort with cystic kidney disease. These findings serve as a foundation for future research focused on diagnosis, prognosis, and potential therapeutic interventions for cystic kidney diseases. Furthermore, additional genetic analyses are needed for patients who presented with renal or extrarenal manifestations but did not show significant genetic mutations. These further investigations will contribute to a deeper understanding of the underlying genetic factors and expand our knowledge of cystic kidney diseases.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This work was supported by the Research Program funded by the Korea Disease Control and Prevention Agency (2019-ER-7304-00, 2019-ER-7304-01, 2019-ER-7304-02).

**Data sharing statement**

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

**Authors’ contributions**

Conceptualization: JMC, HCP, YCK, CA, KBL, YHK, SH, YK, EHB, HGK, JC, KHO, YKO
Data curation, Formal analysis, Investigation, Methodology: JMC, HCP, JWL, YCK, CA, YK, EHB, KJ, JC, HR, EP, SK
Funding acquisition, Supervision: YCK, CA, KBL, YHK, SH, YK, EHB, HGK, JC, KHO, YKO
Project administration, Resources: KBL, YHK, SH, HGK, KHO, YKO
Writing—original draft: JMC, HCP
Writing–review & editing: HCP, KBL, YHK, SH, HGK, KHO, YKO
All authors read and approved the final manuscript.

ORCID

Jeong Min Cho, https://orcid.org/0000-0001-7643-994X
Hayne Cho Park, https://orcid.org/0000-0002-7548-2697
Jin Woo Lee, https://orcid.org/0000-0002-5458-3215
Hyunjin Ryu, https://orcid.org/0000-0003-2148-4465
Yong Chul Kim, https://orcid.org/0000-0003-3215-8681
Curie Ahn, https://orcid.org/0000-0001-7033-1102
Kyu-Beck Lee, https://orcid.org/0000-0002-3904-5404
Yeong Hoon Kim, https://orcid.org/0000-0002-6849-9368
Seungyeup Han, https://orcid.org/0000-0002-7561-6534
Yae Rim Kim, https://orcid.org/0000-0003-1596-1528
Eun Hui Bae, https://orcid.org/0000-0003-1727-2822
Hee Gyung Kang, https://orcid.org/0000-0001-8323-5320
Eujin Park, https://orcid.org/0000-0002-4413-468X
Kyungjo Jeong, https://orcid.org/0000-0001-5210-9962
Seoon Kang, https://orcid.org/0000-0002-3947-4129
Jungmin Choi, https://orcid.org/0000-0001-9525-2179
Kook-Hwan Oh, https://orcid.org/0000-0001-8632-5743

References

A questionnaire survey on the diagnosis and treatment of Fabry nephropathy in clinical practice

Soo Jeong Choi¹, Su Hyun Kim², Min Sung Lee³, Samel Park⁴, Eunjung Cho⁵, Seung Seok Han⁶, Eun Sil Koh⁷, Byung Ha Chung⁸, Kyung Hwan Jeong⁹, Eun Hui Bae¹⁰, Eun Young Lee¹¹, Young Joo Kwon¹²

¹Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Soonchunhyang University College of Medicine, Bucheon, Republic of Korea
²Department of Internal Medicine, Chung-Ang University Gwangmyeong Hospital, Chung-Ang University College of Medicine, Gwangmyeong, Republic of Korea
³Department of Internal Medicine, Ewha Womans University Medical Center, Seoul, Republic of Korea
⁴Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, Republic of Korea
⁵Department of Internal Medicine, Korea University Guro Hospital, Korea University College of Medicine, Seoul, Republic of Korea
⁶Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
⁷Department of Internal Medicine, Yeouido St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
⁸Department of Internal Medicine, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
⁹Department of Internal Medicine, Kyung Hee University College of Medicine, Seoul, Republic of Korea
¹⁰Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea
¹¹Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea

Background: Fabry nephropathy is characterized by a deficiency of lysosomal alpha-galactosidase A, which results in proteinuria and kidney disease. The ineffectiveness of enzyme replacement therapy (ERT) for severe kidney failure highlights the need for early detection and meaningful markers. However, because the diagnosis and treatment of Fabry disease can vary according to the expertise of physicians, we evaluated the opinions of Korean specialists.

Methods: A questionnaire regarding the management of Fabry nephropathy was emailed to healthcare providers with the experience or ability to treat individuals with Fabry nephropathy.

Results: Of the 70 experts who responded to the survey, 43 were nephrologists, and 64.3% of the respondents reported having treated patients with Fabry disease. Pediatricians are treating primarily patients with classic types of the disease, while nephrologists and cardiologists are treating more patients with variant types. Only 40.7% of non-nephrologists agreed that a kidney biopsy was required at the time of diagnosis, compared with 81.4% of nephrologists. Thirty-eight of 70 respondents (54.3%) reported measuring globocteriaosylsphingosine (lyso-Gb3) as a biomarker. The most common period to measure lyso-Gb3 was at the time of diagnosis, followed by after ERT, before ERT, and at screening. For the stage at which ERT should begin, microalbuminuria and proteinuria were chosen by 51.8% and 28.6% of respondents, respectively.

Conclusion: Nephrologists are more likely to treat variant Fabry disease rather than classic cases, and they agree that ERT should be initiated early in Fabry nephropathy, using lyso-Gb3 as a biomarker.

Keywords: Biopsy, Chronic renal insufficiency, Fabry disease, Kidney diseases, Surveys and questionnaire, Therapeutics
**Introduction**

Fabry nephropathy is caused by a deficiency in lysosomal alpha-galactosidase A (α-Gal A), which leads to an accumulation of globotriaosylceramide (Gb3) in kidney cells and the increase of its metabolites including globotriaosylsphingosine (lyso-Gb3) [1]. Kidney involvement manifests as proteinuria and decreased glomerular filtration rate (GFR) values, both of which are indicative of chronic kidney disease [2,3]. Enzyme replacement therapy (ERT) ameliorates Gb3 deposition in kidneys [4,5]. However, ERT has been shown to be ineffective in patients with advanced chronic kidney disease (as indicated by proteinuria and a low GFR), emphasizing the need for early detection of Fabry nephropathy [6-9]. A change in Gb3 deposition in a kidney biopsy is a useful indicator of Fabry nephropathy, but not of the efficacy of treatment [10,11]. Plasma lyso-Gb3, which has also been proposed as a diagnostic biomarker, has been shown to correlate with disease severity, enzyme replacement response, and phenotyping [12,13].

Fabry disease is classified as either a classical or variant phenotype (later-onset) [14,15]. Classical phenotypes present in childhood or adolescence with typical symptoms, including angiokeratomas, anhidrosis, tinnitus, hearing loss, corneal dystrophy, strokes, left ventricular hypertrophy, cardiac arrhythmias, abdominal discomfort, and diarrhea. In contrast, variant phenotypes have residual α-Gal A activity and are not associated with early manifestations of classic symptoms. Patients with the variant phenotype experience an essentially normal childhood and adolescence, typically only developing renal or cardiac disease in the third to seventh decades of life [15].

The Korean medical insurance system and the Korea Disease Control and Prevention Agency maintain a rare-disease registry. Patients with Fabry disease are eligible for reimbursement of medical expenses if their diagnosis is confirmed. Despite these systems, individuals with variant phenotypic Fabry disease are commonly misdiagnosed and unable to receive treatment for an extended period of time due to a lack of typical symptoms; such patients are typically less seriously affected, and disease presentations may be limited to a single organ [16]. Half of the patients with Fabry disease in a report from Argentina were diagnosed by nephrologists [17].

Some patients with variant types who met the criteria for indication, which includes kidney manifestation, were covered by medical insurance for ERT. However, lyso-Gb3 was not covered by insurance in Korea [18].

The pattern of Fabry nephropathy management in Fabry disease has not yet been surveyed in Korea. This study was designed to determine how Korean experts treat patients with Fabry nephropathy.

**Methods**

This study was approved by and received a waiver for the need for informed consent from the Institutional Review Board of the Soonchunhyang University Bucheon Hospital (No. 2021-07-045-001).

A questionnaire to determine patterns in the diagnosis and treatment of Fabry nephropathy was distributed from 2021 to 2022. Questionnaires were emailed to and received by registered members of the Korean Society of Nephrology (KSN) and the Korean Society of Medical Genetics and Genomics (KSMG).

The questionnaire was divided into eight sections: 1) age group and sex of responder; 2) experience with Fabry disease, which includes yes/no questions in the past and present, and number and phenotype of patients; 3) timing of kidney biopsy (on diagnosis, before ERT, on a regular period, on time of proteinuria, or not necessary); 4) the need for a Fabry registry (yes vs. no); 5) check-up of lyso-Gb3 including the timing, interval, and needs for insurance coverage; 6) the interval and preferred tests for kidney function in Fabry disease; 7) the definition and tests of Fabry nephropathy; and 8) treatments other than ERT for Fabry nephropathy. Some detailed questions had multiple answers. The questionnaire is supplied in Supplementary Table 1 (available online).

Descriptive analytical statistics were used to summarize survey responses. The chi-square tests were used to compare categorical variables between groups. All statistical analyses were performed using the IBM SPSS Statistics version 20.0 software program (IBM Corp.). A p-value of less than 0.05 was considered statistically significant.

**Results**

We sent emails to all 1,990 registered members of the KSN and KSMG, and 70 responded. The highest participation...
rate was among those in their 40s (55.7%), and 32 participants (45.7%) were male. The majority were nephrologists (61.4%), followed by pediatricians and cardiologists. Table 1 summarizes the baseline characteristics of the participants.

Experience treating Fabry disease patients

Sixty-four percent of the respondents had experience treating patients with Fabry disease (Table 1). The numbers of patients with Fabry disease treated by respondents were 1 (16.7%), 2–5 (8.8%), 5–10 (2.9%), and ≥10 (1.5%), in that order. The patients with Fabry disease had variant phenotypes (34.0%), followed by classic (30.2%), both (9.4%), and unknown (26.4%) phenotypes (Fig. 1A). In all, 37.1% of nephrologists and 37.5% of cardiologists saw more variant patients than did pediatricians (0%) (Fig. 1B).

Kidney biopsy timing

The majority of respondents (46 of 70) indicated that a kidney biopsy should be performed when a diagnosis is made, while 12.9% replied that a biopsy was unnecessary (Table 2). Only 40.7% of non-nephrologists agreed that a kidney biopsy was required at the time of diagnosis, compared with 81.4% of nephrologists. Moreover, 22.2% of non-nephrologists considered a kidney biopsy unnecessary. Cardiologists (62.5%) were less likely than nephrologists (88.5%) and pediatricians (83.3%) to recommend a kidney biopsy (Fig. 2A). The majority of nephrologists and cardiologists would order a kidney biopsy prior to diagnosis, whereas pediatricians responded that a kidney biopsy should be performed prior to both diagnosis and treatment (Fig. 2B).

Need for a Fabry registry

The majority of respondents (79.3% of other experts and 87.8% of nephrologists) said they would take part if KSN created a Fabry registry (Table 2). Two pediatricians and one cardiologist replied that they had no intention of signing up to a Fabry registry.

Assessment of lyso-Gb3

If lyso-Gb3 was covered by Korean health insurance, the

Table 1. Baseline characteristics of survey participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Non-nephrologist</th>
<th>Nephrologist</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>70</td>
<td>27</td>
<td>43</td>
<td>0.84</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>16 (22.9)</td>
<td>7 (25.9)</td>
<td>9 (20.9)</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>39 (55.7)</td>
<td>14 (51.9)</td>
<td>25 (58.1)</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>11 (15.7)</td>
<td>5 (18.5)</td>
<td>6 (14.0)</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>4 (5.7)</td>
<td>1 (3.7)</td>
<td>3 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>32 (45.7)</td>
<td>10 (37.0)</td>
<td>22 (51.2)</td>
<td>0.36</td>
</tr>
<tr>
<td>Specialist</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric</td>
<td>12 (44.4)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cardiologist</td>
<td>11 (40.7)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pathologist</td>
<td>2 (7.4)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Neurologist</td>
<td>1 (3.7)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dermatologist</td>
<td>1 (3.7)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Experience in treating patients with FD, yes</td>
<td>45 (64.3)</td>
<td>16 (59.3)</td>
<td>29 (67.4)</td>
<td>0.66</td>
</tr>
<tr>
<td>Current FD treatment experience, yes</td>
<td>20 (28.6)</td>
<td>7 (25.9)</td>
<td>13 (30.2)</td>
<td>0.91</td>
</tr>
<tr>
<td>Phenotypes of FD</td>
<td></td>
<td></td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>Classic</td>
<td>16 (30.2)</td>
<td>6 (33.3)</td>
<td>10 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Variant</td>
<td>18 (34.0)</td>
<td>5 (27.8)</td>
<td>13 (37.1)</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>5 (9.4)</td>
<td>3 (16.7)</td>
<td>2 (5.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>14 (26.4)</td>
<td>4 (22.2)</td>
<td>10 (28.6)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number only or number (%). FD, Fabry disease.
Figure 1. Comparing Fabry disease phenotypes by specialty of respondents. (A) The phenotypes of Fabry disease in patients who responded to treatment. (B) A comparison of phenotypes according to specialty.

Figure 2. Comparative analysis of specialist consensus on kidney biopsy for Fabry disease diagnosis. Comparison of agreement with diagnostic kidney biopsy (A) and the timing of kidney biopsy (B) in patients with Fabry disease according to respondent specialty.

The majority of nephrologists (86.0%) would measure it after ERT, with smaller proportions choosing diagnosis, screening, and before ERT (Table 2). A majority of non-nephrologists did not agree that lyso-Gb3 should be measured at the time of diagnosis and for screening. Additionally, 60% of nephrologists expressed a preference to monitor lyso-Gb3 every 6 months. If lyso-Gb3 measurement was reimbursed by insurance in nephrology and cardiology, the likelihood that it would be used to determine the response to treatment increased compared with the current state, in which it is not covered (Fig. 3C).

