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KOREAN SOCIETY OF PEDIATRIC NEPHROLOGY

Childhood Kidney Diseases

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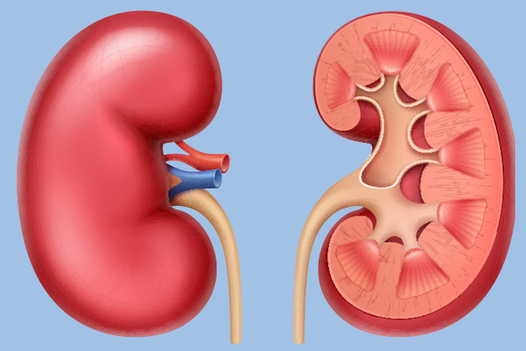

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ChiKD

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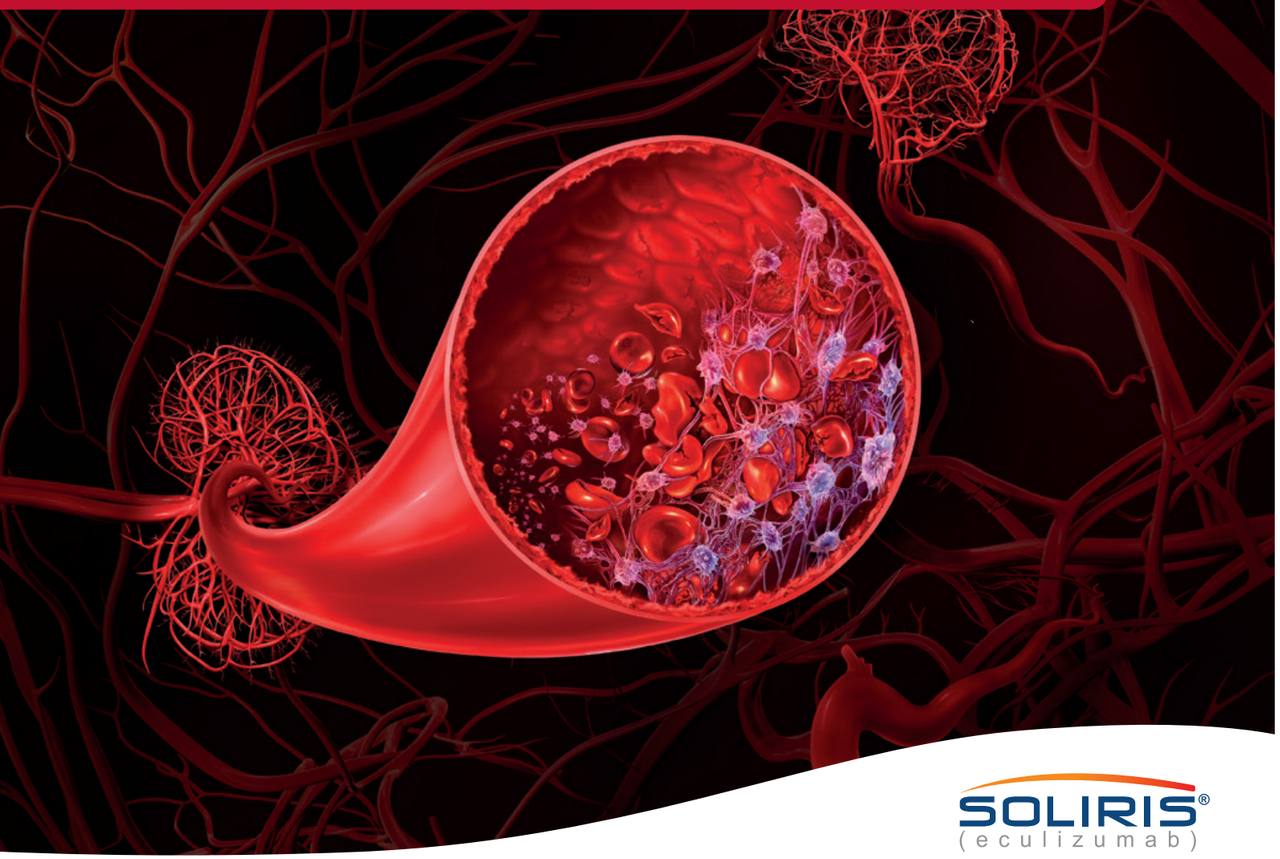
보건의료전문가용

Patients with aHUS can be at continuous risk of the life-threatening consequences of unpredictable complement-mediated TMA[1,2]

Chronic, uncontrolled complement activity in aHUS leads to ongoing endothelial injury, organ damage, and sudden death[2,3]



SOLIRIS®
(eculizumab)

aHUS, atypical Hemolytic Uremic Syndrome; **TMA,** Thrombomicroangiopathy

References: 1. Laurence et al. Atypical Hemolytic Uremic; Essential Aspects of an Accurate Diagnosis. Clin Adv Hematol Oncol. 2016 Nov;14 Suppl 11(11)2-15. **2.** Legendre, C. M. et al. Terminal Complement Inhibitor Eculizumab N Engl J Med N Engl J Med 2013;368 2169-81. **3.** Noris et al. STEC HUS, atypical HUS and TTP are all, Nat. Rev. Nephrol. 2012 8, 622 633

prescribing information

전문의약품

솔리리스주(에쿨리주맙) [성분·함량] 1바이알(30mL)중 유효성분 : 에쿨리주맙(별규) 300mg 첨가제 : 염화나트륨, 인산수소나트륨 칠수화물, 인산이수소나트륨수화물, 주사용수, 폴리소르베이트80 **[효능·효과]** 1) 발작성 야간 혈색소뇨증(PNH : Paroxysmal Nocturnal Hemoglobinuria): 용혈을 감소시키기 위한 발작성 야간 혈색소뇨증(PNH : Paroxysmal Nocturnal Hemoglobinuria) 환자의 치료. 수혈 이력과 관계없이, 높은 질병 활성을 의미하는 임상 증상이 있는 환자의 용혈에 임상적 이익이 확립되었다. 2) 비정형 용혈성 요독 증후군(aHUS : atypical Hemolytic Uremic Syndrome): 보체 매개성 혈전성 미세혈관병증을 억제하기 위한 비정형 용혈성 요독 증후군(aHUS : atypical Hemolytic Uremic Syndrome) 환자의 치료 (사용제한: 시가(Shiga) 특신 생성 대장균에 의한 용혈성 요독 증후군(STEC-HUS) 환자 대상의 적용을 권장하지 않는다. 3) 시신경 척수염 범주 질환(Neuromyelitis optica spectrum disorder): 항아쿠아포린-4(AQP-4) 항체 양성인 환자의 시신경 척수염 범주 질환(NMOSD : Neuromyelitis optica spectrum disorder)의 치료 **[용법·용량]** 심각한 감염에 대한 위험을 줄이기 위해서 환자들은 최신의 백신 접종 지침(Advisory Committee on Immunization Practices(ACIP) recommendations)에 따라 백신 접종을 해야 한다.(사용상의 주의사항 1. 경고 항 참고) 이 약은 정맥투여되어야 하며 급속정맥투여(IV push) 또는 일시정맥투여(IV bolus)로 투여해서는 안된다. <성인> 1) 발작성 야간 혈색소뇨증(PNH) - 첫 4주간은 매 7일마다 600 mg을 투여한다. 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 900 mg을 투여하고, 그 후부터는 매 14일마다 900 mg을 투여한다. 이 약은 권장 투여량과 일정에 맞게 투여, 혹은 예정된 일정의 2일 전/후로 투여 되어야 한다. 2) 비정형 용혈성 요독 증후군(aHUS) 및 시신경 척수염 범주질환(NMOSD) - 첫 4주간은 매 7일마다 900 mg을 투여한다. 네 번째 용량 투여 7일 후에 다섯 번째 용량으로 1200 mg을 투여하고, 그 후부터는 매 14일마다 1200 mg을 투여한다. <소아> 1) 비정형 용혈성 요독증후군(aHUS) 만 18세 미만의 aHUS 환자일 경우, 체중에 따라 다음의 일정으로 투여한다. [표 1] 만 18세 미만 환자에서의 권장 투여법 이 약은 권장 투여일과 일정에 맞게 투여, 혹은 예정된 일정의 2일 전/후로 투여되어야 한다. <혈장교환요법 및 신선 동결혈장투여시> 성인 및 소아 비정형 요독증후군과 성인 시신경 척수염 범주질환 환자에 대해 PE/PI혈장 교환 요법(plasma exchange 또는 plasmapheresis), 또는 신선 동결 혈장 투여(fresh frozen plasma infusion)와 같은 부수적 시술을 받는 경우 추가 용량 투여가 필요하다.

환자 체중	초기 용량	유지 용량
40 kg 이상	4 주간 매 7일마다 900 mg 투여	5주차에 1200 mg, 이후 매 14일마다 1200 mg 투여
30 kg이상 40 kg 미만	2주간 매 7일마다 600 mg 투여	3주차에 900 mg, 이후 매 14일마다 900 mg 투여
20 kg 이상 30 kg 미만	2주간 매 7일마다 600 mg 투여	3주차에 600 mg, 이후 매 14일마다 600 mg 투여
10 kg 이상 20 kg 미만	첫 주에 600 mg 투여	2주차에 300 mg, 이후 매 14일마다 300 mg 투여
5 kg 이상 10 kg 미만	첫 주에 300 mg 투여	2주차에 300 mg, 이후 매 21일마다 300 mg 투여

[표 2] PE/PI 이후 이 약의 추가적 투여법

부수적 시술의 종류	최근 사용한 이 약의 용량	부수적 시술 시 이 약의 추가 투여 용량	추가 투여 시점
혈장 교환 요법 (plasma exchange or plasmapheresis)	300 mg	혈장 교환 요법 시행시마다 300 mg씩	혈장 교환 요법 이후 60분 이내
	600 mg 또는 그 이상	혈장 교환 요법 시행시마다 600 mg씩	
신선 동결 혈장 투여 (fresh frozen plasma infusion)	300 mg 또는 그 이상	신선 동결 혈장 투여 시마다 300 mg씩	신선 동결 혈장 투여 60분 이전

[사용상의 주의사항] 1. 경고 : 중대한 수막구균 감염: 작용기전으로 인하여 이 약의 사용은 중대한 수막구균 감염(패혈증 그리고/또는 뇌수막염)에 대한 환자의 감수성을 증가시킨다. 이 약의 투여 환자에게서 지명적이고 생명을 위협하는 수막구균 감염이 발생하였다. 수막구균 감염은 어느 혈청군에 의해 서도 발생할 수 있지만, 이 약의 투여 환자들은 혼하지 않은 혈청군(X 등)에 의한 질환이 발생할 수 있다. 감염의 위험성을 낮추기 위하여, 이 약의 치료가 지연됨으로 인한 위험성이 수막구균 감염 발생의 위험성보다 큰 경우를 제외하고는 모든 환자들은 반드시 이 약의 투여 시작 최소한 2주 전에 수막구균 백신을 투여 받아야 한다. 만약 접종 받지 않은 환자가 긴급히 이 약의 치료를 받아야 하면, 최대한 빨리 수막구균 백신을 투여 받도록 한다. 수막구균 백신 접종 이후 2주 이내 이 약을 투여할 경우, 4가 수막구균 백신 접종 이후 2주 동안 적절한 예방적 항생요법으로 치료 받아야 한다. 혼한 병원성 수막구균 혈청군을 예방하기 위하여 가능하다면 혈청군 A, C, Y, W135, B에 대한 백신이 권장된다. 환자들은 백신 사용을 위한 최신의 백신 접종 지침(Advisory Committee on Immunization Practices(ACIP) recommendations)에 따라 백신을 접종 혹은 재접종 받아야 한다. 백신 접종은 보체를 더욱 활성화시킬 수 있다. 결과적으로, PNH, aHUS, 불응성 gMG 및 NMOSD를 포함한 보체 매개 질환을 가진 환자들은 용혈(PNH의 경우)이나 혈전성 미세혈관병증(TMA; aHUS의 경우) 또는 중증 근무력증의 악화(불응성 gMG의 경우)또는 재발(NMOSD의 경우)과 같은 그들의 기저 질환의 징후 및 증상이 증가하는 경험을 할 수 있다. 따라서, 지침에 따른 백신 접종 이후 질환의 증상에 대해 면밀히 관찰되어야 한다. 백신 접종은 수막구균 감염 위험을 줄일 수 있지만, 완전히 없애지는 않는다. 적절한 항생제 사용에 대한 공식 지침(예: 국내 성인 세균성 수막염의 임상진료지침 권고안 등)을 고려하여야 한다. 수막구균 감염의 초기 징후나 증상이 나타나는지 면밀히 관찰하고, 감염이 의심되면 즉시 검사받아야 한다. 환자는 이러한 징후와 증상 및 즉시 치료를 받는 절차에 대해 안내 받아야 하며, 담당 의사는 반드시 환자와 이 약의 치료의 위험과 이익을 상의하여야 한다. 수막구균 감염은 초기에 발견하고 치료하지 않으면 급격히 치명적이고 생명을 위협하게 될 수 있다. 중대한 수막구균 감염을 치료받는 환자는 이 약의 투여를 중지하도록 한다. 2. 다음 환자에는 투여하지 말 것 1) 이 약의 주성분, 뮤린 단백질 또는 기타 구성성분에 과민반응이 있는 환자 2) 치료되지 않은 중대한 수막구균(Neisseria meningitidis) 감염 환자 3) 수막구균(Neisseria meningitidis) 백신을 현재 접종하지 않은 환자 또는 백신 접종 이후 2주 동안 적절한 예방적 항생요법으로 치료를 받지 않은 환자(이 약의 치료를 늦추는 것이 수막구균 감염을 일으키는 것보다 중대하지 않은 경우) **[수입자,허가권]** (주) 한독서울특별시 강남구 테헤란로 132전화번호: (02) 527-5114자 개정년월일: 2023년 02월 03일 **※ 보다 자세한 사항은 제품설명서 전문을 참고하시기 바랍니다.** aSOL20230206

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ALEXION

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Aims & Scope

Childhood Kidney Diseases (*Child Kidney Dis, ChiKD;* formerly Journal of the Korean Society of Pediatric Nephrology; ISSN 1226-5292, launched in 1997), the official journal of the Korean Society of Pediatric Nephrology, is a local peer-reviewed journal. It aims to improve kidney health in children and adolescent by covering clinical, and research works relevant to all aspects of pediatric nephrology. Its expected readers are clinicians and researchers around the world, although it has a particular focus on pediatric patients in Asia. Its publication types include reviews, original articles, case reports, editorials, and letters to the editor. The journal aims to serve pediatricians through the prompt publication of significant advances in pediatric nephrology and to rapidly disseminate recently updated knowledge to the public. Additionally, it will initiate dynamic, international, academic discussions concerning the major topics related to pediatric nephrology.

Readership

It is primarily for scientists and clinicians active or interested in the field of pediatric nephrology, but its readership can be expanded to other positions: researchers, clinicians, professors, medical health students, allied health professionals, and policy makers in the field of pediatrics, neonatology, nephrology, urology, pathology, endocrinology, cardiology, neurology, gastroenterology, gynecology/obstetrics, epidemiology, pharmacology, biochemistry, and molecular & cell biology.

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Hyung Eun Yim Department of Pediatrics, Korea University, Republic of Korea

Editorial Office

Korean Society of Pediatric Nephrology
240, Gimpohangang 1-ro, Gimpo 10078, Republic of Korea
Tel: +82-10-4391-0788 E-mail: chikd@chikd.org

Printing Office

M2PI

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

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Review Articles

Original Articles

Case Report

ChiKD CHILDHOOD KIDNEY DISEASES

Review Article

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Antenatally detected urinary tract dilatation: a pediatric nephrologist's point of view

Hyung Eun Yim[1]

[1]Department of Pediatrics, Korea University Ansan Hospital, Ansan, Republic of Korea

Antenatally diagnosed urinary tract dilatation (UTD), previously referred to as antenatal hydronephrosis, is the most commonly detected abnormality by prenatal ultrasonography. Several grading systems have been developed for the classification of antenatal UTD using prenatal and postnatal ultrasonography. UTD comprises a wide variety of congenital abnormalities of the kidney and urinary tract ranging from transient UTD to more significant abnormalities such as vesicoureteral reflux, ureteropelvic junction obstruction, ureterocele, ureterovesical junction obstruction, posterior urethral valves, and non-refluxing megaureter. Optimizing the evaluation of antenatally detected UTD is essential to recognize children with important disorders while avoiding excessive investigations. Conservative approach with close follow-up is increasingly accepted as an appropriate treatment option for patients with asymptomatic vesicoureteral reflux and ureteropelvic junction obstruction in recent years. However, predicting permanent kidney damage in an unselected group of children with antenatal UTD is still challenging. The management and follow-up of children with UTD should be individualized based on recommendations from a pediatric nephrologist, a pediatric urologist, or both. Future research directed at predicting long-term outcomes of children diagnosed with UTD from mild findings to severe disease is needed to refine management for those at higher risk of kidney disease progression.

Keywords: Hydronephrosis; Kidney failure, chronic; Risk management; Urinary tract

Introduction

Antenatal urinary tract dilatation (UTD) is commonly found on routine fetal ultrasound [1,2]. UTD is a preferred term while various terminology such as congenital, fetal, antenatal, or prenatal hydronephrosis has been used for years [3,4]. Given that it is associated with a broad spectrum of conditions ranging from a transient finding to congenital abnormalities of the kidney and urinary tract (CAKUT) leading to chronic kidney disease (CKD), it is important to avoid unnecessary testing and identify cases of significant urinary tract anomaly. Nguyen et al. [4] have recently updated the UTD classification system. A new guideline was subsequently developed for the evaluation of antenatal and postnatal UTD [4]. This review aimed to discuss a clinically significant question evaluating and managing antenatal UTD from a pediatric nephrologist's view and to assist pediatricians in their decision-making about the antenatal UTD.