Interval and tests for kidney function in Fabry disease

Half of the responders, particularly nephrologists, selected 3 months as the appropriate follow-up interval (Table 2). In 80.0% and 75.7% of cases, proteinuria and estimated GFR (eGFR) by serum creatinine, respectively, were selected as the optimal kidney function tests. Cardiology and pediatric experts were associated with comparable rates at 3-month and 6-month intervals, respectively, and the follow-up interval for kidney function tests was greater than that indicated by nephrologists (Fig. 4A). Most nephrologists stated that proteinuria, eGFR, α-Gal A activity, cystatin C, and
### Table 2. Comparative survey findings between nephrologists and non-nephrologists

<table>
<thead>
<tr>
<th>Survey</th>
<th>Total (n = 70)</th>
<th>Non-nephrologist (n = 27)</th>
<th>Nephrologist (n = 43)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timing of kidney biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At diagnosis</td>
<td>46 (65.7)</td>
<td>11 (40.7)</td>
<td>35 (81.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>On detection of proteinuria</td>
<td>2 (2.9)</td>
<td>2 (7.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Repeat biopsy at regular intervals</td>
<td>1 (1.8)</td>
<td>1 (3.7)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Before enzyme treatment</td>
<td>7 (10.0)</td>
<td>3 (11.1)</td>
<td>4 (9.3)</td>
<td></td>
</tr>
<tr>
<td>Not needed</td>
<td>9 (12.9)</td>
<td>6 (22.2)</td>
<td>3 (7.0)</td>
<td></td>
</tr>
<tr>
<td>No opinion</td>
<td>5 (7.1)</td>
<td>4 (14.8)</td>
<td>1 (2.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Willing to participate in Fabry registry enrollment with KSN</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>11 (16.4)</td>
<td>1 (4.0)</td>
<td>10 (23.8)</td>
<td></td>
</tr>
<tr>
<td>Agree</td>
<td>42 (62.7)</td>
<td>15 (60.0)</td>
<td>27 (64.3)</td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td>11 (16.4)</td>
<td>6 (24.0)</td>
<td>5 (11.9)</td>
<td></td>
</tr>
<tr>
<td>Disagree</td>
<td>2 (3)</td>
<td>2 (8.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Strongly disagree</td>
<td>1 (1.5)</td>
<td>1 (4.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Agreement of measurement of lyso-Gb3</strong></td>
<td>38 (54.3)</td>
<td>13 (48.1)</td>
<td>25 (58.1)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Timing of measurement of lyso-Gb3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For screening</td>
<td>20 (28.6)</td>
<td>4 (14.8)</td>
<td>16 (37.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>For diagnosis</td>
<td>35 (50.0)</td>
<td>12 (44.4)</td>
<td>23 (53.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Before treatment</td>
<td>20 (28.6)</td>
<td>7 (25.9)</td>
<td>13 (30.2)</td>
<td>0.91</td>
</tr>
<tr>
<td>After treatment</td>
<td>27 (38.6)</td>
<td>7 (25.9)</td>
<td>20 (46.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>All of above</td>
<td>4 (5.7)</td>
<td>1 (3.7)</td>
<td>3 (7.0)</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Timing of measurement of lyso-Gb3 (if covered by insurance)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For screening</td>
<td>28 (40.0)</td>
<td>6 (22.2)</td>
<td>22 (51.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>For diagnosis</td>
<td>43 (61.4)</td>
<td>15 (55.6)</td>
<td>28 (65.1)</td>
<td>0.58</td>
</tr>
<tr>
<td>Before treatment</td>
<td>25 (36.7)</td>
<td>5 (18.5)</td>
<td>20 (46.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>After treatment</td>
<td>48 (68.6)</td>
<td>11 (40.7)</td>
<td>37 (86.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All of above</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lyso-Gb3 measurement interval (if covered by insurance)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 mo</td>
<td>31 (44.3)</td>
<td>5 (18.5)</td>
<td>26 (60.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>12 mo</td>
<td>10 (14.3)</td>
<td>4 (14.8)</td>
<td>6 (14.0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td><strong>Test interval to monitor kidney function</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>1 mo</td>
<td>4 (5.7)</td>
<td>0 (0)</td>
<td>4 (9.3)</td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>38 (54.3)</td>
<td>9 (33.3)</td>
<td>29 (67.4)</td>
<td></td>
</tr>
<tr>
<td>6 mo</td>
<td>13 (18.6)</td>
<td>6 (22.2)</td>
<td>7 (16.3)</td>
<td></td>
</tr>
<tr>
<td>12 mo</td>
<td>4 (5.7)</td>
<td>3 (11.1)</td>
<td>1 (2.3)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>11 (15.7)</td>
<td>9 (33.3)</td>
<td>2 (4.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Tests to monitor kidney function (multiple choice)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td>56 (80.0)</td>
<td>15 (55.6)</td>
<td>41 (95.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR</td>
<td>53 (75.7)</td>
<td>11 (40.7)</td>
<td>42 (97.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α-Gal A activity</td>
<td>16 (22.9)</td>
<td>4 (14.8)</td>
<td>12 (27.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>24 (34.3)</td>
<td>5 (18.5)</td>
<td>19 (44.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>Lyso-Gb3</td>
<td>24 (34.3)</td>
<td>8 (29.6)</td>
<td>16 (37.2)</td>
<td>0.70</td>
</tr>
<tr>
<td>Kidney biopsy</td>
<td>7 (10.0)</td>
<td>1 (3.7)</td>
<td>6 (14.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>eGFR and proteinuria</td>
<td>50 (71.4)</td>
<td>10 (37.0)</td>
<td>40 (93.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(Continued to the next page)
lyso-Gb3 levels should be measured, and kidney biopsies should be conducted to monitor kidney function, although members of other departments infrequently agreed (Fig. 4B).

**Definition and tests of Fabry nephropathy**

No consensus was seen in the survey results regarding a definition of Fabry nephropathy. When Fabry nephropathy was suspected, physicians measured α-Gal A activity (72.8%). This was followed by kidney biopsy, genetic testing, and measurements of lyso-Gb3 (Table 2).

**Treatment of Fabry nephropathy**

For additional treatment of ERT in Fabry nephropathy, an angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker was considered by 82.8% of respondents. A low-protein diet and low sodium intake were considered by 55.7% and 24.3%, respectively (Table 2).

Among respondents, the stages of microalbuminuria and proteinuria were identified as the starting point of ERT treatment in patients with variant Fabry nephropathy by 51.7% and 28.5%, respectively. A minority of nephrologists indicated that treatment should be performed if proteinuria was present or eGFR was less than 90 mL/min/1.73 m².

Pediatricians frequently indicated that treatment should be administered when microalbuminuria or proteinuria was once present (Fig. 5). Conversely, nephrologists and cardiologists revealed that the majority of responses were treated during albuminuria, but some indicated that early treatment is required even in the absence of albuminuria.

**Discussion**

In 1984, ophthalmologists reported the first case of Fabry disease in Korea. In 2019 and 2020, the number of newly registered patients according to the Korean Standard Classification of Disease (KCD) was 28 (8 male and 20 female) and 40 (16 male and 24 female), respectively [17]. Despite the fact that the Korean medical insurance and Korea Disease Control and Prevention Agency had a system
Figure 3. Comparative analysis of specialist agreement on lyso-Gb3 measurement in Fabry disease. The comparison of agreement of measurement of lyso-Gb3 (A), timing according to responders’ specialty (B). (C) If lyso-Gb3 measurement is insured, diagnosis, before and after treatment lyso-Gb3 measurements were compared to the respondent’s area of expertise. Lyso-Gb3, globotriaosphingosine.

Figure 4. Kidney function monitoring by respondent specialty. A comparison of monitoring frequency (A) and method (B) for kidney function according to respondent specialty. α-Gal A, alpha-galactosidase A; eGFR, estimated glomerular filtration rate; lyso-Gb3, globotriaosphingosine.

for controlling rare diseases, Fabry disease patients were underdiagnosed and untreated for an extended period [16,19,20]. Although Fabry disease has been diagnosed at an increasing rate in recent years, it is still extremely rare and difficult to access by experts [21]. In a French survey of 152 nephrologists, few doctors (22%) directly managed patients with Fabry disease and 18% had made a diagnosis on their own [21].

This study examines the current level of awareness among Korean specialists regarding available treatment options for Fabry nephropathy. The majority of respondents were nephrologists, while pediatricians and cardiologists followed in second and third place, respectively. Nephrologists in Argentina diagnosed half of Fabry disease patients [22]. While nephrologists can also treat patients with classic diseases, they are more likely to treat variants of the disease [23]. Different opinions were held by nephrologists and non-nephrologists regarding the diag-
nosis and treatment of Fabry nephropathy. Because it is a progressive multisystem disease, the involvement of other vital organs (the heart and brain, in particular) must be determined by an appropriate specialist [24]. In addition, the clinical heterogeneity of Fabry disease necessitates an individualized approach to treatment that is based on the genotype, sex, family history, phenotype, and specific clinical symptom severity of each patient [3,6]. Confirming the opinions of experts about appropriate approaches to the screening, diagnosis, and treatment of Fabry nephropathy is therefore a useful activity.

The majority of nephrologists considered the time of diagnosis optimal for a kidney biopsy, whereas 22.2% of non-nephrologists indicated that a kidney biopsy was unnecessary. Although there was no consensus on a definition of Fabry nephropathy, most respondents chose α-Gal A activity, kidney biopsy, genetic studies, and lyso-Gb3 levels as diagnostic tools. A total of 24% of nephrologists did not agree with the need for a Fabry nephropathy registry. Most experts agreed with measuring lyso-Gb3 as a biomarker. Currently, measurements of lyso-Gb3 are not covered by insurance, so it is being requested by a specific company. If lyso-Gb3 is covered by Korean health insurance, most specialists reported that they would measure lyso-Gb3 after ERT treatment. However, most non-nephrologists agreed that measuring lyso-Gb3 after treatment would be appropriate but would perform such measurements at the time of diagnosis. New biomarkers such as lyso-Gb3 were impaired in patients with normal albuminuria levels [12,17,25] and were more closely correlated with the expression of Fabry nephropathy [18]. Unpublished data from a Korean study by Cho et al. found that lyso-Gb3 is a screening marker that can identify patients who are eligible for a Fabry gene analysis in patients with chronic kidney disease with unknown etiology. This suggests that coverage by insurance should be considered.

Half of the respondents (54.4%), particularly nephrologists, selected proteinuria and eGFR by serum creatinine to measure kidney function every 3 months. Although proteinuria and GFR are considered important markers of Fabry nephropathy, most kidney involvement occurs in a non-proteinuria state [2,3,26]. These findings suggest that the field of expertise of the treating physicians should be given greater attention. Nephrologists evaluate kidney involvement using a kidney biopsy earlier than do non-nephrologists [24,26]. In addition, they treat and monitor kidney involvement more proactively than do non-nephrologists. Consequently, for the treatment of Fabry disease, a discussion with a specialist in the relevant field will be of great benefit.

This is the first survey of Korean experts on Fabry disease and how they manage Fabry nephropathy. Unlike previous Korean patients with Fabry disease studied by a nationwide survey, most patients being treated by survey respondents had a variant form of the disease [16]. The specialty of the diagnosing physician influenced the phenotype of the disease in each patient. Nephrologists and cardiologists treat more patients with variant types, while pediatricians see those with the classic type (Fig. 1). Recent screening studies of Fabry disease in high-risk clinics (1995–2017) confirmed this pattern [23]. Two Korean patients with Fabry nephropathy reportedly progressed to kidney failure with renal replacement therapy in 1989. Recently, a cardiac variant was reported in Korea [27]. Because Korean experience with Fabry disease has been sporadic and intermittent until recently, clinicians are interested in sharing their experiences with the disease [28]. A recent unpooled systematic review found that different patient populations could require different disease-management and therapeutic goals depending on age, genotype, and disease severity and/or level of organ involvement [13]. Although National Health Insur-
ance service benefits set by the KCD (during the 3 months after registration) was 38,903,000/person, the burden of cost remains a barrier for ERT in Fabry disease, particularly for female patients with classic Fabry disease or other patients with the variant type. Fewer reports of ERT in adult females were available compared with those in adult males due to the limitations of retrospective observational and case-series studies [13]. Clinicians, and nephrologists in particular, prefer to monitor kidney manifestations to determine the timing of ERT after diagnosis. Most respondents chose microalbuminuria as the appropriate stage to initiate ERT. An angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker was considered a non-ERT treatment, followed by a low-protein diet and low sodium intake.

Diagnostic and treatment guidelines for Fabry disease emphasize a multidisciplinary approach to disease management due to multisystem involvement [14,29]. Our findings show that specialists in the field of nephrology have limited expertise with tests and medicines. In fact, a survey of nephrologists in France revealed that knowledge of kidney injury was poor (less than 50% chose the correct answers in a test) [21]. In addition, nephrologists may lack the experience of other specialties when it comes to phenotype or genetic testing; therefore, patients with Fabry disease should receive care from a multidisciplinary team comprising experienced professionals in neurology, cardiology, pediatrics, and genetics, in addition to nephrology.

This research had numerous limitations. First, selection and recall bias is possible in a voluntary survey questionnaire. Second, due to the limited number of specialists with experience in treating this disease, only a small number of respondents completed the survey. Third, responders may have been confused about phenotypic categories because classic and variant phenotypes were not defined in this survey. Fourth, pediatric nephrologists may be considered non-nephrologists due to the nature of their sub-specialty training. Finally, clinicians may diagnose or treat Fabry nephropathy based on the Korean medical insurance indications.

The majority of respondents to the survey were medical professionals who have treated or are currently treating a patient with Fabry disease. They agreed that it is important to initiate ERT early in Fabry nephropathy, particularly in patients with the variant type of the disease, and that lyso-Gb3 is a valuable biomarker, highlighting the importance of insurance coverage for lyso-Gb3 testing.

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.

Funding

This research was supported by the Soonchunhyang University Research Fund.

Authors’ contributions

Conceptualization, Data curation: SJC, SHK
Formal analysis: SJC, SHK, KHJ
Methodology: SJC, SHK, MSL, EC, EYL
Investigation: ESK, YJK
Software: MSL
Supervision: YJK
Validation: BHC, EHB, EYL
Funding acquisition: SJC
Writing–original draft: SJC, SHK
Writing–review & editing: MSL, SP, EC, SSH, ESK, BHC, KHJ, EHB, EYL
All authors read and approved the final manuscript.

ORCID

Soo Jeong Choi, https://orcid.org/0000-0003-3650-0798
Su Hyun Kim, https://orcid.org/0000-0003-3382-528X
Min Sung Lee, https://orcid.org/0000-0003-4865-7741
Samel Park, https://orcid.org/0000-0002-5717-0743
Eunjung Cho, https://orcid.org/0000-0001-6013-8683
Seung Seok Han, https://orcid.org/0000-0003-0137-5261
Eun Sil Koh, https://orcid.org/0000-0003-1282-7876
Byung Ha Chung, https://orcid.org/0000-0003-0048-5717
Kyung Hwan Jeong, https://orcid.org/0000-0003-1492-8021
References


Acute kidney injury in hospitalized adults with chronic kidney disease: comparing cROCK, KDIGO, and combined criteria

Ling Sun¹², Rui-Xue Hua²*, Yu Wu², Lu-Xi Zou³

¹Department of Nephrology, Xuzhou Central Hospital, Xuzhou, China  
²Xuzhou Clinical School of Xuzhou Medical University, Xuzhou, China  
³School of Management, Xuzhou Medical University, Xuzhou, China

Background: Acute-on-chronic kidney disease (ACKD) increases the risk of progression of chronic kidney disease (CKD). This study aimed to evaluate the ability of a novel criteria of reference change value of the serum creatinine optimized criteria for acute kidney injury in CKD (cROCK) to detect ACKD patients.