Q1. What is the definition of UTD?

Different grading systems for UTD have been developed for a long time. The Society for Fetal Urology (SFU) system and renal pelvis anterior-posterior diameter (APD) measurements for grading UTD are commonly used [5]. However, the recently

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Correspondence to
Hyung Eun Yim
Department of Pediatrics, Korea University Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Republic of Korea
E-mail: ped7427@korea.ac.kr

updated UTD classification [4] seems to be appropriate for predicting long-term renal outcome and need for surgery in addition to identifying postnatal CAKUT [6-8]. According to the UTD classification system [4], antenatal UTD is defined as a renal pelvis APD of ≥4 mm in the second trimester (<28 weeks) and/or ≥7 mm in the third trimester (≥28 weeks). The new system focuses not only on the kidney, but also on the entire urinary tract system, classifying UTD into two antenatal categories (UTD A1, UTD A2-3) and three postnatal categories (UTD P1, P2, and P3). UTD A1 is considered at low risk for postnatal CAKUT based on APD of 4 to <7 mm at <28 weeks and APD of 7 to <10 mm at ≥28 weeks. Abnormal kidney parenchyma (cortical thinning, hyperechogenicity, cystic dysplasia, or indistinct corticomedullary differentiation), calyces, ureters, bladder (wall thickening, ureterocele, or dilated posterior urethra), or amniotic fluid accompanied with a renal APD of ≥7 mm at <28 weeks or ≥10 mm at ≥28 weeks corresponds to antenatal UTD A2-3, which is considered to be at an increased risk for postnatal CAKUT. At least 48 hours after birth, the presence of a renal pelvis APD of < 10 mm without other abnormalities (no calyceal or ureteral dilation, no abnormalities of renal parenchyma or bladder) is defined as normal in the UTD classification system. In this system, an APD of 10 to 15 mm or central calyx dilatation is defined as UTD P1 (low risk) and an APD of ≥15 mm or peripheral calyceal dilatation or dilated ureter of >4 mm with an APD of ≥10 mm or calyceal dilatation is defined as UTD P2 (intermediate risk). The presence of renal parenchymal abnormality, bladder abnormality, or oligohydramnios combined with an APD of ≥10 mm or any calyceal dilatation is classified as UTD P3 (high risk) [3,4]. UTD P1, UTD P2, and UTD P3 are comparable to SFU grade I-II, SFU grade III, and SFU grade IV, respectively (Fig. 1) [9].

Q2. What causes UTD?

The main etiologies of antenatally diagnosed antenatal UTD can be grouped into three broad categories: (1) physiologic or transient dilation; (2) vesicoureteral reflux (VUR); and (3) obstructive uropathy. As the degree of antenatal and postnatal UTD increases, there is an increased risk of CAKUT except VUR [10]. A recent paper reported that one-third of children with antenatal UTD had the UTD before birth and that the UTD was resolved or stabilized by the end of 2 to 3 years for another third of children while UTD persisted or CAKUT was diagnosed for the remaining cases [3]. Transient or physiologic UTD might be associated with hydration status, bladder filling, transient narrowing of the ureteropelvic junction (UPJ), or delayed maturation of ureteral peristalsis [11]. UPJ obstruction and VUR are the two most common CAKUT conditions, both of which can be diagnosed in 10% to 12% of cases [1,12]. Other CAKUT conditions causing UTD include ureterovesical junction obstruction, primary non-refluxing megaureter, bladder outlet obstruction including posterior urethral valve (PUV) or ureterocele, duplex collecting system, multicystic dysplastic kidney, and so on [3,11].

	Antenatal UTD		Postnatal UTD			SFU grading			
	UTD A1	UTD A2-3	UTD P1	UTD P2	UTD P3	I	II	III	IV
Renal pelvic APD (mm)	4 to <7 (<28 wk) 7 to <10 (≥28 wk)	≥7 (<28 wk) ≥10 (≥28 wk)	10 to <15	≥15	≥10	Pelvis splitting	Pelvis widening		
		Or	Or	Or	Or				
Calyceal dilatation		Any	Central	Peripheral	Any		Major	Grade II + minor	
		Or		Or					
Ureter dilatation (mm)		Any[a]		≥4[b]					
		Or			And				
Parenchymal or bladder abnormality or oligo-hydramnios		Yes[a]			Yes				Grade III + cortex thinning

Fig. 1. UTD classification system. UTD, urinary tract dilatation; SFU, Society for Fetal Urology; APD, anterior-posterior diameter. [a]With renal pelvic APD ≥4 mm or calyceal dilatation. [b]Renal pelvic APD ≥10 mm or calyceal dilatation. Parenchymal abnormality includes cortical thinning, increased echogenicity, indistinct corticomedullary differentiation, or cystic dysplasia. Bladder abnormality includes bladder wall thickening, ureterocele, or dilated posterior urethra. For a more complete understanding of UTD classification system, please refer to the images in the article of Nguyen et al. [4].

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Q3. What is the optimal evaluation of UTD?

Extensive investigation for UTD is being proposed for cases with moderate or severe dilatation (UTD A2-3, P2, and P3). Nonetheless, it is recommended that all antenatal UTDs (UTD A1, A2-3) should be validated by two serial postnatal ultrasonography (US) at >48 hours after birth and a few weeks or months later [3,13]. For cases with suspected bladder outlet obstruction, kidney and bladder US should be checked as soon as possible postnatally and renal function should be checked together with indwelling urinary catheter. With urologic consultation, a follow-up US should be performed sooner [3,13]. For UTD P1, a follow-up US is recommended at 3, 6, and 12 months of life [3]. If there are only renal pelvis APD of <10 to 15 mm and/or central calyceal dilatation (UTD P1), further evaluation is not recommended [3]. However, peripheral calyceal dilation has been reported to increase the risk of a diagnosis of CAKUT [3,4]. If there are worsening findings on serial postnatal US, further work-up including renal function tests, voiding cystourethrogram (VCUG) or mercaptoacetyltriglycine (MAG3) scintigraphy (or diuretic renal scan) and urologic consultation should be considered. For UTD P2-3, a follow-up US in 1 to 3 months is suggested. Evaluation with renal function tests (especially serum creatinine, electrolytes, and blood gas analysis), VCUG, and MAG3 scan are considered [3,14]. Diuretic renal scan is usually performed from 6 to 8 weeks of age [3]. Measurement of serum cystatin C instead of creatinine may offer significant advantages in neonates and young infants given that serum cystatin C levels are less affected by age, sex, dietary protein intake, and muscle mass compared to creatinine [15]. Consultation to a pediatric urologist and the use of prophylactic antibiotics can be considered based on the severity of clinical conditions. Supplementary comments for the antibiotic prophylaxis will be mentioned in the following subject (Q5). A follow-up US at 6–12 months even after initial resolution could be recommended for some cases with UTD P2-3 since a recurrence of significant UTD has been reported in patients with spontaneous improvement (Fig. 2) [14,16].

Q4. Who will need a urologic intervention?

The exact indications and suitable time for surgical intervention remain controversial. About 50% of postnatal UTDs will resolve and the remaining 40% to 45% will show improvement or stabilization of UTD within the first 3 years of life [3,14,17]. General indications for surgery include bladder outlet obstruction with oligohydramnios and recurrent urinary tract infections (UTIs) with VUR or UPJ obstruction. Increasing dilatation and/or decreasing split function (<40% with impaired renal drainage or >10% of renal function deterioration on a follow-up renal scan) are also indicative of the need for surgery [3,13,14]. The cumulative incidence of needing surgery was about 20% to 30% children with antenatal UTD in long-term studies [17-19], while another study reported a far smaller proportion of patients undergoing any surgical procedure [20]. Yang et al. [18] have found that the ipsilateral differential renal function is preserved only in the early pyeloplasty group, implying that early surgical treatment is