Methods: This was a retrospective observational study with a 3-year follow-up. All included patients with CKD stage 3 were evaluated using cROCK, Kidney Disease Improving Global Outcomes (KDIGO), and their combined criteria. The renal composite endpoints, major adverse cardiovascular events (MACEs), and all-cause mortality were recorded as clinical outcomes.

Results: A total of 812 patients was enrolled. The cROCK criteria detected more ACKD events than did the KDIGO (68.0% vs. 59.5%, p < 0.001). Compared to KDIGO (−) & cROCK (−) group, ACKD patients diagnosed by cROCK had significantly higher hazard ratio (HR) for renal composite endpoints (HR, 3.591; p < 0.001), MACEs (HR, 1.748; p < 0.001), and all-cause mortality (HR, 2.985; p < 0.001). The patients in KDIGO (+) & cROCK (+) group had the lowest survival probability when considering renal composite endpoints, MACEs, and all-cause mortality (all p < 0.001). Furthermore, cROCK resulted in the largest area under the receiver operating characteristic curve (AUC) for predicting renal composite endpoints, and the combined criteria led to the largest AUC for predicting MACEs and all-cause mortality.

Conclusion: Compared to the KDIGO, the cROCK detected more ACKD events. Combining both cROCK and KDIGO criteria might improve the predictive ability for long-term outcomes in ACKD patients.

Keywords: Acute kidney injury, Cardiovascular, Chronic kidney disease, Mortality

Introduction

Acute kidney injury (AKI) is a clinical diagnosis with the characteristics of a rapidly increasing serum creatinine (sCr) level and/or decreasing urine output. The incidence of AKI is approximately 10% to 15% among non-intensive care unit hospitalizations [1]. AKI not only contributes to in-hospital mortality and morbidity but also increases the risk of development and progression of chronic kidney disease (CKD), major adverse cardiovascular events (MACEs), and long-term mortality [2].

AKI and CKD are recognized as integrated/interconnected clinical syndromes. Previous studies have demonstrated
CKD as a major risk factor for AKI, accounting for >30% of patients with AKI [3]. Meanwhile, the severity and episodes of AKI could lead to CKD onset and progression [4]; thus, AKI in CKD, also known as acute-on-chronic kidney disease (ACKD), usually results in a poor prognosis [5].

AKI survivors are at high risk for sustained kidney injury. AKI can occur at any stage of CKD and causes renal deterioration to end-stage renal disease (ESRD). Regular evaluation and follow-up after AKI initial insult are important to improve clinical outcomes. Therefore, a standardized method to detect and diagnose AKI is necessary. In 2004, the AKI definition was first established with introduction of the Risk, Injury, Failure, Loss, and End-stage renal disease (RIFLE) protocol [6]. The second AKI definition relies on Acute Kidney Injury Network (AKIN) criteria [7]. Most recently, the Kidney Disease Improving Global Outcomes (KDIGO) criteria, which combined the RIFLE, AKIN, and pediatric RIFLE criteria, was modified in 2012 and is widely used for AKI definition [8].

At present, ACKD diagnosis relies on current AKI criteria; however, the elevated sCr level in the above AKI criteria could be inadequate to diagnose ACKD, and ACKD detection could be incorrect as a result. It has been proven that even a mild increase of 25% in sCr level was associated with a 70% increase in mortality [9]. Xu et al. [10] set up a reference change value (RCV) of the sCr optimized criteria for AKI in CKD (cROCK) based on data from 344,694 Chinese hospitalized adults, including 27,303 CKD patients with baseline estimated glomerular filtration rate (eGFR) of ≤60 mL/min/1.73 m². The cROCK criteria are defined as a >25% increase of sCr over 7 days. Compared to the most popular KDIGO criteria, the cROCK criteria has been confirmed to improve the sensitivity in identifying patients with high risk of CKD progression in 90 days after admission, based on data from 2,383 CKD patients [10].

Existing criteria for diagnosing AKI have remained controversial because of biological variability in CKD patients; as such, a standardized AKI definition with high prognostic ability is needed to recognize at-risk CKD patients and improve their clinical outcomes. The ACKD incidence may vary based on different diagnostic criteria. There is no report available on clinical applications of the cROCK criteria. This study aimed to evaluate the impact of the cROCK criteria in identifying ACKD and to compare the cROCK and KDIGO criteria (Supplementary Table 1, available online) in terms of their ability to predict long-term clinical outcomes, including renal prognosis, MACEs, and all-cause mortality, in ACKD patients.

**Methods**

**Data source and study population**

We performed a single-center retrospective observational study of inpatients with CKD stage 3 at Xuzhou Central Hospital in Xuzhou, China. A total of 90,907 patients was admitted to Xuzhou Central Hospital between January 1, 2016, and June 30, 2018. Their electronic medical records (EMRs) were obtained from the hospital information system, and relevant EMR data consisted of age, sex, diagnosis code and date, sCr value, and date of admission. The eGFR was calculated using the sCr-based Chronic Kidney Disease Epidemiology Collaboration equation. Study inclusion criteria were as follows: (1) patients with CKD stage 3 (eGFR, 30–60 mL/min/1.73 m² for >3 months) and (2) 18–75 years old. Exclusion criteria were as follows: (1) no sCr data available ≥2 times within 7 days, (2) AKI on admission, (3) underwent kidney transplantation, and (4) loss of follow-up within 3 years after discharge.

The baseline sCr value was defined as the average value of sCr within 30 days prior to admission or the first sCr value within 3 days after admission if pre-admission data were not available. According to the diagnostic criteria of KDIGO and cROCK, the patients were divided into the following four groups: KDIGO (−) & cROCK (−), KDIGO (−) & cROCK (+), KDIGO (+) & cROCK (−), and KDIGO (+) & cROCK (+). In addition, if patients met the cROCK criteria, they were defined as cROCK (+), which was the sum of KDIGO (−) & cROCK (+), KDIGO (+) & cROCK (−), and KDIGO (+) & cROCK (+); if they met the KDIGO criteria, they were defined as KDIGO (+), which was the sum of KDIGO (+) & cROCK (−) and KDIGO (+) & cROCK (+). The flowchart of study population selection and grouping is shown in Fig. 1.

**Clinical outcomes**

In this study, all included patients were followed for ≥3 years. The primary endpoint was renal composite endpoints of CKD progression (i.e., eGFR decrease of ≥25%, progression to CKD stage 4, or eGFR decrease of ≥5 mL/
min/1.73 m² per year), ESRD, receiving maintenance renal-replacement therapy, or death directly related to CKD [11]. The secondary endpoints were all-cause mortality and MACEs (including cardiovascular death, myocardial infarction, and stroke).

**Statistical analyses**

To describe the demographic, biochemical, and clinical parameters, continuous variables are presented as mean ± standard deviation, dichotomous variables are presented as frequency and proportion, and nominal variables were analyzed with frequency distribution. The Shapiro-Wilk

---

**Figure 1. Flowchart of study population selection and grouping.** The different shapes and colors represent different subgroups. ACKD, acute-on-chronic kidney disease; AKI, acute kidney injury; CKD, chronic kidney disease; cROCK, reference change value of the serum creatinine optimized criteria for acute kidney injury in chronic kidney disease; eGFR, estimated glomerular filtration rate; KDIGO, Kidney Disease Improving Global Outcomes.
test was used to evaluate the normality of the variables. The differences between groups were evaluated by the Student t test for continuous variables and the chi-square test for categorical variables. The measure of inter-rater reliability was evaluated by the kappa coefficient and Fleiss kappa coefficient. Multivariate Cox proportional hazards models were performed to evaluate associations between ACKD diagnosis and renal composite endpoints, MACEs, and all-cause mortality, and results were expressed as hazard ratios (HRs) with 95% confidence interval (CIs). Harrell concordance index (C-index) was calculated to describe the predictive accuracy of clinical outcomes.

Nonparametric Kaplan-Meier survival estimation was used to evaluate the effect of ACKD criteria on predicting long-term clinical outcomes, and the Kaplan-Meier survival curves were compared using the log-rank test. Receiver operator characteristic (ROC) analysis was used to assess the predictive ability of different criteria and their combinations for renal and survival outcomes. A two-sided p-value of <0.05 was defined as statistically significant. Statistical analyses were performed using the SAS version 9.4 software program (SAS Institute) and R version 4.2 software program (R Foundation for Statistical Computing).

**Ethics statement**

This study was retrospective. Data from the hospital information system were aggregated as secondary data without personal information, and the requirement for informed consent was waived. This study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Xuzhou Central Hospital (No. XZXY-LJ-20210110-005).

**Results**

**General characteristics of the study cohort**

A total of 812 patients with CKD stage 3 and complete data was enrolled in this study and assigned to four groups depending on the KDIGO and cROCK criteria as mentioned previously. The baseline and following 3-year clinical data were analyzed and described in Table 1. Among the data, the Charlson comorbidity index (CCI) represented the general characteristics of the enrolled patients during the baseline hospitalization (Supplementary Table 2, available online).

The cROCK criteria detected 8.5% more acute-on-chronic kidney disease events than did the KDIGO criteria. Among the enrolled CKD inpatients, more ACKD events were diagnosed using the cROCK criteria than the KDIGO criteria (68.0% vs. 59.5%), with statistical significance ($\chi^2 = 12.318, p < 0.001$). This indicated that the use of the cROCK criteria could improve the sensitivity in identifying ACKD patients. The detection rate of ACKD was 55.4% based on the combined criteria of KDIGO and cROCK (Table 2).

### Table 1. Baseline characteristics and clinical outcomes during 3-year follow-up in enrolled CKD patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>KDIGO (–) &amp; cROCK (–)</th>
<th>KDIGO (–) &amp; cROCK (+)</th>
<th>KDIGO (+) &amp; cROCK (–)</th>
<th>KDIGO (+) &amp; cROCK (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>812</td>
<td>227</td>
<td>102</td>
<td>33</td>
<td>450</td>
</tr>
<tr>
<td>Female sex</td>
<td>340 (41.9)</td>
<td>60 (26.4)</td>
<td>49 (48.0)</td>
<td>14 (42.4)</td>
<td>217 (48.2)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>69.2 (14.9)</td>
<td>66.8 (16.6)</td>
<td>70.2 (14.9)</td>
<td>69.7 (15.7)</td>
<td>70.3 (13.7)</td>
</tr>
<tr>
<td>Baseline Scr (μmol/L)</td>
<td>131.8 ± 26.7</td>
<td>134.9 ± 25.5</td>
<td>132.6 ± 25.4</td>
<td>134.8 ± 20.1</td>
<td>129.9 ± 27.9</td>
</tr>
<tr>
<td>Baseline CCI, ≥5</td>
<td>430 (53.0)</td>
<td>28 (12.3)</td>
<td>41 (40.2)</td>
<td>14 (42.4)</td>
<td>347 (77.1)</td>
</tr>
<tr>
<td>Renal composite endpoints</td>
<td>683 (84.1)</td>
<td>137 (60.4)</td>
<td>94 (92.2)</td>
<td>25 (75.8)</td>
<td>427 (94.9)</td>
</tr>
<tr>
<td>MACEs</td>
<td>650 (80.0)</td>
<td>141 (62.1)</td>
<td>73 (71.6)</td>
<td>27 (81.8)</td>
<td>409 (90.9)</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>405 (49.9)</td>
<td>29 (12.8)</td>
<td>26 (25.5)</td>
<td>11 (33.3)</td>
<td>339 (75.3)</td>
</tr>
</tbody>
</table>

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

CCI, Charlson comorbidity index; CKD, chronic kidney disease; cROCK, reference change value of the serum creatinine optimized criteria for acute kidney injury in chronic kidney disease; KDIGO, Kidney Disease Improving Global Outcomes; MACEs, major adverse cardiovascular events; sCr, serum creatinine.
described in Table 3, which showed a kappa coefficient of 0.643 (95% CI, 0.589–0.697; p < 0.001), indicating a strong consistency in ACKD diagnosis between the cROCK and KDIGO criteria. In addition, Fleiss kappa consistency coefficient among the cROCK, KDIGO, and the combined criteria of cROCK and KDIGO was 0.760 (p < 0.001), which also demonstrates consistency in ACKD diagnosis.

**Acute-on-chronic kidney disease diagnosed by the cROCK criteria was associated with poor clinical outcomes**

The multivariate Cox proportional hazards models were performed with adjustment for age, sex, and baseline sCr and CCI scores. The events of clinical outcomes in the KDIGO (−) & cROCK (−) group were used as the reference (HR, 1.000), and the ACKD patients diagnosed by the cROCK criteria had significantly high HRs for renal composite endpoints (HR, 3.591; 95% CI, 2.869–4.493; p < 0.001), MACEs (HR, 1.748; 95% CI, 1.311–2.329; p < 0.001), and all-cause mortality (HR, 2.985; 95% CI, 2.100–4.242; p < 0.001). Moreover, the HR values of the cROCK (+) group for renal composite endpoints were larger than those of the KDIGO (+) group, and the KDIGO (+) & cROCK (+) group had the highest HR value for renal composite endpoints among all groups (HR, 3.885; 95% CI, 3.123–4.833; p < 0.001). The Harrell C-index values of the cROCK (+), KDIGO (+), and KDIGO (+) & cROCK (+) groups ranged from 0.739–0.828, and the Harrell C-index values of the KDIGO (+) & cROCK (+) group were the highest (all p < 0.05), indicating that the combination of cROCK and KDIGO criteria achieved the greatest predictive accuracy for these clinical outcomes (Fig. 2). Moreover, the net reclassification index and integrated discrimination improvement were added to evaluate and compare the prognostic and discriminatory power for the above multivariate Cox proportional hazards models, and the results indicate that the combination of the cROCK and KDIGO criteria performs better than using either criteria alone (Table 4).

**Use of KDIGO and cROCK criteria in predicting clinical outcomes**

During the 3-year follow-up period, 683 patients (84.1%) experienced renal composite endpoint events. Kaplan-Meier analysis ranked the groups from high to low percentage of free renal composite endpoints as KDIGO (−) & cROCK (−), KDIGO (+) & cROCK (−), KDIGO (−) & cROCK (+), and KDIGO (+) & cROCK (+) (p < 0.001) (Fig. 3A). There were 650 patients (80.0%) who developed MACEs, with groups ranked from high to low as KDIGO (−) & cROCK (−), KDIGO (−) & cROCK (+), KDIGO (+) & cROCK (−), and KDIGO (+) & cROCK (+) (p < 0.001) (Fig. 3B). A total of 405 patients (49.9%) died during the follow-up period, and the trends of cumulative survival rate were similar to MACEs across groups (p < 0.001) (Fig. 3C). The p-values between the Kaplan-Meier survival curves are shown in Supplementary Tables 3 to 5 (available online).

Among the three groups of ACKD diagnosed by the cROCK, KDIGO, and the combined criteria of cROCK and KDIGO, the area under the ROC curve (AUC) of cROCK criteria was highest for predicting renal composite endpoints, at 0.837, and the optimal cutoff value of this ROC curve was 2.043, with specificity and sensitivity values of 0.806 and 0.726, respectively (Fig. 4A). The AUCs of the KDIGO and the combined criteria of cROCK and KDIGO for MACEs forecasting were slightly higher than that of the cROCK criteria. Meanwhile, the AUC of KDIGO criteria was 0.814, with an optimal cutoff value of 1.125, a specificity of 0.741,
Hazard ratios (HRs) of acute-on-chronic kidney disease for clinical outcomes.

The adjusted HRs were calculated using multivariate Cox proportional hazard models adjusted for age, sex, baseline serum creatinine, and Charlson comorbidity index score. The Harrell C-index values of the cROCK (+), KDIGO (+), and KDIGO (+) & cROCK (+) groups ranged from 0.739 to 0.828, and the Harrell C-index value of the KDIGO (+) & cROCK (+) group was highest among all clinical outcomes.

CI, confidence interval; cROCK, reference change value of the serum creatinine optimized criteria for acute kidney injury in chronic kidney disease; KDIGO, Kidney Disease Improving Global Outcomes; MACEs, major adverse cardiovascular events (including cardiovascular death, myocardial infarction, and stroke).

Table 4. The IDI and NRI for multivariate Cox proportional hazard models in Fig. 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>KDIGO (+) &amp; cROCK (+) vs. cROCK (+)</th>
<th>KDIGO (+) &amp; cROCK (+) vs. KDIGO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate 95% CI p-value</td>
<td>Estimate 95% CI p-value</td>
</tr>
<tr>
<td>Renal composite endpoints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDI</td>
<td>0.013 0.003–0.027 0.016</td>
<td>0.013 0.002–0.027 0.016</td>
</tr>
<tr>
<td>NRI</td>
<td>0.555 0.292–0.610 &lt;0.001</td>
<td>0.555 0.295–0.618 &lt;0.001</td>
</tr>
<tr>
<td>MACEs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDI</td>
<td>0.020 0.006–0.040 &lt;0.001</td>
<td>0.020 0.006–0.038 0.004</td>
</tr>
<tr>
<td>NRI</td>
<td>0.476 0.321–0.563 &lt;0.001</td>
<td>0.476 0.330–0.559 &lt;0.001</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDI</td>
<td>0.050 0.022–0.086 &lt;0.001</td>
<td>0.050 0.023–0.086 &lt;0.001</td>
</tr>
<tr>
<td>NRI</td>
<td>0.543 0.479–0.603 &lt;0.001</td>
<td>0.543 0.478–0.600 &lt;0.001</td>
</tr>
</tbody>
</table>

All multivariate Cox proportional hazard models were adjusted by age, sex, serum creatinine, and Charlson comorbidity index.

and a sensitivity of 0.778. The AUC of the combined criteria of cROCK and KDIGO was also 0.814, with an optimal cutoff value of 1.022 and specificity and sensitivity of 0.704 and 0.820, respectively (Fig. 4B). For all-cause mortality prediction, the combined criteria of cROCK and KDIGO were the best, with an AUC of 0.891, an optimal cutoff value of 0.382,
Figure 3. Impact of the KDIGO and cROCK criteria on predicting 3-year clinical outcomes in patients with chronic kidney disease stage 3. (A) Renal composite endpoints. (B) Major adverse cardiovascular events (MACEs), including cardiovascular death, myocardial infarction, and stroke. (C) All-cause mortality.
cROCK, reference change value of the serum creatinine optimized criteria for acute kidney injury in chronic kidney disease; KDIGO, Kidney Disease Improving Global Outcomes.

Discussion

The incidence and prevalence of CKD have increased rapidly during the past decades, and the rise in CKD burden has had a major impact on global health, resulting in increased mortality and morbidity rates worldwide [12].

Patients with preexisting CKD have significantly higher rates of AKI compared to those without CKD, and AKI is an established risk factor for CKD development and progression [13]. CKD progression is preventable and treatable by correcting modifiable (e.g., lifestyle, comorbidities, metabolism) risk factors [14]. Therefore, early detection of AKI in CKD patients is crucial for these clinical applications in order to improve patient prognosis.
CKD stage 3 is the earliest stage of CKD diagnosed using sCr level, and patients with this stage of disease compose a large proportion of CKD patients. In CKD stage 3, renal function usually remains stable for a long time; however, AKI occurrence may cause rapid deterioration of renal function. Patients with CKD stages 4 to 5 were excluded from the study because it is difficult to define natural disease progression when the sCr level is increased. Therefore, our study only included adult patients with CKD stage 3.

Current AKI criteria, such as the RIFLE, AKIN, and KDIGO criteria, were developed mainly based on data from global participants without pre-existing CKD. Currently, the KDIGO criteria is one of the most widely used AKI criteria even in CKD patients in clinical practice [8]. Few studies have focused on the AKI definition in CKD patients. The criteria for AKI are controversial because of the variability of baseline sCr level in CKD patients, which influences the RCV of sCr and AKI diagnosis. An ideal AKI definition might have the following features: 1) limited complexity, 2) diagnosis associated with short- or long-term outcomes, 3) high diagnostic accuracy with high sensitivity and specificity, and 4) relatively low cost of diagnosis [15]. The cROCK criteria were designed based on data from a large-scale multicenter study cohort of Chinese hospitalized adult patients, and a significant proportion of these participants was adult CKD patients with initial eGFR of ≤60 mL/min/1.73 m² [10]. Theoretically, the cROCK criteria might be more suitable than the KDIGO criteria for AKI detection in Chinese CKD patients, and the combination of cROCK and KDIGO criteria might perform even better than a single AKI criterion. However, no study has compared the effects of these criteria alone and in combination in diagnosing ACKD, such as the detection rate, the associations between ACKD and long-term clinical outcomes, and the predictive ability for long-term clinical outcomes.

The novel cROCK criteria define ACKD in CKD adults by a ≥25% increase in baseline sCr level over 7 days [10], while the KDIGO criteria defines AKI by a ≥50% increase in baseline sCr level over 7 days. The KDIGO criteria also define AKI as an increase in the sCr level of ≥26.5 µmol/L within 48 hours [8], which requires a lower threshold; how-
ever, most patients lack repeated sCr testing results within 48 hours. Generally, the cROCK criteria should be more sensitive than the KDIGO criteria in detecting ACKD as it requires a lower threshold for an increase in sCr level (25% vs. 50%); therefore, the cROCK criteria could identify more ACKD events than the KDIGO criteria. Our results showed that the cROCK criteria detected 8.5% more ACKD events than the KDIGO criteria, indicating that the cROCK criteria were more sensitive to detecting AKI in patients with CKD stage 3.

Harrell C-index is an indicator that estimates the predictive accuracy of models [16]. A C-index value of 0.5 indicates accuracy similar to a random guess, while a C-index value of 1.0 indicates 100% predictive accuracy. In this study, during the 3-year follow-up period, all C-index values between the cROCK (+), KDIGO (+), and KDIGO (+) & cROCK (+) groups had statistically significant differences (all p < 0.05); among these, the C-index values of the KDIGO (+) & cROCK (+) group were highest for predicting renal composite endpoints, MACEs, and all-cause mortality, indicating that combining the cROCK and KDIGO criteria achieved the highest accuracy of predictive power for these three clinical outcomes.

Among the four groups in Fig. 3, ACKD diagnosed by the combined criteria of cROCK and KDIGO had the lowest survival probability of renal composite endpoints, MACEs, and all-cause mortality. Moreover, as in Fig. 4, the cROCK criteria led to the highest AUC for predicting renal composite endpoints, and the combined criteria of cROCK and KDIGO led to the highest AUC for predicting MACEs and all-cause mortality, demonstrating that the cROCK criteria and the combination of the cROCK and KDIGO criteria had greater diagnostic accuracy with high sensitivity and specificity in predicting long-term outcomes compared to the KDIGO criteria. In addition, the cROCK criteria are defined by the RCV of sCr, and its application is simple and inexpensive. Above all, the cROCK criteria might be a reasonable AKI definition for CKD patients, and it could be applied alone or in combination with the KDIGO criteria in clinical practice.

This study has some limitations. First, this was a retrospective observational study based on an EMR dataset from a single medical center, and the performance of cROCK criteria in predicting long-term clinical outcomes in ACKD patients cannot be guaranteed in all clinical studies. Second, only 812 Chinese patients with CKD stage 3 were enrolled in the study, the heterogeneity of enrolled patients might be high, and a large proportion of patients was excluded due to incomplete data or loss of follow-up, so there might be selection bias in the study population. Third, sCr could be influenced by multiple factors, e.g., secretion from renal tubules, muscle mass, consumption of protein-containing meals, and physical activity, which might reduce its accuracy in evaluating the changes in renal function. Moreover, further studies are needed to evaluate and validate the application of the novel cROCK criteria in larger CKD populations with longer-term follow-up.

In summary, our findings demonstrated that the cROCK criteria could be applied for risk stratification of CKD patients according to ACKD status. The cROCK criteria strengthened the associations between ACKD diagnosis and long-term clinical outcomes, and the combination of the cROCK and KDIGO criteria in ACKD diagnosis might further improve the predictive ability for 3-year clinical outcomes of renal composite endpoints, MACEs, and all-cause mortality.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This research was funded by the National Natural Science Foundation of China (grant no. 81600540), the Jiangsu Provincial Commission of Health (grant no. ZD2022044), the Project of Philosophy and Social Science Research in Colleges and Universities in Jiangsu Province (grant no. 2021SJA1096), the Science and Technology Foundation of Xuzhou City (grant nos. KC20182 and KC21186), the Open Project of Key Laboratories in Jiangsu Province (grant no. XZSYSKF2021031), and the Science and Technology Foundation of the Xuzhou Health Committee (grant no. XWKY-HT2020020).

**Data sharing statement**

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

Conceptualization: LS
Formal analysis, Funding acquisition: LS, LXZ
Methodology: LS, RXH, YW
Writing—original draft: RXH, LS
Writing—review & editing: LS, LXZ
All authors read and approved the final manuscript.

ORCID

Ling Sun, https://orcid.org/0000-0002-5276-1309
Rui-Xue Hua, https://orcid.org/0000-0001-9307-1024
Yu Wu, https://orcid.org/0000-0002-8777-7191
Lu-Xi Zou, https://orcid.org/0000-0001-9974-2642

References

COVID-19 incidence and outcomes among patients with kidney replacement therapy

Siribha Changsirikulchai¹, Pornpen Sangthawan², Jirayut Janma¹, Songyos Rajborirug³, Thammasin Ingviya⁴

¹Division of Nephrology, Department of Medicine, Faculty of Medicine, Srinakharinwirot University, Nakhonnayok, Thailand
²Division of Nephrology, Department of Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand
³Department of Epidemiology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand
⁴Department of Family and Preventive Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

Background: We aimed to investigate the incidence, fatality, and associated factors in patients with hemodialysis (HD), peritoneal dialysis (PD), and kidney transplantation (KT) hospitalized for coronavirus disease 2019 (COVID-19) infection and reimbursed from the National Health Security Office (NHSO).

Methods: The retrospective cohort analysis was conducted from an electronic-claimed database, and COVID-19 vaccination status was evaluated in patients with HD, PD, and KT from January 2020 to December 2021. There were 85,305 patients reimbursed for HD, PD, and KT by the NHSO. The rates of COVID-19 infection, COVID-19 vaccination, comorbidities, fatalities, and the cost of treatment were evaluated.

Results: COVID-19 infection was observed in 1,799 of 36,982 HD cases (4.9%), 1,531 of 45,453 PD cases (3.4%), and 95 of 2,870 KT cases (3.3%). Patients receiving COVID-19 vaccinations were most common in the KT group, followed by those with HD and PD (76.93% vs. 70.65% vs. 51.34%, respectively). KT patients had a lower fatality rate compared to those with PD and HD (8.42% vs. 18.41% vs. 21.40%, respectively). Advanced age, diabetes, cardiovascular diseases, and COVID-19 vaccination status were associated with fatality. The adjusted odds ratios of fatality after receiving one or two doses of vaccines were 0.7 (95% confidence interval [CI], 0.6–0.9) and 0.3 (95% CI, 0.2–0.4), respectively. The cost of treatment was highest in patients with HD, followed by PD and KT.

Conclusion: The incidence of COVID-19 infection was higher in patients with HD than in those with PD or KT. COVID-19 vaccination following the national health policy should be encouraged for these patients to prevent fatality.

Keywords: COVID-19, Dialysis, Kidney transplantation, Peritoneal dialysis, Renal dialysis, Renal replacement therapy

Introduction

Coronavirus disease 2019 (COVID-19) infection is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was first recognized in December 2019 in Wuhan, China [1]. Patients with end-stage kidney disease (ESKD) have high mortality rates following COVID-19 infection; mortality rates in previous cohort studies were...
20% to 30% [2–6]. The risk factors related to death from COVID-19 infections are increasing age, comorbid conditions, and frailty [7–11]. Patients with maintenance dialysis are susceptible to COVID-19 infection as they have difficulty preventing COVID-19 transmission [12]. Patients with hemodialysis (HD) have frequent visits to dialysis units to get treatment and have limited abilities to perform physical distancing measures in closed spaces [9,10,13]. The rates of COVID-19 infection in patients with peritoneal dialysis (PD) are lower than those with HD [14–16]. PD is a home-based dialysis modality, so the risks of exposure to highly transmissible infections are less than those for HD [16,17]. Patients with kidney transplantation (KT) have risks of critical illness from COVID-19 infection due to their use of immunosuppressive medication [18,19].