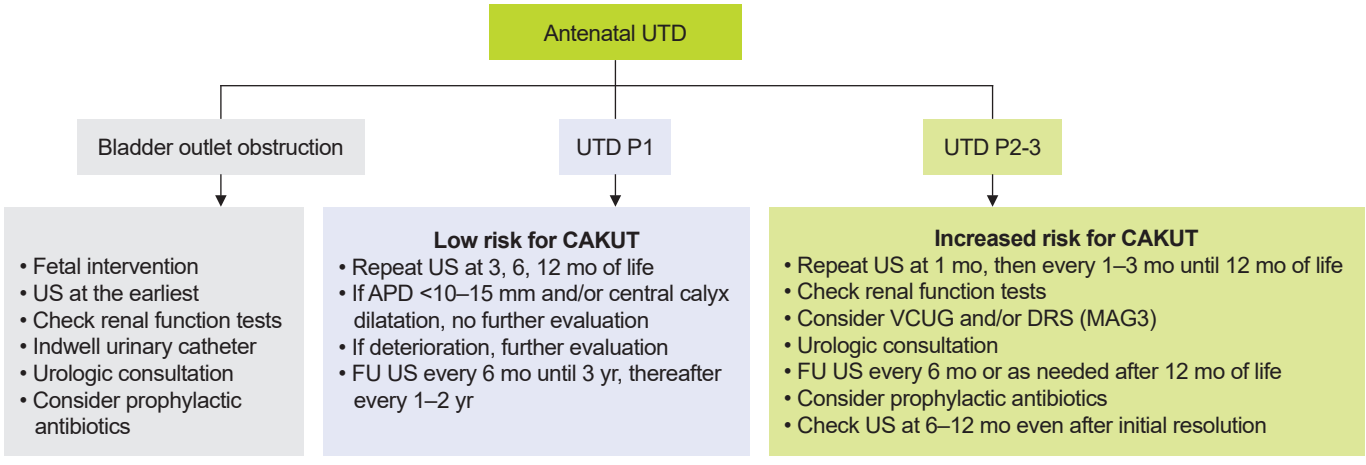

Fig. 2. Evaluation, management, and follow-up for antenatal and postnatal UTD. UTD, urinary tract dilatation; US, ultrasonography; CAKUT, congenital abnormalities of the kidney and urinary tract; APD, anterior-posterior diameter; FU, follow-up; VCUG, voiding cystourethrogram; DRS, diuretic renal scan; MAG3, mercaptoacetyltriglycine. Adapted from Herthelius. Pediatr Nephrol 2023;38:3221-7 [3].

important to preserve renal function in patients with persistent UTD P2-3 (SFU grade III or IV). In addition, several predictors for surgical intervention have been suggested, including initial postnatal APD, renal pyramidal thickness, delayed cortical tissue transit time on diuretic renal scan, and renal parenchyma-to-hydronephrosis area ratio [21-24].

Q5. What is the risk of UTI? Do we need an antibiotic prophylaxis?

In general, children with antenatally diagnosed UTD are at an increased risk of UTI. Infants with antenatal UTD are more likely to have acute pyelonephritis within the first year of life than those without UTD [25]. Several studies have shown a cumulative incidence of 7% to 14% for UTI during infancy [26-28]. While some studies reported higher incidence of 14% to 40% for UTI especially in cases with moderate or severe UTD [29,30], others revealed lower occurrence of UTI (3.3% to 6.83%) in children with antenatal diagnosis of UTD [31,32]. Among underlying uropathies, VUR has been shown to be the most important risk factor for UTI within the UTD population [28]. UTI rates were 3- to 6-fold higher in patients with hydroureteronephrosis than in those with isolated hydronephrosis according to a systematic review by Braga et al. [33]. For a long time, the use of continuous antibiotic prophylaxis (CAP) has been a challenging issue. A systematic review [34] has shown that uncircumcised boys and children with ureteral dilatation and/or high-grade UTD are more prone to develop UTI and that CAP is recommended for these subgroups of patients. However, benefits of CAP are limited in infants with mild to moderate UTD since the protective effect of CAP against UTI has not been revealed yet [34,35]. The use of CAP for UTI prevention in infants with prenatal UTD has been acknowledged as a low level of evidence by the American Urological Association, the SFU, and the Canadian Urological Association [33].

Q6. What is the long-term outcome of antenatally detected UTD?

While permanent kidney damage is known to occur in about 40% of children with moderate or severe UTD [3], only a few studies have provided long-term outcomes of antenatally detected UTD [18,20,36,37]. Costa et al. [19] have reported the development of a composite event of hypertension, proteinuria, and/or reduced estimated glomerular filtration rate (eGFR) in 5% of a cohort of 447 children with isolated antenatal APD ≥5 mm at a median follow-up of 6.4 years. However, children with mild UTD did not have any chronic kidney damage during the follow-up period. Another study by Herthelius et al. [20] has shown that none of the children with antenatally detected UTD has proteinuria or reduced eGFR during 12 to 15 years of follow-up. Among confirmed cases with postnatal renal APD >7 mm and/or kidney parenchyma, calyces, ureters, or bladder pathology, persistent UTD occurred in 15% and persistent kidney damage assessed by renal DMSA (technetium 99m dimercaptosuccinic acid) scan or US was developed in 32% to 39%. They have concluded that it is unnecessary to perform long-term follow-up or use CAP in children with postnatal APD ≤7 mm and normal renal parenchyma, calyces, ureters, and bladder. According to a recent report by Herthelius [3], CAKUT is less likely to be diagnosed afterward in children older than 1 year who have a renal APD <15 mm without other abnormal findings on repeated exams. In contrast, there is a different story for a much longer follow-up of children with CAKUT [38,39]. In a study performed by Sanna-Cherchi et al. [38], renal deterioration was not evident until late adolescence apart from PUVs and bilateral hypodysplasia. However, 58 (18.6%) of 312 patients with CAKUT had started dialysis by 30 years of age. Patients with single kidney and those with renal hypodysplasia combined with PUVs were at increased risks for dialysis (hazard ratios: 2.43 and 5.1, respectively) compared to those with renal hypodysplasia, multicystic kidney, or horseshoe kidney [38]. Another study has also revealed that end-stage kidney disease caused by CAKUT is developed more often in adult age than in pediatric age [39]. Using data on the incidence and prevalence of renal replacement therapy (RRT) in a total of 212,930 patients, the median age at RRT start was found to be 31 years for patients with CAKUT and 61 years for those with non-CAKUT [39]. Patients with renal dysplasia required RRT at a very young age (median, 16 years) compared with those in other CAKUT categories. The incidence of RRT due to reflux-associated pyelonephritis increased sharply during the first two decades, reaching its peak in the early 20s. However, 50% of patients with CAKUT did not start RRT before turning 40s [39]. These studies suggest that ongoing loss of remnant nephrons can lead to CKD progression across the entire age range. Mild forms of CAKUT, including low nephron endowment at birth, seem to be more frequent than expected and be revealed in later adulthood [40]. Effective transition strategies from pediatric to adult nephrology service would be essential to achieve disease-specific good-quality

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care for this group of patients [39]. Individualized follow-up and management plans for children with UTD and/or CAKUT should be applied based on recommendations from a pediatric nephrologist or urologist, or both.

Q7. What do we research for the optimal management of UTD?

Since 2014, many studies have been performed to validate the correlation between the UTD classification system and clinical outcomes [6-9,20]. For predicting various outcomes such as surgical intervention, UTI risk, and chronic kidney damage, further extensive evaluation regarding the grading system would be necessary to assess its utility. Meanwhile, over the years, numerous urinary and serum biomarkers for UPJ obstruction and VUR have been studied, including neutrophil gelatinase-associated lipocalin [41-43], cystatin C [41], kidney injury molecule-1 [44], monocyte chemoattractant protein-1 [44], β2-microglobulin [43], and so on. Further studies are also needed to confirm the efficacy of these biomarkers to predict the development and progression of CKD as well as CAKUT itself. In addition, in line with the artificial intelligence era, investigations for grading UTD using machine learning algorithms (automated convolutional neural network model) have been reported. It was reported that a machine learning model classified 94% to 97.6% of patients correctly or within one grade of the diagnosis of radiologists of UTD [45,46]. Deep learning model could also predict renal complications in children with antenatal UTD concerning UPJ obstruction [47]. These models may offer great promise in their ability to affect clinical decision-making with a large amount of supplemental analytical data. Since genetic and environmental contributions for CAKUT have been identified, long-term prospective studies of patients with CAKUT coupled with comprehensive genomic analysis, functional validation of genetic variants, and an in-depth assessment of the in utero or perinatal environment are needed [40,48]. Recent advances in genetics, epigenetics, and molecular medicine might also offer an opportunity to expand our knowledge on the development of CAKUT and the proper management of patients with this condition.

Conclusion

Optimal evaluation of antenatal and/or postnatal UTD is essential as children with clinically significant abnormalities need to be identified while avoiding unnecessary testing. While most children with antenatal UTD have a favorable long-term outcome with a low risk of kidney disease progression, a greater portion of children with CAKUT need RRT during adulthood than during childhood. There is no definite answer to the question of at what time point we can stop the follow-up safely in a child with persistent UTD. In children with persistent moderate or severe UTD (UTD P2-P3, SFU III-IV), a non-negligible risk of permanent kidney damage exists. To improve the evaluation and management of these patients, future research studies should perform additional risk stratification and develop evidence-based interventions.

Conflicts of interest

Hyung Eun Yim is an editorial-in-chief of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflict of interest relevant to this article was reported.

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Author contributions

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References

1. Ismaili K, Hall M, Donner C, Thomas D, Vermeylen D, Avni FE, et al. Results of systematic screening for minor degrees of fetal renal pelvis dilatation in an unselected population. Am J Obstet Gynecol 2003;188:242-6.
2. Feldman DM, DeCambre M, Kong E, Borgida A, Jamil M, McKenna P, et al. Evaluation and follow-up of fetal hydronephrosis. J Ultrasound Med 2001;20:1065-9.
3. Herthelius M. Antenatally detected urinary tract dilatation: long-term outcome. Pediatr Nephrol 2023;38:3221-7.
4. Nguyen HT, Phelps A, Coley B, Darge K, Rhee A, Chow JS. 2021 update on the urinary tract dilation (UTD) classification system: clarifications, review of the literature, and practical suggestions. Pediatr Radiol 2022;52:740-51.