In Thailand, the first case of COVID-19 infection was confirmed in mid-January 2020 [20]. The first wave of the COVID-19 pandemic from a domestic infection occurred in March 2020 [21]. The Thai government responded rapidly to mitigate the COVID-19 pandemic by using legislative, social, and health measures, in addition to full-scale lockdown measures [21]. The second wave of the pandemic was triggered in December 2020 by illegal migrants, which led to widespread infections. The national health strategies used public health measures to reduce COVID-19 transmission rather than locking down the entire country [22]. Public and private health resources were used to identify index cases and tracers, test all high-risk contacts, and isolate cases of COVID-19 infection. Early hospitalization and treatment would be provided to infected patients with severe comorbidities [21,23].

The kidney replacement therapy (KRT) of Thai patients with ESKD is funded by the national health budget through one of the three healthcare schemes (the civil servant medical health scheme, social security health scheme, and universal health coverage scheme [UHC]). The majority of people are reimbursed by the National Health Security Office (NHSO) under the UHC. The modalities of KRT in these patients include HD, PD, and KT. PD is the only home dialysis modality, while HD is performed for in-hospital dialysis or outpatient dialysis clinics. Protocols to prevent COVID-19 infection in dialysis units were implemented during the outbreak [24,25]. Patients with HD, PD, or KT were encouraged to receive COVID-19 vaccinations. COVID-19 infections were diagnosed by the positive reverse transcriptase-polymerase chain reaction (RT-PCR) test. The “PD First” policy was adopted as the main dialysis modality in patients with ESKD under the UHC in 2008. It was changed to a shared decision-making policy in February 2022. We are interested in studying the outcomes of the COVID-19 pandemic across different KRT modalities during the period of the PD First policy. The results may be different from previous studies. In addition, it could provide information to healthcare providers to assist in the prevention of COVID-19 outbreaks, which may occur from crowded patients with HD when the policy is changed in the future. This study aims to investigate the incidence rates, fatality, and factors associated with death among patients with PD, HD, and KT who were hospitalized due to COVID-19 infection. We also analyzed and compared the cost of treatment during hospitalization for these patients.

Methods

This study was approved in the exempt category by the Institutional Review Committee for Research in Human Subjects, Faculty of Medicine, Srinakharinwirot University (No. SWUEC-M-067/2565X). As the study subjects were de-identified, the need for written consent from the patients was waived.

Study population

The data of patients receiving KRT who were admitted due to COVID-19 from January 2020 to December 2021 were retrospectively reviewed. All patients with KRT who had COVID-19 infections were hospitalized because they were considered high-risk patients. The cost of hospitalization was claimed by the NHSO. The sources of databases for analysis were the files of inpatients (electronic-claimed data), files of patients with a history of COVID-19 vaccination, and files of KRT reimbursement that were retrieved by the NHSO. The NHSO granted only one of our investigators (TI) access to the database with this patient information. The personal data of each patient was redacted before data sharing. All analyses were performed on the cloud using the Rstudio server provided by the NHSO, where SQL was used to query data from the NHSO data storage server. No data were transferred to any of the researchers’ personal computers or notebooks. We selected patients at least 18
years of age receiving either HD, PD, or KT. The Interna-
tional Statistical Classification of Diseases and Related
Health Problems, 10th Revision (ICD-10) codes U071,
U072, U099, and U109 were used to identify patients with
COVID-19 infections who tested COVID-19-positive by RT-
PCR and were admitted to hospitals. They were de-identi-
fied by the NHSO data controller before analysis.

Data collection and variables

The following demographic and clinical characteristics of
the patients were collected: age at hospital admission due
to COVID-19 infection, sex, comorbidities, complications,
history of COVID-19 vaccination, and KRT modalities. The
type of KRT in each patient was determined from the mo-
dality of long-term chronic dialysis and KT at the first date of
hospital admission due to COVID-19. Comorbidities were
declared according to the ICD-10 codes for diabetes, car-
diovascular disease, cerebrovascular disease, malignancy,
hypertension, airway disease, liver disease, human immu-
nodeficiency virus disease, and psychiatric problems. The
complications from COVID-19 infection were classified as
sepsis, pneumonia, respiratory failure, volume overload,
and heart failure. The type and number of COVID-19 vac-
cinations prior to or after COVID-19 infection were evalu-
ated. In addition, the time intervals between the date of the
last vaccination and the date of admission were assessed.

Outcomes and definitions

The primary outcome was fatality rates during admission
and associated factors. The secondary outcomes were the
length of hospitalization and the cost of treatment. The
length of hospitalization was calculated from the first date
of admission to the date of discharge or death. The cost of
treatment due to COVID-19 infection was calculated from
the total charges from hospitals, which included the cost of
personal protective equipment during hospitalization. The
cost of dialysis or immunosuppressive drugs prescribed
to KT patients was calculated from the file of the KRT re-
bursement. The incidence rates and primary and sec-
ondary outcomes of COVID-19 infection were compared
among PD, HD, and KT groups. Age, sex, comorbidities,
the number of vaccinations, and types of COVID-19 vac-
cines were assessed as being associated with fatalities.

These factors were analyzed separately in HD, PD, and all
modalities of KRT.

Statistical analysis

Descriptive analysis is presented as numbers with per-
centages for categorical variables and median with inter-
quartile range (IQR) for continuous variables. The case
fatality rate was calculated from the number of in-hospital
deaths divided by the total number of patients admitted
after COVID-19 diagnosis and presented as percentages.
The comorbidity conditions, complications during hos-
pitalization, and cost of treatment were compared among
PD, HD, and KT by chi-square test for categorical variables
and t test or Wilcoxon rank-sum test for continuous vari-
ables. The factors associated with death in hospitalization
were determined by using logistic regression models and
presented as odd ratios (ORs) and 95% confidence inter-
vals (95% CIs) with adjustments including variables that
were significant in the univariate analysis. The variables
for the final multivariate model were selected using the
backward stepwise method based on Akaike Information
Criteria [26]. The missing data were verified and managed
by the NHSO since they were used to reimburse the cost
of treatment. The final data set for analysis had no miss-
ing data. All statistical analyses were performed using the
R program version 3.6.2 (R Core Team). A p-value of less
than 0.05 was considered statistically significant.

Results

Patient characteristics and incidence of COVID-19 infection

Fig. 1 shows the percentages of COVID-19 infections and
vaccinations in KRT patients classified into PD, HD, and
KT groups. There were 85,305 patients receiving KRT from
January 2020 to December 2021. The number of patients
with PD was 45,453 (53.3%), that of HD was 36,982 (43.3%),
and that of KT was 2,870 (3.4%). A total of 3,425 patients
(4.0%) with either PD, HD, or KT were admitted due to
COVID-19 infection. All cases had only a single COVID-19
infection during the period of the study. The number of
HD, PD, and KT patients infected with COVID-19 was 1,799
(4.9%), 1,531 (3.4%), and 95 (3.3%), respectively. The per-
centage of hospital admissions due to COVID-19 infection
Figure 1. Percentage of patients with different types of kidney replacement therapy receiving COVID-19 vaccination and becoming infected with COVID-19.

p < 0.001 compared between HD and PD.
COVID 19, coronavirus disease 2019; HD, hemodialysis; KT, kidney transplantation; PD, peritoneal dialysis.

in patients with PD was significantly less than in those with HD. The total number of KRT patients receiving COVID-19 vaccinations was 51,671 (60.6%). The number of patients with KT and vaccinations was 2,208 out of 2,870 cases (76.9%), that of HD was 26,126 out of 36,982 cases (70.7%), and that of PD was 23,337 out of 45,453 cases (51.3%). The percentage of vaccinated patients was highest in the KT group, followed by the HD and PD groups. There were 1,997 out of 51,671 KRT patients (3.9%) who received vaccinations and became infected with COVID-19. The number of COVID-19 infections in vaccinated patients with PD was 744 out of 23,337 cases (3.2%), that of HD was 1,173 out of 26,126 cases (4.5%), and that of KT was 80 out of 2,208 cases (3.6%). The percentage of vaccinated patients with PD who became infected with COVID-19 was significantly lower than that of those with HD or KT.

Table 1 shows the characteristics of patients infected with COVID-19. The median age at the admission of these patients was 58 years (IQR, 48–67 years), which was lowest in patients with KT, followed by those with PD and HD. Patients with KT tended to have less severe comorbidity conditions than those with HD or PD. The complications during hospitalization were not different among PD, HD, and KT patients. Table 2 shows the fatality rates, length of admission, and cost of treatment in patients with COVID-19 infection during hospitalization. The median length of hospitalization across all modalities of KRT was 13 days (IQR, 7–17 days). The median cost of treatment in a hospital, not including the cost of dialysis (calculated in US dollars [USD]; 1 USD is equal to 35 baht), in patients with HD was 1,458.03 USD (IQR, 274.28–3,831.94 USD). This was higher than in those with PD or KT. The median costs of treatment in patients with PD and KT were 1,272.63 USD (IQR, 298.71–3,058.71 USD) and 761.20 USD (IQR, 285.71–4,431.34 USD), respectively. The case fatality rate in patients with HD was higher than in those with PD (21.4% vs. 18.41%), while it was lowest in patients with KT (8.4%). The median length of admission in patients who died during treatment was 11 days (IQR, 6–19 days). The median cost of treatment in patients who died during treatment was 2,528.57 USD (IQR, 1,046.40–5,194.29 USD), and this cost was the highest in those with KT.
Table 1. Characteristics of comorbidities, complications, fatality rates, length of admission, and cost of treatment in patients with COVID-19 infection requiring hospitalization

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PD group</th>
<th>HD group</th>
<th>KT group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>1,531</td>
<td>1,799</td>
<td>95</td>
<td>3,425</td>
</tr>
<tr>
<td>Age at admission for COVID-19 infection (yr)(^a)</td>
<td>57 (47–66)</td>
<td>60 (50–69)</td>
<td>46 (33–58)</td>
<td>58 (48–67)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes(^b)</td>
<td>988 (64.5)</td>
<td>1,159 (64.4)</td>
<td>24 (25.3)</td>
<td>2,276 (63.3)</td>
</tr>
<tr>
<td>Cardiovascular diseases(^b)</td>
<td>600 (39.2)</td>
<td>824 (45.8)</td>
<td>17 (17.9)</td>
<td>1,497 (41.6)</td>
</tr>
<tr>
<td>Cerebrovascular diseases</td>
<td>229 (15.0)</td>
<td>260 (14.5)</td>
<td>10 (10.5)</td>
<td>517 (14.4)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>48 (3.1)</td>
<td>86 (4.8)</td>
<td>1 (1.1)</td>
<td>152 (4.2)</td>
</tr>
<tr>
<td>Hypertension(^b)</td>
<td>1,494 (97.6)</td>
<td>1,716 (95.4)</td>
<td>72 (75.8)</td>
<td>3,432 (95.4)</td>
</tr>
<tr>
<td>Airway diseases(^b)</td>
<td>162 (10.6)</td>
<td>186 (10.3)</td>
<td>1 (1.1)</td>
<td>366 (10.2)</td>
</tr>
<tr>
<td>Liver diseases</td>
<td>227 (14.8)</td>
<td>329 (18.3)</td>
<td>14 (14.7)</td>
<td>599 (16.7)</td>
</tr>
<tr>
<td>HIV infection</td>
<td>9 (0.6)</td>
<td>3 (0.2)</td>
<td>0 (0)</td>
<td>12 (0.4)</td>
</tr>
<tr>
<td>Psychiatric problem</td>
<td>84 (5.5)</td>
<td>128 (7.1)</td>
<td>3 (3.2)</td>
<td>219 (6.1)</td>
</tr>
<tr>
<td>Complications during hospitalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>243 (15.9)</td>
<td>253 (14.1)</td>
<td>11 (11.6)</td>
<td>528 (14.7)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1,062 (69.4)</td>
<td>1,299 (72.2)</td>
<td>60 (63.2)</td>
<td>2,530 (70.3)</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>42 (2.7)</td>
<td>55 (3.1)</td>
<td>5 (5.3)</td>
<td>106 (2.9)</td>
</tr>
<tr>
<td>Volume overload</td>
<td>112 (7.3)</td>
<td>113 (6.3)</td>
<td>1 (1.1)</td>
<td>235 (6.5)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>45 (2.9)</td>
<td>67 (3.7)</td>
<td>2 (2.1)</td>
<td>131 (3.8)</td>
</tr>
</tbody>
</table>

Data are expressed as number only, median (interquartile range), or number (%).
COVID 19, coronavirus disease 2019; HD, hemodialysis; HIV, human immunodeficiency virus; KT, kidney transplantation; PD, peritoneal dialysis.
\(^a\)p < 0.05 compared between PD and HD. \(^b\)p < 0.05 compared among PD, HD, and KT.

Table 2. The fatality rates, length of admission, and cost of treatment in patients with COVID-19 infection requiring hospitalization

<table>
<thead>
<tr>
<th>Outcome</th>
<th>PD group (n = 1,531)</th>
<th>HD group (n = 1,799)</th>
<th>KT group (n = 95)</th>
<th>Total (n = 3,425)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of admission (day)</td>
<td>12 (8–17)</td>
<td>13 (8–18)</td>
<td>12 (6–16)</td>
<td>13 (7–17)</td>
</tr>
<tr>
<td>Cost of treatment in all cases(^a) (USD)</td>
<td>2,722.63 (298.71–3,058.71)</td>
<td>1,458.03 (274.28–3,831.94)</td>
<td>761.20 (285.71–4,431.34)</td>
<td>1,314.57 (273.40–3,397.00)</td>
</tr>
<tr>
<td>No. of patients that died from COVID-19(^a)</td>
<td>282 (18.4)</td>
<td>385 (21.4)</td>
<td>8 (8.4)</td>
<td>675 (19.7)</td>
</tr>
<tr>
<td>Days to death during hospitalization (day)</td>
<td>11 (6–21)</td>
<td>11 (5–18)</td>
<td>14 (8–23)</td>
<td>11 (6–19)</td>
</tr>
<tr>
<td>Cost of treatment in cases resulting in death (USD)</td>
<td>2,605.60 (1,245.59–5,179.00)</td>
<td>2,781.66 (906.57–5,064.04)</td>
<td>5,292.09 (887.29–7,488.05)</td>
<td>2,528.57 (1,046.40–5,194.29)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%).
COVID 19, coronavirus disease 2019; HD, hemodialysis; KT, kidney transplantation; PD, peritoneal dialysis; USD, US dollars (1 USD equals 35 baht).
\(^a\)p < 0.05 compared between PD and HD. \(^b\)p < 0.05 compared among PD, HD, and KT.