5. Kim SY, Kim MJ, Yoon CS, Lee MS, Han KH, Lee MJ. Comparison of the reliability of two hydronephrosis grading systems: the Society for Foetal Urology grading system vs. the Onen grading system. Clin Radiol 2013;68:e484-90.
6. Melo FF, Mak RH, Simoes E Silva AC, Vasconcelos MA, Dias CS, Rosa LC, et al. Evaluation of urinary tract dilation classification system for prediction of long-term outcomes in isolated antenatal hydronephrosis: a cohort study. J Urol 2021;206:1022-30.
7. Melo FF, Vasconcelos MA, Mak RH, Silva AC, Dias CS, Colosimo EA, et al. Postnatal urinary tract dilatation classification: improvement of the accuracy in predicting kidney injury. Pediatr Nephrol 2022;37:613-23.
8. Hwang J, Kim PH, Yoon HM, Song SH, Jung AY, Lee JS, et al. Application of the postnatal urinary tract dilation classification system to predict the need for surgical intervention among neonates and young infants. Ultrasonography 2023;42:136-46.
9. Nguyen HT, Benson CB, Bromley B, Campbell JB, Chow J, Coleman B, et al. Multidisciplinary consensus on the classification of prenatal and postnatal urinary tract dilation (UTD classification system). J Pediatr Urol 2014;10:982-98.
10. Lee RS, Cendron M, Kinnamon DD, Nguyen HT. Antenatal hydronephrosis as a predictor of postnatal outcome: a meta-analysis. Pediatrics 2006;118:586-93.
11. Jain S, Chen F. Developmental pathology of congenital kidney and urinary tract anomalies. Clin Kidney J 2018;12:382-99.
12. Mallik M, Watson AR. Antenatally detected urinary tract abnormalities: more detection but less action. Pediatr Nephrol 2008;23:897-904.
13. Aksu N, Yavascan O, Kangin M, Kara OD, Aydin Y, Erdogan H, et al. Postnatal management of infants with antenatally detected hydronephrosis. Pediatr Nephrol 2005;20:1253-9.
14. Deshpande AV. Conversations for the future in the follow-up of antenatally diagnosed renal pelvicalyceal dilatation. Pediatr Nephrol 2021;36:5-8.
15. Roos JF, Doust J, Tett SE, Kirkpatrick CM. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children: a meta-analysis. Clin Biochem 2007;40:383-91.
16. Matsui F, Shimada K, Matsumoto F, Takano S. Late recurrence of symptomatic hydronephrosis in patients with prenatally detected hydronephrosis and spontaneous improvement. J Urol 2008;180:322-5.
17. Zee RS, Herndon CD, Cooper CS, Kim C, McKenna PH, Khoury A, et al. Time to resolution: a prospective evaluation from the Society for Fetal Urology hydronephrosis registry. J Pediatr Urol 2017;13:316.
18. Yang Y, Hou Y, Niu ZB, Wang CL. Long-term follow-up and management of prenatally detected, isolated hydronephrosis. J Pediatr Surg 2010;45:1701-6.
19. Costa FP, Simoes E Silva AC, Mak RH, Ix JH, Vasconcelos MA, Dias CS, et al. A clinical predictive model of renal injury in children with isolated antenatal hydronephrosis. Clin Kidney J 2019;13:834-41.
20. Herthelius M, Axelsson R, Lidefelt KJ. Antenatally detected urinary tract dilatation: a 12-15-year follow-up. Pediatr Nephrol 2020;35:2129-35.
21. Dias CS, Silva JM, Pereira AK, Marino VS, Silva LA, Coelho AM, et al. Diagnostic accuracy of renal pelvic dilatation for detecting surgically managed ureteropelvic junction obstruction. J Urol 2013;190:661-6.
22. Song SH, Park S, Chae SY, Moon DH, Park S, Kim KS. Predictors of renal functional improvement after pyeloplasty in ureteropelvic junction obstruction: clinical value of visually assessed renal tissue tracer transit in 99mTc-mercaptoacetyltriglycine renography. Urology 2017;108:149-54.
23. Hodhod A, Capolicchio JP, Jednak R, Eid H, El-Doray AE, El-Sherbiny M. Is the renal pyramidal thickness a good predictor for pyeloplasty in postnatal hydronephrosis? J Pediatr Urol 2018;14:277.
24. Rickard M, Lorenzo AJ, Braga LH. Renal parenchyma to hydronephrosis area ratio (PHAR) as a predictor of future surgical intervention for infants with high-grade prenatal hydronephrosis. Urology 2017;101:85-9.
25. Walsh TJ, Hsieh S, Grady R, Mueller BA. Antenatal hydronephrosis and the risk of pyelonephritis hospitalization during the first year of life. Urology 2007;69:970-4.
26. Zareba P, Lorenzo AJ, Braga LH. Risk factors for febrile urinary tract infection in infants with prenatal hydronephrosis: comprehensive single center analysis. J Urol 2014;191:1614-8.
27. Zee RS, Herbst KW, Kim C, McKenna PH, Bentley T, Cooper CS, et al. Urinary tract infections in children with prenatal hydronephrosis: a risk assessment from the Society for Fetal Urology Hydronephrosis Registry. J Pediatr Urol 2016;12:261.
28. Visuri S, Jahnukainen T, Taskinen S. Incidence of urinary tract infections in infants with antenatally diagnosed hydronephrosis: a retrospective single center study. J Pediatr Surg 2017;52:1503-6.
29. Lee JH, Choi HS, Kim JK, Won HS, Kim KS, Moon DH, et al. Nonrefluxing neonatal hydronephrosis and the risk of urinary tract infection. J Urol 2008;179:1524-8.
30. Coelho GM, Bouzada MC, Pereira AK, Figueiredo BF, Leite MR, Oliveira DS, et al. Outcome of isolated antenatal hydronephrosis: a prospective cohort study. Pediatr Nephrol 2007;22:1727-34.
31. Sencan A, Carvas F, Hekimoglu IC, Caf N, Sencan A, Chow J, et al. Urinary tract infection and vesicoureteral reflux in children with mild antenatal hydronephrosis. J Pediatr Urol 2014;10:1008-13.
32. Pennesi M, Amoroso S, Bassanese G, Pintaldi S, Giacomini G, Barbi E. Frequency of urinary tract infection in children with antenatal diag-

https://doi.org/10.3339/ckd.24.002

nosis of urinary tract dilatation. Arch Dis Child 2020;105:260-3.
33. Braga LH, Easterbrook B, Jegatheeswaran K, Lorenzo AJ. From research question to conducting a randomized controlled trial on continuous antibiotic prophylaxis in prenatal hydronephrosis: a rational stepwise process. Front Pediatr 2016;4:27.
34. Silay MS, Undre S, Nambiar AK, Dogan HS, Kocvara R, Nijman RJ, et al. Role of antibiotic prophylaxis in antenatal hydronephrosis: a systematic review from the European Association of Urology/European Society for Paediatric Urology Guidelines Panel. J Pediatr Urol 2017;13:306-15.
35. Rianthavorn P, Phithaklimnuwong S. The role of antibiotic prophylaxis in mild to moderate isolated hydronephrosis detected in antenatal screening. Investig Clin Urol 2020;61:200-6.
36. Nef S, Neuhaus TJ, Sparta G, Weitz M, Buder K, Wisser J, et al. Outcome after prenatal diagnosis of congenital anomalies of the kidney and urinary tract. Eur J Pediatr 2016;175:667-76.
37. Sarhan OM, Helaly AE, Al Otay A, Ghanbar MA, Nakshabandi Z. Isolated low grade prenatally detected unilateral hydronephrosis: do we need long term follow-up? Int Braz J Urol 2018;44:812-8.
38. Sanna-Cherchi S, Ravani P, Corbani V, Parodi S, Haupt R, Piaggio G, et al. Renal outcome in patients with congenital anomalies of the kidney and urinary tract. Kidney Int 2009;76:528-33.
39. Wuhl E, van Stralen KJ, Verrina E, Bjerre A, Wanner C, Heaf JG, et al. Timing and outcome of renal replacement therapy in patients with congenital malformations of the kidney and urinary tract. Clin J Am Soc Nephrol 2013;8:67-74.
40. Murugapoopathy V, Gupta IR. A primer on congenital anomalies of the kidneys and urinary tracts (CAKUT). Clin J Am Soc Nephrol 2020;15:723-31.
41. Pavlaki A, Printza N, Farmaki E, Stabouli S, Taparkou A, Sterpi M, et al. The role of urinary NGAL and serum cystatin C in assessing the severity of ureteropelvic junction obstruction in infants. Pediatr Nephrol 2020;35:163-70.
42. Gavrilovici C, Dusa CP, Iliescu Halitchi C, Lupu VV, Spoiala EL, Bogos RA, et al. The role of urinary NGAL in the management of primary vesicoureteral reflux in children. Int J Mol Sci 2023;24:7904.
43. Kazlauskas V, Bilius V, Jakutis V, Komiagiene R, Burnyte B, Verkauskas G. Urine biomarkers combined with ultrasound for the diagnosis of obstruction in pediatric hydronephrosis. Front Pediatr 2022;9:762417.
44. Karakus S, Oktar T, Kucukgergin C, Kalelioglu I, Seckin S, Atar A, et al. Urinary IP-10, MCP-1, NGAL, Cystatin-C, and KIM-1 levels in prenatally diagnosed unilateral hydronephrosis: the search for an ideal biomarker. Urology 2016;87:185-92.
45. Smail LC, Dhindsa K, Braga LH, Becker S, Sonnadara RR. Using deep learning algorithms to grade hydronephrosis severity: toward a clinical adjunct. Front Pediatr 2020;8:1.
46. Ostrowski DA, Logan JR, Antony M, Broms R, Weiss DA, Van Batavia J, et al. Automated Society of Fetal Urology (SFU) grading of hydronephrosis on ultrasound imaging using a convolutional neural network. J Pediatr Urol 2023;19:566.
47. Weaver JK, Logan J, Broms R, Antony M, Rickard M, Erdman L, et al. Deep learning of renal scans in children with antenatal hydronephrosis. J Pediatr Urol 2023;19:514.
48. Son MH, Park E, Yim HE, Nam YJ, Lee YS, Choi EK, et al. Maternal exposure to airborne particulate matter during pregnancy and lactation induces kidney injury in rat dams and their male offspring: the role of vitamin D in pregnancy and beyond. Kidney Res Clin Pract 2024 Jan 2 [Epub]. https://doi.org/10.23876/j.krcp.23.106

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Navigating the landscape of clinical genetic testing: insights and challenges in rare disease diagnostics

Soo Yeon Kim[1]

[1]Department of Genomic Medicine, Seoul National University Hospital, Seoul, Republic of Korea

With the rapid evolution of diagnostic tools, particularly next-generation sequencing, the identification of genetic diseases, predominantly those with pediatric-onset, has significantly advanced. However, this progress presents challenges that span from selecting appropriate tests to the final interpretation of results. This review examines various genetic testing methodologies, each with specific indications and characteristics, emphasizing the importance of selecting the appropriate genetic test in clinical practice, taking into account factors like detection range, cost, turnaround time, and specificity of the clinical diagnosis. Interpretation of variants has become more challenging, often requiring further validation and significant resource allocation. Laboratories primarily classify variants based on the American College of Medical Genetics and Genomics and the Association for Clinical Genomic Science guidelines, however, this process has limitations. This review underscores the critical role of clinicians in matching patient phenotypes with reported genes/variants and considering additional factors such as variable expressivity, disease pleiotropy, and incomplete penetrance. These considerations should be aligned with specific gene-disease characteristics and segregation results based on an extended pedigree. In conclusion, this review aims to enhance understanding of the complexities of clinical genetic testing, advocating for a multidisciplinary approach to ensure accurate diagnosis and effective management of rare genetic diseases.

Keywords: Genetic diseases; Genetic testing; Rare diseases

Introduction

The landscape of diagnostic methodologies for genetic diseases has undergone a remarkable transformation, leading to the discovery of thousands of genetic conditions. Currently, around 7,000 rare diseases have been identified, with an estimated 80% attributed to genetic factors, and 50% manifesting during childhood [1-4]. Early genetic diagnosis has proven particularly beneficial for pediatric patients, offering significant cost savings and enabling long-term disease management [5]. Recent technological advancements and cost reductions have made various genetic tests integral to diagnostic evaluations in clinical practice. Next-generation sequencing (NGS) stands out due to its ability to simultaneously identify variants across multiple genes. This capability has led to cost efficiency and high diagnostic rates, especially for diseases characterized by genetic heterogeneity. Nevertheless, the challenge of interpreting the vast array of variants generated by NGS is non-trivial and often requires additional resources for validation. NGS may not be the optimal choice for single-gene diseases distinguishable by characteristic clinical features, and it is not considered the gold standard for detecting certain genetic variations such as copy number varia-

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Correspondence to
Soo Yeon Kim
Department of Genomic Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea
E-mail: idue0209@snu.ac.kr

tions (CNVs) or short tandem repeats (STRs). Therefore, a critical aspect of the diagnostic process is accurate clinical assessment, followed by the judicious choice of testing methodology and careful interpretation of test reports by clinicians.

This review is divided into two sections. The first section covers various clinical genetic testing methodologies, discussing the types of tests and factors influencing test selection. The second section focuses on the interpretation of germline sequence variants, a common outcome of NGS. This structure allows for a comprehensive exploration of clinical genetic testing, ranging from broad methodologies to the detailed interpretation of specific variants.

Comprehensive review of clinical genetic testing in rare genetic diseases

Overview of genetic testing modalities

Clinical genetic testing has become a pivotal component in diagnosing rare diseases, offering a broad range of tests available in clinical settings. Each genetic test is performed based on specific principles and has corresponding indications. There are three primary types of genetic testing: cytogenetic (chromosomal), DNA (molecular), and biochemical. Chromosomes, thread-like structures made up of DNA can be observed under a microscope after specific staining since the 1980s [6]. Microarray techniques, designed to identify small-unbalanced rearrangements, had developed and now feature diverse established platforms [7-9]. This method is recognized as a first-tier cytogenetic test for patients with developmental delay/intellectual disabilities, multiple congenital anomalies, and autism spectrum disorder [10-13]. Fluorescence *in situ* hybridization provides a unique advantage by visually mapping genetic material within a cell, facilitating the identification of structural chromosomal abnormalities [14,15]. Molecular genetic testing, the most frequently performed category, assesses single DNA loci, single genes, or multiple genes. Sanger sequencing, a traditional method and the gold standard for identifying single nucleotide variations is renowned for its high accuracy in analyzing short DNA sequences [16]. The polymerase chain reaction (PCR), a versatile tool widely used to amplify small DNA segments, is essential in various genetic tests, including those for infectious diseases [17-19]. Multiplex ligation-dependent probe amplification (MLPA), a robust method for detecting deletions and duplications of up to 50 nucleic acid sequences, proves invaluable for diagnosing various genetic disorders [20,21]. Some biochemical genetic tests, such as Southern blotting, while less common, still play a role in identifying specific DNA sequences in larger DNA samples [22,23]. NGS, a revolutionary form of molecular genetic testing, enables the rapid sequencing of large stretches of DNA or RNA, dramatically transforming the fields of genomics and molecular biology and facilitating a broad range of applications [24-27].

Critical factors in genetic test selection

In clinical practice, each genetic testing method is tailored to a specific indication. In terms of the detection range, some tests focus on single loci, while others, like NGS, can cover the entire genome. However, NGS is not the gold standard for detecting CNVs or STRs. Despite a decrease in cost and processing time, NGS remains more expensive and has a longer turnaround time (TAT) compared to traditional tests. Clinicians must take these factors into account when selecting tests (Table 1). An accurate and specific clinical diagnosis is crucial, as it guides the identification of potential causative genes and common types of genetic variation. For example, in cases clinically diagnosed with Fragile X syndrome, the first-tier confirmatory tests are Southern blotting or PCR targeting *FMR1*. Similarly, for pathologically confirmed Alport syndrome with a family history of X-linked inheritance, testing for the *COL4A5* gene using sequencing and MLPA is advisable, as 10% to 15% of these cases involve exon-level deletions or duplications [28,29]. A precise clinical diagnosis facilitates targeted testing, thereby reducing both the length of the diagnostic journey and associated costs. Although essential, a detailed clinical assessment, including examination findings, routine laboratory tests, and specific biomarkers such as pathological findings, is not extensively discussed in this article.

The urgency of diagnosis is vital in certain cases, necessitating consideration of clinical severity and TAT. Many inherited metabolic disorders can lead to irreversible damage if not promptly managed [30]. In cases of serious and rapidly progressive illnesses, quick decision-making is essential. Some patients may find themselves in a situation where they have a serious and rapidly progressive illness, requiring swift decision-making. In such cases, opting for tests with the fastest available results rather than the most cost-effective sequence of tests may be clinically justified. Rapid genomic sequencing, which significantly shortens TAT, is increasingly used for patients with suspected medically actionable disorders or those in intensive care units [31-33].