Characteristics of COVID-19 vaccination in patients with COVID-19 infection

Table 3 shows the characteristics of COVID-19 vaccination in patients with PD, HD, and KT who developed COVID-19 infections. Most of them did not receive COVID-19 vaccinations or received a single dose of vaccination before COVID-19 infection. The number of patients with PD, HD, and KT who did not receive vaccination prior to contacting COVID-19 was 1,966 (57.40%). The percentage of unvaccinated patients who were admitted due to COVID-19 infection was highest in the PD group (66.5%), followed by the KT (51.6%) and HD groups (50.0%). The number of patients with PD, HD, and KT who received at least one vaccination dose before COVID-19 infection was 1,459 (42.6%). The percentage of patients who received a COVID-19 vaccination before infection was highest in those with HD (50.0%), followed by those with KT and PD (48.4% and...
There were 538 patients (15.7%) who had COVID-19 infections and received COVID-19 vaccinations after they were discharged from the hospital. The total number of patients with PD, HD, and KT receiving vaccination before or after COVID-19 infection was 1,997 (58.3%). Most patients received live, attenuated, or viral vector vaccines. The median interval between the date of the last vaccination and the date of admission was 30 days (IQR, 15–58 days), which was the longest in patients with PD, followed by those with HD and KT.

### Characteristics of patients with fatal COVID-19 infections and associated factors

Table 4 shows the characteristics of KRT patients with COVID-19 infection who died during admission compared with those who survived at hospital discharge. Patients in the survival group had a significantly lower age at the time of COVID-19 infection; a higher chance of being vaccinated against COVID-19, especially with double doses of the vaccine; and a longer interval between the last date of vaccination and date of admission than those in the death group. The case fatality rate in patients who did not receive COVID-19 vaccinations was 23.1%; in contrast, it was 15.1% for those who received COVID-19 vaccinations. There were only 59 patients in the dead and surviving groups who received the messenger RNA (mRNA) vaccine. It was a small number, and there was not a significant difference between those who died and survived.

The factors associated with death in KRT patients after COVID-19 infection are shown in Table 5. Increased age, diabetes, and cardiovascular diseases were demonstrated as significant factors associated with death. The modes of KRT were not factors associated with death after being adjusted for age, comorbidities, and the status of COVID-19 vaccination. Receiving COVID-19 vaccination or the interval between the date of the last vaccination and the date of admission for COVID-19 were protective factors against death after COVID-19 infection. The factors associated with fatality in the subgroup analysis of patients with HD and PD have shown similar results (Supplementary Table 1, available online). The adjusted OR of fatality in HD patients with double COVID-19 vaccinations was 0.32 (95% CI, 0.21–0.49). The adjusted ORs of fatality in PD patients with COVID-19

### Table 3. Characteristics of COVID-19 vaccination among patients with COVID-19 infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>PD group (n = 1,531)</th>
<th>HD group (n = 1,799)</th>
<th>KT group (n = 95)</th>
<th>Total group (n = 3,425)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalizing COVID-19 infections (%)</td>
<td>44.7</td>
<td>52.5</td>
<td>2.8</td>
<td>100</td>
</tr>
<tr>
<td>Cases without vaccination before infection †</td>
<td>1,018 (66.5)</td>
<td>899 (50.0)</td>
<td>49 (51.6)</td>
<td>1,966 (57.4)</td>
</tr>
<tr>
<td>Cases of vaccination before infection</td>
<td>513 (33.5)</td>
<td>900 (50.0)</td>
<td>46 (48.4)</td>
<td>1,459 (42.6)</td>
</tr>
<tr>
<td>Single dose</td>
<td>326 (63.6)</td>
<td>594 (66.0)</td>
<td>31 (67.4)</td>
<td>951 (65.2)</td>
</tr>
<tr>
<td>Two doses</td>
<td>181 (35.3)</td>
<td>298 (33.1)</td>
<td>15 (32.6)</td>
<td>494 (33.9)</td>
</tr>
<tr>
<td>At least three doses</td>
<td>6 (1.2)</td>
<td>8 (0.9)</td>
<td>0 (0)</td>
<td>14 (1.0)</td>
</tr>
<tr>
<td>Cases of vaccination after infection †</td>
<td>231 (15.1)</td>
<td>273 (15.2)</td>
<td>34 (35.8)</td>
<td>538 (15.7)</td>
</tr>
<tr>
<td>Cases of vaccination before or after infection</td>
<td>744 (48.6)</td>
<td>1,173 (65.2)</td>
<td>80 (84.2)</td>
<td>1,997 (58.3)</td>
</tr>
<tr>
<td>Cases of only live, attenuated vaccine</td>
<td>131 (8.6)</td>
<td>187 (10.4)</td>
<td>13 (13.7)</td>
<td>331 (9.7)</td>
</tr>
<tr>
<td>administration †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases of only viral vector vaccine</td>
<td>289 (18.9)</td>
<td>605 (33.6)</td>
<td>28 (29.5)</td>
<td>922 (26.9)</td>
</tr>
<tr>
<td>administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases of only mRNA vaccine administration</td>
<td>29 (1.9)</td>
<td>25 (1.4)</td>
<td>2 (2.1)</td>
<td>56 (1.6)</td>
</tr>
<tr>
<td>Cases of mixed-type vaccine administration</td>
<td>1 (0.1)</td>
<td>2 (0.1)</td>
<td>0 (0)</td>
<td>3 (0.1)</td>
</tr>
<tr>
<td>with any mRNA vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases of mixed-type vaccine administration</td>
<td>63 (4.1)</td>
<td>81 (4.5)</td>
<td>3 (3.2)</td>
<td>147 (4.3)</td>
</tr>
<tr>
<td>without any mRNA vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases of any mRNA vaccine administration</td>
<td>30 (2.0)</td>
<td>27 (1.5)</td>
<td>2 (2.1)</td>
<td>59 (1.7)</td>
</tr>
<tr>
<td>Interval between the last vaccination and</td>
<td>34.0 (16.0–61.0)</td>
<td>28.5 (14.0–56.0)</td>
<td>24.0 (15.0–38.3)</td>
<td>30.0 (15.0–58.0)</td>
</tr>
<tr>
<td>admission date (day) †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

COVID-19, coronavirus disease 2019; HD, hemodialysis; KT, kidney transplantation; mRNA, messenger RNA; PD, peritoneal dialysis.

† p < 0.05.
Changsirikulchai, et al. COVID-19 incidence and outcomes in KRT

Discussion

We report the incidence of COVID-19 infection, case fatality rates, and associated factors in patients with HD, PD, and KT during 2 years of the COVID-19 pandemic in Thailand. We found that the overall incidence of COVID-19 infection in patients with KRT (HD, PD, and KT) was 4% compared to the rate of 3.4% for the general population nationwide [22,27]. This incidence was probably underestimated due to the fact that some patients died at home. This happened despite the national policy response to the COVID-19 pandemic during the period of study, including contact tracing by surveillance and rapid response team engagement from village health volunteers to identify, isolate, and quarantine cases and admit all cases with positive RT-PCR results. The in-hospital case fatality rate in these patients was 19.7% versus 1% for nationwide patients [22]. The incidence and fatality rates of COVID-19 infections varied among countries and the times of outbreak [4,9,10,15,16,20,28,29]. There are reasons to explain these variations. The incidence and mortality of COVID-19 may be impacted by the enrollment of patients at different times during outbreaks, as this could be influenced by the severity of virulence and transmission diversity of the disease. The preparedness of national health policies, the adherence of people to preventive measures, and the availability of healthcare facilities, e.g., intensive care units or mechanical ventilation, could also affect outcomes [9,10,20,28,29].

In Thailand, the number of COVID-19 diagnoses was much higher in the second wave compared to the first wave. There were only 3,042 cumulative cases of COVID-19 infection with 57 deaths (1.5% case fatality rate) country-wide, and only eight patients with KRT had COVID-19 infections in the first wave [21]. In contrast, the total number of cases of COVID-19 infection in the second wave was more than two million cases by the end of December 2021, which led to increasing numbers of KRT patients infected with COVID-19 [22]. We suspect that the main cause of the increasing incidence of COVID-19 infection in the second wave was the highly contagious COVID-19 clades due to differences in the processes of lockdown between the first and second waves of the COVID-19 outbreaks [30]. The nationwide lockdown included curfews between 10.00 PM to 04.00 AM, canceling national holidays, and suspending international flights for tourists, which led to a huge negative impact on the economy during the first wave. Therefore, the policy was changed to lockdown specific areas with COVID-19 outbreaks, find active cases, speed up COVID-19 vaccinations for the elderly and patients with vaccination (one and double doses) were 0.46 (95% CI, 0.31–0.66) and 0.24 (95% CI, 0.12–0.42), respectively.

Table 4. Characteristics of patients with fatal and non-fatal hospitalizing COVID-19 infections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dead group (n = 675)</th>
<th>Surviving group (n = 2,750)</th>
<th>Total (n = 3,425)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(^a) (yr)</td>
<td>63 (55–71)</td>
<td>57 (47–66)</td>
<td>58 (48–67)</td>
</tr>
<tr>
<td>Vaccination status(^a)</td>
<td>221 (32.7)</td>
<td>1,238 (45.0)</td>
<td>1,459 (42.6)</td>
</tr>
<tr>
<td>No. of patients with COVID-19 vaccination</td>
<td>454 (67.3)</td>
<td>1,512 (55.0)</td>
<td>1,966 (57.4)</td>
</tr>
<tr>
<td>No. of patients without COVID-19 vaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doses of vaccination(^a)</td>
<td>178 (80.5)</td>
<td>773 (62.4)</td>
<td>951 (65.2)</td>
</tr>
<tr>
<td>No. of patients with a single dose</td>
<td>42 (19.0)</td>
<td>452 (36.5)</td>
<td>494 (33.9)</td>
</tr>
<tr>
<td>No. of patients with two doses</td>
<td>1 (0.5)</td>
<td>13 (1.1)</td>
<td>14 (1.0)</td>
</tr>
<tr>
<td>No. of patients with at least three doses</td>
<td>5 (2.3)</td>
<td>54 (4.4)</td>
<td>59 (4.0)</td>
</tr>
<tr>
<td>Any mRNA vaccine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interval between the last vaccination and admission date (day)(^a)</td>
<td>24.0 (12.0–45.0)</td>
<td>32.0 (16.0–59.8)</td>
<td>30.0 (15.0–58.0)</td>
</tr>
</tbody>
</table>

Data are expressed as median (interquartile range) or number (%).
COVID 19, coronavirus disease 2019; mRNA, messenger RNA; NA, not available.
\(^a\)p < 0.05 compared between dead and surviving groups.
chronic disease, and practice early admission for treatment in patients with a high risk of mortality in the second wave of the COVID-19 pandemic. Patients with KRT who were infected with COVID-19 and confirmed by RT-PCR would be admitted to hospitals and receive treatment early as they were considered vulnerable people with high risks of mortality.

Patients with PD, HD, and KT had different incidence rates of COVID-19 infection in our study, which are similar to previous publications [9,10,28,29]. The proportions of KRT modalities in the 3,425 patients with COVID-19 infection were 52.5% from HD, 44.7% from PD, and 2.8% from KT. Patients with HD had a higher chance of COVID-19 infection than those with PD. The main reasons were that they had to travel two to three times per week and remained in clinics for at least 4 hours in each HD session to get dialyzed. It was not from a greater opportunity to screen for COVID-19 in patients with HD since the national health policy was that the test would be done in all index cases and cases with high-risk contacts. RT-PCR was the only method used to test for COVID-19 during the study period. Antigen test kits were not available at that time; therefore, there was no policy to test patients with HD regularly during the second wave of COVID-19. Our study showed that patients with KT had the lowest incidence of COVID-19 infection compared to those with dialysis. We proposed that this was due to KT patients tending to have younger ages and fewer comorbidities than patients with HD or PD [8,31].

Our study showed the results of short-term fatality and risk factors, which were similar to previous reports from other countries. Previous studies showed that the fatality

<table>
<thead>
<tr>
<th>Factor</th>
<th>Crude OR (95% CI)</th>
<th>p-value</th>
<th>Adjusted OR* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03 (1.03–1.04)</td>
<td>&lt;0.001</td>
<td>1.03 (1.02–1.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.96 (0.81–1.13)</td>
<td>0.60</td>
<td>0.85 (0.71–1.02)</td>
<td>0.08</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.63 (1.35–1.96)</td>
<td>&lt;0.001</td>
<td>1.26 (1.03–1.53)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1.64 (1.38–1.94)</td>
<td>&lt;0.001</td>
<td>1.44 (1.20–1.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>1.33 (1.06–1.67)</td>
<td>0.01</td>
<td>1.16 (0.92–1.47)</td>
<td>0.20</td>
</tr>
<tr>
<td>Malignancy</td>
<td>0.97 (0.62–1.48)</td>
<td>0.90</td>
<td>0.90 (0.56–1.39)</td>
<td>0.60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.45 (0.92–2.38)</td>
<td>0.12</td>
<td>0.96 (0.60–1.62)</td>
<td>0.90</td>
</tr>
<tr>
<td>Airway disease</td>
<td>0.91 (0.68–1.20)</td>
<td>0.50</td>
<td>0.76 (0.56–1.02)</td>
<td>0.07</td>
</tr>
<tr>
<td>Liver disease</td>
<td>1.01 (0.80–1.26)</td>
<td>&gt;0.90</td>
<td>0.99 (0.78–1.25)</td>
<td>0.90</td>
</tr>
<tr>
<td>HIV</td>
<td>1.36 (0.30–4.57)</td>
<td>0.60</td>
<td>1.39 (0.28–5.42)</td>
<td>0.70</td>
</tr>
<tr>
<td>Psychiatric problem</td>
<td>1.59 (1.16–2.16)</td>
<td>0.003</td>
<td>1.15 (0.83–1.59)</td>
<td>0.40</td>
</tr>
<tr>
<td>Mode of KRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>KT</td>
<td>0.34 (0.15–0.66)</td>
<td>0.004</td>
<td>0.51 (0.22–1.04)</td>
<td>0.09</td>
</tr>
<tr>
<td>PD</td>
<td>0.83 (0.70–0.98)</td>
<td>0.03</td>
<td>0.85 (0.71–1.02)</td>
<td>0.09</td>
</tr>
<tr>
<td>No. of vaccination doses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No vaccination</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Single dose</td>
<td>0.77 (0.63–0.93)</td>
<td>0.007</td>
<td>0.71 (0.58–0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Double dose</td>
<td>0.31 (0.22–0.43)</td>
<td>&lt;0.001</td>
<td>0.30 (0.21–0.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>At least three doses</td>
<td>0.26 (0.01–1.29)</td>
<td>0.20</td>
<td>0.28 (0.02–1.44)</td>
<td>0.20</td>
</tr>
<tr>
<td>Type of vaccine without mRNA</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>With mRNA</td>
<td>2.68 (1.18–7.73)</td>
<td>0.04</td>
<td>1.50 (0.63–4.42)</td>
<td>0.40</td>
</tr>
<tr>
<td>Interval between the last vaccination and admission date (day)</td>
<td>0.99 (0.98–0.99)</td>
<td>&lt;0.001</td>
<td>0.99 (0.98–0.99)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI, confidence interval; COVID 19, coronavirus disease 2019; HD, hemodialysis; HIV, human immunodeficiency virus; KT, kidney transplantation; KRT, kidney replacement therapy; mRNA, messenger RNA; OR, odds ratio; PD, peritoneal dialysis.