Table 1. Comparative characteristics of different genetic testing methodologies

Method	Range	Common indication	TAT	Cost	Example
Karyotype	Genome-wide	CNVs Other structural variations	<1 mo	Low	Down syndrome
Chromosomal microarray	Genome-wide	CNVs UPD (SNP platform)	< 1 mo	Average	Challenging cases
FISH	Targeted	CNVs Other structural variations	<1 wk	Low	Angelman's syndrome
Target PCR	Targeted	SNVs Repeat expansions	<1 wk	Low	
MLPA	Targeted	Small CNV (exon level)	>1 mo	Low	Duchenne muscular dystrophy
Southern blot	Targeted	Small CNV Repeat expansions	>1 mo	Low	Fragile X syndrome
Sanger sequencing	Targeted	SNVs	>1 mo	Average	Cystic fibrosis
Gene panel	Targeted (wide)	SNVs	>2 mo	High	Long QT syndrome
Exome/genome sequencing	Genome-wide	SNVs CNVs (possible only in genome sequencing)	>2 mo	High	Challenging cases

TAT, turn-around-time; CNV, copy number variation; UPD, uniparental disomy; SNP, single nucleotide polymorphism; FISH, fluorescence *in situ* hybridization; PCR, polymerase chain reaction; SNV, single nucleotide variation; MLPA, multiplex ligation-dependent probe amplification.

Finally, cost-effectiveness is a critical factor in healthcare, particularly for the diagnosis of rare diseases. The introduction of advanced genomic technologies like NGS has broadened our diagnostic scope, but their higher initial costs necessitate careful test selection. Cost-effectiveness typically involves starting with less expensive tests, followed by more comprehensive and sensitive yet costlier methods if the initial results are inconclusive. This tiered approach balances the need for thorough genetic analysis with budget constraints and enhances patient care by providing efficient and economically viable genetic testing strategies. Economic considerations in genetic testing go beyond cost reduction; they focus on maximizing the value that each test brings in terms of clinical outcomes and informed healthcare decisions. Although recent studies have demonstrated the cost-effectiveness of genome sequencing as a primary diagnostic tool in certain rare disease groups [34-36], the heterogeneity in study inclusions and cost-effectiveness parameters cast doubt on the generalizability of these results, highlighting the need for further well-designed studies. Additionally, costs and availability differ by country; for example, in Korea, the current insurance system officially covers only limited multi-gene panel tests, necessitating a different approach to the diagnostic strategy.

Deciphering genetic variants: analysis and clinical correlation

Various genetic variants contribute to the development of rare genetic disorders. However, this section focuses specifically on germline sequence variants, which have become increasingly significant due to the rise in NGS utilization. This technology generates numerous variants of uncertain clinical significance. The process of identifying variants through NGS data involves the following sequential steps: variant calling, annotation, and the evaluation of disease causality. In this section, we will delve into the process of evaluating the final disease causality of a variant as reported in the test, particularly from the clinician's perspective.

Germline variant classification

Classifying germline sequence variants is a crucial step in genetic testing, guided by comprehensive criteria established by leading organizations such as the American College of Medical Genetics and Genomics (ACMG) and the Association for Clinical Genomic Science (ACGS). The widely recognized and utilized ACMG guideline categorizes variants into five groups: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. This classification relies on various factors, including the population database, gene characteristics, prior reports, segregation results, computational predictions, and

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functional studies [37]. ACGS provides a framework aligned with ACMG, underscoring the significance of clinical context and multidisciplinary expert consensus in variant interpretation [38]. Typically, laboratory physicians report pathogenic and likely pathogenic variants and occasionally variants of uncertain significance based on their policies. Despite global application, these guidelines are not without limitations. Variability in interpretation among clinicians and laboratories may result in inconsistent variant classification [39]. The databases crucial for variant interpretation are still evolving and may not sufficiently cover population-specific variations. Furthermore, these guidelines often prioritize molecular characteristics over the complete clinical profile of the patient, potentially leading to less personalized assessments. There is a growing demand for clearer variant interpretation, prompting efforts to modify and update these guidelines to better suit specific genetic or clinical subgroups [40-44]. However, studies based on large-scale cohorts have yet to provide universally applicable criteria for variant interpretation. Consequently, the final interpretation of variants, particularly those of uncertain significance, remains challenging when relying solely on guidelines. Achieving accurate interpretation and application of these findings necessitates a comprehensive, patient-centric approach by clinicians [45-48].

Clinical correlation and personalized interpretation of variants

Before finalizing the results for a patient, clinicians must consider various factors in alignment with the reported genes/variants. The first factor is key phenotypes which include the age of onset, primary clinical symptoms, and disease progression. While theoretically, patients with the same variant may exhibit similar clinical symptoms, it is common to find individuals with the same variant presenting a wide range of diverse phenotypes, even within the same family (Fig. 1). This necessitates careful consideration [49]. For instance, the autosomal dominant polycystic kidney disease, is well known for various phenotypes, ranging from simple cyst to early end-stage renal disease, illustrating variable expressivity (Fig. 1A) [50]. The *GLB1* gene, known to cause GM1-gangliosidosis with symptoms including progressive cerebral degeneration and developmental regression, is also responsible for Morquio disease. Morquio disease is characterized by multiple skeletal abnormalities and coarse facial features without clear neurological symptoms, exemplifying phenotypic variation or pleiotropy (Fig. 1B) [51,52]. A detailed family history assessment is crucial for patients with genetic disorders. The inheritance patterns of the identified genes should align with the family history and segregation results. If a gene associated with an autosomal dominant Mendelian disorder is documented, the variant should not be present in the asymptomatic parents, indicating de novo variation. Family test results, reflecting inheritance patterns, are critical

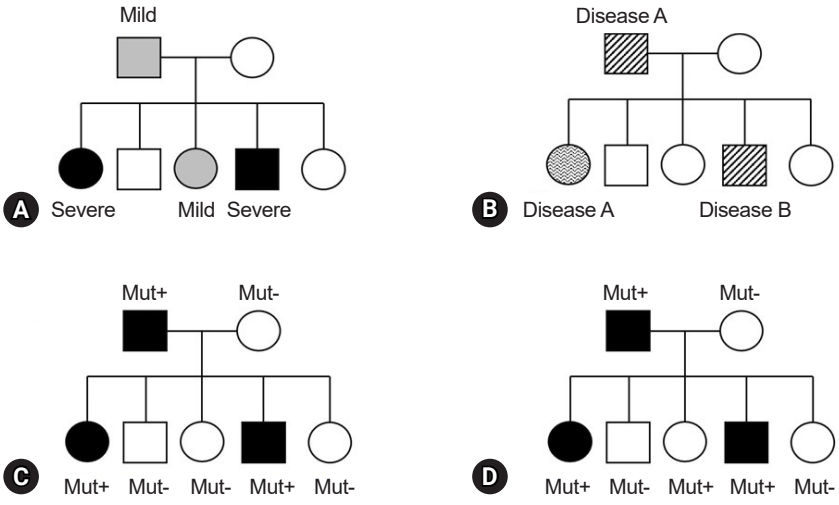

Fig. 1. Conceptual representation of expressivity, pleiotropy, and penetrance in autosomal dominant genetic disorders. (A) A pedigree displaying an autosomal dominant genetic with varying levels of disease expressivity among family members. (B) A pedigree illustrating an autosomal dominant genetic disorder demonstrating disease pleiotropy within family members. (C) A pedigree of autosomal dominant genetic disorder exhibiting complete penetrance. (D) A pedigree of autosomal dominant genetic disorder exhibiting incomplete penetrance. Mut, mutation.

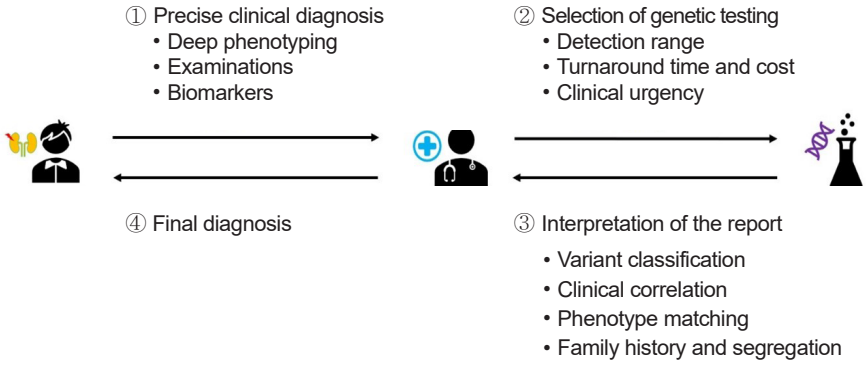

Fig. 2. Physician's guide to genetic testing in rare diseases.

and are incorporated into the ACMG and ACGS guidelines [37,38]. Unmatched results necessitate a reevaluation of the diagnosis. Notably, some diseases exhibit incomplete or reduced penetrance, explaining why asymptomatic family members carry the causative variant (Fig. 1D) [53,54]. Factors influencing penetrance include variant types, gene expression levels, epigenetic changes, gene-environment interactions, and genetic modifiers [55]. However, these theoretical factors often provide limited practical insight in clinical settings. Clinicians primarily rely on previous clinical reports and databases for practical information. For example, consider the case of the *COL1A1* gene, associated with osteogenesis imperfecta, a rare connective tissue disorder. Osteogenesis imperfecta is known for its variable expression and incomplete penetrance, as documented in several clinical studies [56,57]. When a variant of the *COL1A1* is identified in a family, and an asymptomatic family member carries the variant, it can be confirmed as causative if the phenotype matches the disease and co- segregation results for the rest of the family members (ideally from an extended pedigree) align with the known inheritance pattern, given the recognized incomplete penetrance of the gene. Similarly, specific diseases or gene subgroups, such as inherited retinal disease, hereditary spastic paraplegia, polycystic kidney disease, and renal agenesis/hypoplasia, are known to exhibit incomplete penetrance [58-61]. In summary, clinicians can ascertain the final causality of a variant using case-level clinical correlations. This determination hinges on a comprehensive assessment in which all pieces of the puzzle, including phenotypic consistency with the gene, family test results based on an extended pedigree, and research findings from existing databases, fit together harmoniously. If any of these factors are missing or inconclusive, a conservative interpretation approach should be adopted.