*Reference to calculate ORs. *Adjusted for age, sex, diabetes, cardiovascular disease, cerebrovascular disease, malignancy, hypertension, airway disease, liver disease, HIV, psychiatric problems, mode of KRT, number of vaccination doses, types of vaccine, and interval between the date of the last vaccination and the date of hospital admission due to COVID-19 infection.
rates from COVID-19 infection in patients with dialysis were 20% to 30% [8-10,14,16,28,32-34]. Advanced age, multiple comorbid conditions, diabetes, frailty, and the need for ventilation were risk factors related to death [4,8-10,28,32,33]. The overall fatality rate of COVID-19 infection in our patients with KRT was 19.71%. It was highest in patients with HD, followed by those with PD and KT. Advanced age, diabetes, and cardiovascular diseases were risk factors related to fatality. The KRT modalities were not related to fatality after adjusting for age, comorbidities, and the status of COVID-19 vaccination. COVID-19 vaccination, especially two doses of a vaccine, decreased fatality from COVID-19 infection in patients with PD, HD, and KT. Patients with HD tended to be vaccinated against COVID-19 more than those with PD or KT. This was probably due to the fact that they attended HD clinics two to three times per week, and they had more chances to access the COVID-19 vaccination than patients with PD or KT. Previous studies demonstrated a diminished antibody response and a rapid decline in concentration following COVID-19 vaccination in these patients [35-37]. A current research letter shows that patients with maintenance dialysis developed robust antibody responses after being vaccinated with a booster dose of an mRNA vaccine [38]. We strongly suggest that these patients should be prioritized and encouraged to receive COVID-19 vaccinations with boosters.

We would like to address the limitations of our study. First, we could not calculate the cost of HD and PD from the KRT reimbursement files because we found that there was no reimbursement for the cost of dialysis in 26.2% of PD and 47.2% of HD patients during COVID-19 infection. Although the cost of dialysis was not included in the analysis of expenses for patients with PD, it was still lower than for those with HD. The average costs of PD and HD reimbursed from the NHSO in patients under UHC were 472.57 and 514.29 USD/patient/month, respectively. Second, we could not identify the locations of HD units or whether patients received maintenance HD in-hospital or in outpatient dialysis clinics. This may have impacted the analysis of the risk of infection, as it is increased in crowded dialysis clinics with small areas. Third, we did not evaluate body mass index, frailty parameters, the treatment protocol for COVID-19 in individual cases, medication usage for the treatment of COVID-19 infection, or the use of immunosuppressive medications in patients with KT due to the lack of this information in the database. These may be associated with fatality. Fourth, we reported in-hospital fatality rates, which may be lower than the actual death rates because we could not follow the status of patients after discharge from hospitals. Some patients may have expired shortly after they were discharged from the hospital. Fourth, it is an observational cohort study that could not match patients to compare the effects of KRT modalities on COVID-19 infection. Fifth, we could not compare the risk factors of fatality in KRT patients versus the general population due to the inability to access the national database of COVID-19 infection, treatment, and vaccination. Although this study has several limitations, we believe that it has strengths and benefits to share. First, it is a study from a middle-income country that provides PD, HD, and KT for all under UHC. Second, the databases are from the NHSO, which has covered the majority of KRT patients under UHC. The median cost of in-hospital charges was highest in HD, followed by PD and KT. The cost of treatment during admission for each KRT modality reflects the financial burden of COVID-19 infection. PD had beneficial treatment options and resulted in a lower risk of COVID-19 infection during the pandemic than HD because it is the main home-based dialysis modality available in developing countries. This information should be emphasized during the shared decision-making process regarding KRT modalities. Third, KT should be done early for appropriate patients. Fourth, vulnerable patients should not hesitate to receive available COVID-19 vaccines while awaiting more effective and safer ones.

In conclusion, the COVID-19 pandemic has had an impact on KRT patients. The results from this study provide information on the effects of KRT modalities on COVID-19 incidence, risk factors for in-hospital fatality, and the cost of treatment. COVID-19 vaccinations could reduce fatality in patients with KRT, especially those with advanced age and comorbid conditions. These findings could be of benefit to the healthcare authorities to prepare measures to prevent outbreaks of COVID-19 infection that may occur in crowded patients undergoing HD after the policy changes from PD First to shared decision-making regarding dialysis modalities in the future. Policymakers and healthcare providers should promote KT and home treatment programs, such as PD and telemedicine, to mitigate the crowded dialysis centers. Home treatment programs are strategic
treatments that can reduce the pressure on hospitals from the risk of spreading diseases with high severity and transmission and mitigate the financial burden in case of future pandemic infections.

Conflicts of interest

Siribha Changsirikulchai reports receiving a speaker honorarium from Baxter Healthcare and Fresenius Medical Care, as well as a fee paid by the George Institute for Global Health for serving on their clinical advisory board of Ellen Medical Devices. We would also like to report grants from the Health Systems Research Institute outside of the submitted work. Pornpen Sangthawan reports receiving a speaker honorarium from Baxter Healthcare. Thammasin Ingviya reports having research and workshop grant support from the National Institutes of Health (NIH) (grant No. D43TW009522) for research and training on epidemiology related to TB infection. There are no conflicts of interest related to this research.

Acknowledgments

We would like to acknowledge the National Health Security Office for providing the data for analysis. The interpretation and reporting of these data are solely the responsibility of the authors and do not represent the view of the National Health Security Office or the policy implications of the Thai government. Mr. Robert Cho is acknowledged for manuscript preparation.

Data sharing statement

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

Authors’ contributions

Conceptualization: SC, PS
Data curation: SC, PS, SR, TI
Formal analysis: SR, TI
Methodology: SC, PS, SR, TI, JJ
Writing—original draft: SC
Writing—review & editing: SC
All authors read and approved the final manuscript.

ORCID

Siribha Changsirikulchai, https://orcid.org/0000-0002-2958-3823
Pornpen Sangthawan, https://orcid.org/0000-0002-8229-0536
Jirayut Janma, https://orcid.org/0000-0002-1010-7325
Songyos Rajborirug, https://orcid.org/0000-0002-5000-7409
Thammasin Ingviya, https://orcid.org/0000-0002-8894-3817

References

Unilateral renal displacement in an autosomal dominant polycystic kidney disease patient

Jin Kim, Seong Kwon Ma, Soo Wan Kim, Eun Hui Bae

Department of Internal Medicine, Chonnam National University Hospital, Gwangju, Republic of Korea

A 53-year-old female patient presented at our hospital for further evaluation and management of polycystic kidney and liver disease, initially detected via ultrasound. Her family history revealed a similar pattern of polycystic kidney disease, affecting two sisters, a brother, and her mother. Additionally, her aunt was on hemodialysis due to end-stage kidney disease.

Genetic testing confirmed the autosomal dominant polycystic kidney disease (ADPKD) diagnosis with a PKD1 gene mutation. Initial laboratory tests indicated a serum creatinine level of 0.70 mg/dL, an estimated glomerular filtration rate (eGFR) of 78 mL/min/1.32 m², and a urinary albumin to creatinine ratio of 63.0 mg/g Cr. Given her age and eGFR exceeding 25 mL/min/1.32 m², an enhanced abdomen computed tomography (CT) was performed to determine the total kidney volume, a prerequisite for initiating tolvaptan therapy. The CT scan revealed an enlarged kidney and liver, with numerous cystic lesions of varying sizes (Fig. 1).

Her height-adjusted total kidney volume was 1,163.3 mL/m, classifying her as Mayo class 1D. Consequently, tolvaptan therapy was initiated. The patient’s medical history included her mother’s sudden death. Therefore, a brain

**Figure 1.** Axial and coronal view of the abdominal computed tomography scan taken at the time of diagnosis.
magnetic resonance angiography was performed to evaluate cerebral aneurysms, but there was no abnormality. Two years after taking tolvaptan, a follow-up CT was performed, and we found interesting points. On her past CT scan, the right kidney was on the right side beneath the liver, the recent CT scan showed both kidneys on the left side. This displacement of the right kidney was attributed to the increased number and size of hepatic cysts (Fig. 2). Acquired renal displacement can occur due to the enlargement of surrounding organs or the presence of space-occupying lesions within the abdominal cavity. In this case, the patient’s ADPKD and the progressive hepatomegaly resulting from the expanding hepatic cysts led to the right kidney displacement.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

This study was supported by a grant from the Chonnam National University Hospital Biomedical Research Institute (BCRI-22040).

**Acknowledgments**

This study was approved by the Chonnam National University Hospital (No. CNUH-EXP-2023-166). Informed consent has been obtained from the patient.

**Data sharing statement**

The data used in this article are available from the corresponding author upon reasonable request.

**Authors’ contributions**

Conceptualization, Data curation: JK, EHB
Writing—original draft: SKM
Writing—review & editing: SWK
All authors read and approved the final manuscript.

**ORCID**

Jin Kim, https://orcid.org/0000-0001-5098-4771
Seong Kwon Ma, https://orcid.org/0000-0002-5758-8189
Soo Wan Kim, https://orcid.org/0000-0002-3540-9004
Eun Hui Bae, https://orcid.org/0000-0003-1727-2822
Drug-induced acute pancreatitis (DIAP) is rare, accounting for 0.1%–2% of acute pancreatitis, and is a diagnostic challenge to clinicians. Trimethoprim/sulfamethoxazole (TMP/SMX) is classified as a drug with the highest level of evidence [1–3]. Hypersensitivity is suggested as a possible mechanism and can be confirmed by the in vitro proliferation of T cells to the drug in the lymphocyte transformation test (LTT).

Kidney transplant recipients (KTRs) receive TMP/SMX in the early days of transplantation and after antirejection treatment for Pneumocystis jiroveci pneumonia (PJP) prophylaxis [4]. However, no case of TMP/SMX-associated acute pancreatitis in a KTR has been reported yet. Here, we describe a case of TMP/SMX-induced pancreatitis confirmed by rechallenging and LTT in a KTR. This report was approved by the Institutional Review Board of Seoul St. Mary’s Hospital (No. KC23ZASI0083) and informed consent from the patient discussed in the report was obtained.

A 64-year-old man was admitted for preemptive living donor kidney transplantation (KT) preparation. He was treated with plasmapheresis plus intravenous immunoglobulin for desensitization due to ABO and human leukocyte antigen incompatibility. Seven days prior to KT, TMP/SMX at 80/400 mg twice daily was started with immunosuppressants (tacrolimus, mycophenolate mofetil, and prednisolone) for PJP prophylaxis. On the day of the planned KT, he complained of severe epigastric pain, requiring prompt evaluation and postponement of KT (Fig. 1).

On physical examination, tenderness was found in the epigastrium with muscle guarding. The laboratory workup showed a white blood cell count of 17,880/mm$^3$, a C-reactive protein level of 0.08 mg/dL (reference, 0–0.5 mg/dL), and amylase and lipase levels of 1,742.0 U/L and 2,139.4 U/L, respectively (reference: amylase, 28–100 U/L; lipase, 13.0–60.0 U/L). Liver function tests were within normal limits. Serum triglyceride and immunoglobulin G4 (IgG4) levels were 45 mg/dL and 16.12 mg/dL (reference of IgG4, 3.9–86.4 mg/dL), respectively. Abdominal computed tomography showed peripancreatic fat infiltration with minimal parenchymal swelling. Therefore, the diagnosis...
of acute pancreatitis was made. Because there was no obvious cause of acute pancreatitis, DIAP was highly suspected. Then, possible etiologic drugs, such as TMP/SMX (class Ia), prednisolone (class II), and tacrolimus (class III) were withheld. After discontinuing the drugs, the patient’s abdominal pain subsided, along with a decrease in serum amylase and lipase levels.

To confirm TMP/SMX as the culprit drug, we performed in vitro tests using peripheral blood sampled from the patient and one healthy donor as the negative control (Fig. 2A). Skin test was unavailable due to taking systemic steroid. Lymphocyte proliferation (stimulation index, 1.91 ± 0.44) was enhanced in the concomitant incubation of TMP (100 μg/mL) and SMX (500 μg/mL) with peripheral blood mononuclear cells (PBMCs) from the patient (Fig. 2B).

The diagnosis of TMP/SMX-associated acute pancreatitis was made for the patient. Therefore, he underwent KT without PJP prophylaxis after pancreatitis resolved. However, he developed PJP 2 months after transplant, and intravenous TMP/SMX was restarted. Five days after reexposure, serum amylase and lipase levels increased.

Acute pancreatitis is a rare but life-threatening complication in patients with transplanted kidneys if not properly managed. Even though drugs are rare causes of acute pancreatitis, a recent systematic review reported that more than 150 drugs are known to cause DIAP. TMP/SMX is classified as class Ia, with the highest level of evidence, but the evidence was mostly based on cause-and-effect relationships and repeated episodes of adverse events. Our case confirmed TMP/SMX as the culprit drug of acute pancreatitis episodes in a KTR through both positive re-challenge with the drug and lymphocyte stimulation.

The possible mechanisms of DIAP include hypersensitivity reactions, cellular toxicity of the drug or metabolites, and spasm of the sphincter of Oddi. Among them, the immunological basis of hypersensitivity reactions starts with the development of drug-specific memory T cells from naïve T cells after recognition of the drug peptide by major histocompatibility molecules. Then, when antigen-presenting cells present the specific drug peptide to memory T cells, the drug-specific T cells are activated and expanded [5].

The LTT is an in vitro test for T cell-mediated drug hypersensitivity, measuring the proliferation of drug-specific memory T cells following the coincubation of patient PBMCs with the offending drug [6]. Although the drug rechal-

---

**Figure 1. Clinical course of acute pancreatitis in the presence of TMP/SMX exposure.** ABOi, ABO incompatible; CP, clindamycin primaquine; FK, tacrolimus; HLAi, HLA incompatible; KT, kidney transplantation; MMF, mycophenolate mofetil; PJP, Pneumocystis jiroveci pneumonia; TMP/SMX, trimethoprim/sulfamethoxazole.
Lymphocyte transformation test is the gold standard for evaluating drug hypersensitivity, it is time-consuming and can carry risks for the patient. The above patient was diagnosed with TMP/SMX-induced pancreatitis by reexposure and positive LTT results. This indicates that TMP/SMX-induced DIAP could be associated with T cell-mediated hypersensitivity reactions, and LTT is helpful in confirming DIAP when the diagnosis is uncertain.

Our case uniquely reports TMP/SMX as a drug eliciting acute pancreatitis in a KTR. Moreover, the evidence was based on relapse after reexposure and positive LTT results. The results suggest that TMP/SMX should be considered a causative drug when acute pancreatitis occurs in KTRs, and LTT is helpful in diagnosing DIAP.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Acknowledgments**

The authors thank Mr. So Young Park for her help with lymphocyte transformation test data.

**Data sharing statement**

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

**Authors’ contributions**

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

**ORCID**

Hanbi Lee, https://orcid.org/0000-0001-7326-0602
Hyung Duk Kim, https://orcid.org/0000-0003-0484-3550
Chul Woo Yang, https://orcid.org/0000-0001-9796-636X
Sook Young Lee, https://orcid.org/0000-0002-3346-3664
Hwa Young Lee, https://orcid.org/0000-0002-1582-2256
Byung Ha Chung, https://orcid.org/0000-0003-0048-5717
References


Asahi KASEI

REXEED™ Series
Hemodialyzer / REXEED™

Our Perfect Solution for HD
Combining the Best with a Large Line-UP
WET TYPE Polysulfone

Fluoroplastic Dialysis Catheter Supercath CLS

Minimize Risk of Damage on Vessel Wall
Can be used on NON IDEAL Access Site
Patients may MOVE their Arms during Dialysis Session
Good Alternative for Patients with Metal Allergy

MDS-101
Asahi Dialysis System MDS-101
Dialysis Equipment

Slim & Smart
High visibility and Simplified procedures
Secured ultrafiltration system
Easy maintenance

Exclusive Distributor: AY Trading Co., Ltd. TEL: 02-585-7661 / FAX: 02-585-7664
BLOG web page https://blog.naver.com/ayt0717 YouTube search “AY Trading”
Improving lives together

Fresenius Medical Care is the world’s leading provider of dialysis products and services, offering life-sustaining care for people living with chronic kidney failure.

In Asia Pacific, we draw on our decades of experience and expertise to deliver our vision - Creating a future worth living. For patients. Worldwide. Every day.
Glucose Control & CV Event Reduction!
제2형 당뇨병 환자를 위한
새로운 정면승부

- 심혈관계 사망 위험 38% 감소
- 우수한 HbA1c 강하 효과

제2형 당뇨병 환자가 표준치료를 받고 있다면도 심혈관계 감소를 동반한
제2형 당뇨병 환자들은 이전히 심혈관계 사망 위험에 노출되어 있습니다. 4

SP-MACE, 3-point major adverse cardiovascular events. 4P-MACE, 4-point major adverse cardiovascular events. CI, confidence interval. CV, cardiovascular. HbA1c, glycosylated hemoglobin. RAA5, renin-angiotensin-aldosterone system.

자리안경 (제한관리용) 10mg, 15mg
시바이의 전문적인 분석에 따르면, 자리안경은 당뇨병 환자에게 특히 유용한 치료입니다. 자리안경을 사용한 환자들의 HbA1c 수치는 상승한 것이나, 자리안경을 사용하지 않은 환자들의 HbA1c 수치는 상승한 것으로 나타났습니다. 자리안경의 사용으로 인해 HbA1c 수치는 유의하게 감소했습니다. 자리안경은 당뇨병의 관리에 있어 매우 효과적인 치료로 알려져 있습니다. 자리안경의 사용은 당뇨병 환자의 건강을 개선하고 질병의 발생을 방지하는 데 도움이 될 수 있습니다.

자리안듀오 (제한관리용) 5mg/5mg, 10mg/10mg, 15mg/15mg, 20mg/20mg
자리안듀오는 자리안경과 함께 사용할 수 있는 치료입니다. 자리안듀오의 사용으로 인해 HbA1c 수치는 유의하게 감소했습니다. 자리안듀오는 당뇨병의 관리에 있어 매우 효과적인 치료로 알려져 있습니다. 자리안듀오의 사용은 당뇨병 환자의 건강을 개선하고 질병의 발생을 방지하는 데 도움이 될 수 있습니다.
미쎄라®와 렌벨라®가 한독으로 하나가 되었습니다.

Stay stable, Mircera®
CKD 환자의 안정적인 Hb level 관리를 위해

Real Value, Renvela®
체내 흡수 및 축적되지 않는 비칼슘제일 인긴합제®

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌벨라 정(세ベル라탄산염) 렌벨라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

CKD 환자의 질환 치료를 위해

미쎄라®와 렌벨라®가 한독으로 하나가 되었습니다.

Stay stable, Mircera®
CKD 환자의 안정적인 Hb level 관리를 위해

Real Value, Renvela®
체내 흡수 및 축적되지 않는 비칼슘제일 인긴합제®

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベルラ 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌벨라 정(세ベル라탄산염) 렌벨라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌벨라 정(세ベル라탄산염) 렌벨라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌ベル라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)

---

미쎄라 프리필드주 국내허가사항 (as of 2022-05-10) 2. 렌벨라 정(세ベル라탄산염) 렌ベル라

3) 발작 환자 4) 수혈이 필요한 출혈환자 또는 최근에 출혈이 있었던 환자 5) 혈소판 수치가 500 X 10^9/L 보다 높은 환자

1) 보다 자세한 제품 문의 또는 제품 관련 부작용 보고는 (주)한국로슈(02-)
INDICATIONS
1. New anemia
2. Chemotherapy induced anemia in solid cancer patients

DOSE AND ADMINISTRATION
- Hemodialysis patients
  - Initial dose
    The usual dose of NESP in adult patients is 20 μg, to be administered as a single intravenous injection once weekly.
  - Initial dose at the switching from erythropoietin preparations: See Precautions related to Design and Administration
- Maintenance dose
  When correction of anemia is achieved, the usual dose of NESP in adult patients is 15-40 μg as darbepoetin alfa (intravenous bolus), to be administered at a single intravenous injection once weekly. If correction of anemia is maintained by once weekly injection, the frequency of administration can be changed to every two weeks with an initial dose set to be two-fold of the dose in the once weekly injection. In this case, the usual dose in adult patients is 10-120 μg administered as a single intravenous injection once every two weeks. In all cases, the dose should be adjusted in view of the degree of anemia symptoms and the patient's age, and should not exceed 180 μg as a single injection. The target of anemia correction is around 11 g/dl of hemoglobin level.
- Hemodialysis patients and patients with chronic kidney disease not on dialysis
  - Initial dose
    The usual dose of NESP in adult patients is 30 μg to be administered as a single injection once every two weeks subcutaneously or intravenously.

Precautions related to Design and Administration
1. Initial dose at the switching from erythropoietin preparations: See Precautions related to Design and Administration
2. Maintenance dose
  When correction of anemia is achieved, the usual dose of NESP in adult patients is 30-120 μg as darbepoetin alfa (intravenous bolus), to be administered as a single injection once every two weeks subcutaneously or intravenously. If correction of anemia is maintained by once every two weeks injection, the frequency of administration can be changed to once every four weeks with a initial dose set to be two-fold of the dose in the once every two weeks injection. In this case, the usual dose in adult patients is 60-180 μg administered as a single injection once every four weeks subcutaneously or intravenously. In all cases, the dose should be adjusted in view of the degree of anemia symptoms and the patient’s age, and should not exceed 180 μg as a single injection. The target of anemia correction is around 11 g/dl of hemoglobin level.

PRECAUTIONS
See the package insert.

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

PACKAGING
1 μg/kg, 5 μg/kg
for NESP 20μg, 50μg, 40μg, 60μg, 120μg, respectively

MANUFACTURED BY:
Takeda Pharmaceutical Co., Ltd.
1040-22 Matsubara-cho, Takamatsu-ku, Osaka, Japan

IMPORTED BY:
Kyowa Hakko Kirin Co., Ltd.
150-1 Nihonbashimachi, Talisan-kōen, Chuo-ku, Tokyo, Japan

http://www.kyowa-kirin-korea.com
At B. Braun, we don't just develop products. We provide solution for life.

Diacap Pro
THE TRUSTED PERFORMER

Dialog+
THE POWER OF FLEXIBILITY
Astellas, only PROgraf

프로그램의 환자 생명 연장을 위한 동행은 앞으로도 계속됩니다.
Homechoice Claria enabled by Sharesource
from pediatric to elderly population

Homechoice Claria | SIMPLE & SMART APD for your patient to maintain daily life

Sharesource

ON-DEMAND ACCESS to treatment data by GREAT VISIBILITY in APD

- Intuitive Triage Dashboard
- Patient Snapshot
- Treatment Summary
Boryung Renal Business Unit provides **TOTAL RENAL CARE**

<table>
<thead>
<tr>
<th>Boryung Renal Business Unit</th>
<th>Boryeong Building, 136 Changuyeonggung-ro, Jongno-gu, Seoul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Customer Service Center</td>
<td>Tel 080.708.8088  Fax 02.741.5291  <a href="http://www.boryung.co.kr">www.boryung.co.kr</a></td>
</tr>
</tbody>
</table>
Initiate early with

ACERTIL®
ACERTIL® PLUS
ARGININE
FLUDEX® SR

for your Hypertension patients!


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

[Importer/Seller] SERVIER KOREA LTD. 23th fl. 24 Yeoui-daero, Yeongdeungpo-gu, Seoul, 07320 Tel. 02-3415-8500 (www.servier.co.kr) [Medical Information Inquiry] Tel. 02-3415-8500 e-mail: medinfo.korea@servier.com
1. Manuscript Submission

Manuscripts for *Kidney Research and Clinical Practice* (KRCP) should be submitted online at https://www.editorialmanager.com/krcp. All submissions to KRCP must conform to the International Committee of Medical Journal Editors (ICMJE) uniform requirements for manuscripts submitted to biomedical journals. Our requirements reflect those of the ICMJE, although we also have specific requirements for different types of article. For editorial questions, please contact us via e-mail (registry@ksn.or.kr), telephone (+82-2-3486-8736), or fax (+82-2-3486-8737).

Important information

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

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

1. A cover letter. It must include your name, address, telephone and fax numbers, e-mail address, and state that all authors have contributed to the paper and have never submitted the manuscript, in whole or in part, to other journals.
2. A conflict of interest disclosure statement (see relevant section 4.2 below).
3. All studies involving human subjects, human data or any material derived from human must be approved by the relevant review or ethics committee. Articles must include a statement on ethics approval, the name of the relevant committee that approved the study and the committee’s approval number. Manuscripts may be rejected at any time if the authors of the research fail to provide the approval number validated by the relevant committee (see relevant section 4.1 below).
4. Articles covering the use of animals in experiments must be approved by the relevant authorities.
5. Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified.
6. The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants, the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).
7. Clinical trials should be registered at a primary national clinical trial registration site such as www.clinicaltrials.gov, https://cris.nih.go.kr/cris/index.jsp, or other sites accredited by the World Health Organization or the International Committee of Medical Journal Editors.
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.
11. The copyright transfer agreement has been incorporated into KRCP submission system to collect digital signatures from each author. Upon submission of a manuscript, an email will be sent to each author for electronic signature prior to starting review process. The manuscript will not be reviewed as planned until all signatures are received. The paper submitted without the signatures of all authors on all statements will be finally removed from the system without further notice.

2. Types of Articles

2.1. Original Articles

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

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

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

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

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

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

3. Manuscript Preparation

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

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

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

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

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

Journal articles:

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”).
3.9. Certificate of English editing
All submitted manuscripts should be written in clear, correct English. Non-native English-speaking authors are required to attach an English language editing certificate when submitting their manuscript in order to undergo further review. For authors who use English as their native language, please upload an empty file with the filename “Certificate of English Editing (empty).”

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.

4.4. Redundant Publication or Duplicate Submission
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:
• Avoid excessive detail and clutter and keep text to a minimum.
• Any descriptive text should be at least 12 pt font size.
• The visual abstract should be saved as an editable PowerPoint 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:
• Select bold, solid color icons
• Avoid highly detailed icons as the intricacy may be lost in the small format
• Exclude trade names, logos, or images of trademarked items.
• Graphics should be 440 pixels wide by 350-365 pixels tall.

7. Peer Review
This journal operates blind review processes. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. For more information, please refer to Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (Available at: http://www.icmje.org/icmje-recommendations.pdf).

8. Copyright
KRCP is the official peer-reviewed publication of the Korean Society of Nephrology. Manuscripts published in the Journal become the permanent property the Korean Society of Nephrology. All articles published in the Journal are protected by copyright, which covers the exclusive rights to reproduce and distribute the article, as well as translation rights. No KRCP article, in part or whole, cannot be reproduced, stored, or transmitted for commercial purposes, without prior written permission from the Korean Society of Nephrology.

9. Similarity Check
Similarity Check is a multi-publisher initiative to screen published and submitted content for originality. To find out more about Similarity Check, visit http://www.crossref.org/crosscheck/index.html. All manuscripts submitted to KRCP may be screened, using the iThenticate tool, for textual similarity to other previously published works.

10. Open Access Policy
Every peer-reviewed research article in this journal is freely available via our website (https://www.krcp-ksn.org). Articles published in KRCP are distributed under the terms of the Creative Commons Attribution Non-Commercial and No Derivatives License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits unrestricted non-commercial use, distribution of the material without any modifications, and reproduction in any medium, provided the original works properly cited. ANY USE of the open access version of this Journal in whole or in part must include the customary bibliographic citation, including author and publisher attribution, date, article title, Kidney Research and Clinical Practice (Kidney Res Clin Pract), and the URL https://www.krcp-ksn.org and MUST include a copy of the copyright notice. If an original work is subsequently reproduced or disseminated not in its entirety but
only in part or as a derivative work this must be clearly indicated. For any commercial use of material from the open access version of the journal, permission MUST be obtained from KRCP. If necessary, please contact the Editorial Board through our editorial office (registry@ksn.or.kr). Proprietary rights notice for KRCP online were available at: https://www.krcp-ksn.org/authors/permission.php

11. Data Sharing Policy

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:

- Original Article, Review Article, Special Article, and Study Protocol: KRW 1,000,000 (Korea) / USD 1,000 (rest of world)
- Correspondence, Image in Practice: KRW 300,000 (Korea) / USD 300 (rest of world).

There are no additional charges based on color, length, figures or other elements. The publication costs for invited papers such as editorials, some reviews and special articles are covered by the Korean Society of Nephrology. Payments are processed by a department unconnected to KRCP’s editorial board.

- 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.