Conclusion

This review highlights the complexities and advancements in clinical genetic testing for rare diseases, emphasizing significant strides in diagnostic tool development, particularly NGS. While NGS has greatly improved our ability to identify genetic diseases, it has also necessitated meticulous validation. The selection of an appropriate genetic test in clinical practice requires careful consideration of factors such as detection range, cost, and clinical specificity. The role of germline variant classification, guided by the ACMG and ACGS guidelines, is crucial, but it faces limitations, including subjectivity and insufficient coverage of population-specific variations. To ensure accurate diagnosis and effective management, clinicians must meticulously align patient phenotypes with reported genes/variants, taking into consideration the variability in disease expression and extensive family histories (Fig. 2). This holistic and detailed approach is essential for enhancing patient care within the complex landscape of rare genetic diseases.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References

1. Boycott KM, Vanstone MR, Bulman DE, MacKenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nat Rev Genet 2013;14:681-91.
2. Orphanet activity report. Orphanet reports series/procedures [Internet]. Orphanet; 2022 [cited 2024 Jan 11]. Available from: https://www.orpha.net/consor/cgi-bin/Education_Home.php?lng=EN
3. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet 2020;28:165-73.
4. Global Genes. RARE disease facts [Internet]. Global Genes; 2020 [cited 2024 Jan 11]. Available from: https://globalgenes.org/rare-facts/
5. Lalonde E, Rentas S, Lin F, Dulik MC, Skraban CM, Spinner NB. Genomic diagnosis for pediatric disorders: revolution and evolution. Front Pediatr 2020;8:373.
6. Yunis JJ. Mid-prophase human chromosomes: the attainment of 2000 bands. Hum Genet 1981;56:293-8.
7. Kallioniemi OP, Kallioniemi A, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization: a rapid new method for detecting and mapping DNA amplification in tumors. Semin Cancer Biol 1993;4:41-6.
8. Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 1997;20:399-407.
9. Albertson DG, Pinkel D. Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 2003;12 Spec No 2:R145-52.
10. Ahn JW, Bint S, Bergbaum A, Mann K, Hall RP, Ogilvie CM. Array CGH as a first line diagnostic test in place of karyotyping for postnatal referrals: results from four years' clinical application for over 8,700 patients. Mol Cytogenet 2013;6:16.
11. Battaglia A, Doccini V, Bernardini L, Novelli A, Loddo S, Capalbo A, et al. Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. Eur J Paediatr Neurol 2013;17:589-99.
12. Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. Annu Rev Med 2010;61:437-55.
13. Vissers LE, de Vries BB, Veltman JA. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. J Med Genet 2010;47:289-97.
14. Rudkin GT, Stollar BD. High resolution detection of DNA-RNA hybrids in situ by indirect immunofluorescence. Nature 1977;265:472-3.
15. Iqbal MA, Ulmer C, Sakati N. Use of FISH technique in the diagnosis of chromosomal syndromes. East Mediterr Health J 1999;5:1218-24.
16. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74:5463-7.
17. Goossens M. The amplification of nucleotide sequences by PCR and the new technics for molecular diagnosis. Reprod Nutr Dev 1990;Suppl 1:117s-124s.
18. Ben-Ezra JM. Amplification methods in the molecular diagnosis of genetic diseases. Clin Lab Med 1995;15:795-815.
19. Erlich HA, Arnheim N. Genetic analysis using the polymerase chain reaction. Annu Rev Genet 1992;26:479-506.
20. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002;30:e57.
21. Stuppia L, Antonucci I, Palka G, Gatta V. Use of the MLPA assay in the molecular diagnosis of gene copy number alterations in human genetic diseases. Int J Mol Sci 2012;13:3245-76.
22. Borst M, Miller DM. DNA isolation and Southern analysis: a clinician's view. Am J Med Sci 1990;299:356-60.
23. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503-17.
24. Reuter JA, Spacek DV, Snyder MP. High-throughput sequencing technologies. Mol Cell 2015;58:586-97.
25. Pettersson E, Lundeberg J, Ahmadian A. Generations of sequencing technologies. Genomics 2009;93:105-11.
26. Fernandez-Marmiesse A, Gouveia S, Couce ML. NGS technologies as a turning point in rare disease research, diagnosis and treatment. Curr Med Chem 2018;25:404-32.
27. Chung CC, Hue SP, Ng NY, Doong PH; Hong Kong Genome Project; Chu AT, et al. Meta-analysis of the diagnostic and clinical utility of exome and genome sequencing in pediatric and adult patients with rare diseases across diverse populations. Genet Med 2023;25:100896.
28. Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European Community Alport Syndrome Concerted Action" study. J Am Soc Nephrol 2003;14:2603-10.
29. Bekheirnia MR, Reed B, Gregory MC, McFann K, Shamshirsaz AA, Masoumi A, et al. Genotype-phenotype correlation in X-linked Alport syndrome. J Am Soc Nephrol 2010;21:876-83.
30. Rao AN, Kavitha J, Koch M, Suresh Kumar V. Inborn errors of metabolism: review and data from a tertiary care center. Indian J Clin Biochem 2009;24:215-22.
31. Kim MJ, Kim SY, Lee JS, Kang S, Park LJ, Choi W, et al. Rapid targeted sequencing using dried blood spot samples for patients with suspected actionable genetic diseases. Ann Lab Med 2023;43:280-9.

32. Owen MJ, Niemi AK, Dimmock DP, Speziale M, Nespeca M, Chau KK, et al. Rapid sequencing-based diagnosis of thiamine metabolism dysfunction syndrome. N Engl J Med 2021;384:2159-61.
33. Wojcik MH, Callahan KP, Antoniou A, Del Rosario MC, Brunelli L, ElHassan NO, et al. Provision and availability of genomic medicine services in level IV neonatal intensive care units. Genet Med 2023;25:100926.
34. Incerti D, Xu XM, Chou JW, Gonzaludo N, Belmont JW, Schroeder BE. Cost-effectiveness of genome sequencing for diagnosing patients with undiagnosed rare genetic diseases. Genet Med 2022;24:109-18.
35. Runheim H, Pettersson M, Hammarsjo A, Nordgren A, Henriksson M, Lindstrand A, et al. The cost-effectiveness of whole genome sequencing in neurodevelopmental disorders. Sci Rep 2023;13:6904.
36. Yeung A, Tan NB, Tan TY, Stark Z, Brown N, Hunter MF, et al. A cost-effectiveness analysis of genomic sequencing in a prospective versus historical cohort of complex pediatric patients. Genet Med 2020;22:1986-93.
37. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
38. Ellard S, Baple EL, Callaway A, Berry I, Forrester N, Turnbull C, et al. ACGS best practice guidelines for variant classification in Rare Disease 2020 [Internet]. Association for Clinical Genomic Science; 2020 [cited 2024 Jan 11]. Available from: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf
39. Amendola LM, Jarvik GP, Leo MC, McLaughlin HM, Akkari Y, Amaral MD, et al. Performance of ACMG-AMP variant-interpretation guidelines among nine laboratories in the clinical sequencing exploratory research consortium. Am J Hum Genet 2016;98:1067-76.
40. Inoue Y, Machida O, Kita Y, Yamamoto T. Need for revision of the ACMG/AMP guidelines for interpretation of X-linked variants. Intractable Rare Dis Res 2022;11:120-4.
41. Patel MJ, DiStefano MT, Oza AM, Hughes MY, Wilcox EH, Hemphill SE, et al. Disease-specific ACMG/AMP guidelines improve sequence variant interpretation for hearing loss. Genet Med 2021;23:2208-12.
42. Strande NT, Brnich SE, Roman TS, Berg JS. Navigating the nuances of clinical sequence variant interpretation in Mendelian disease. Genet Med 2018;20:918-26.
43. Gelb BD, Cave H, Dillon MW, Gripp KW, Lee JA, Mason-Suares H, et al. ClinGen's RASopathy Expert Panel consensus methods for variant interpretation. Genet Med 2018;20:1334-45.
44. Savige J, Storey H, Watson E, Hertz JM, Deltas C, Renieri A, et al. Consensus statement on standards and guidelines for the molecular diagnostics of Alport syndrome: refining the ACMG criteria. Eur J Hum Genet 2021;29:1186-97.
45. Chen E, Facio FM, Aradhya KW, Rojahn S, Hatchell KE, Aguilar S, et al. Rates and classification of variants of uncertain significance in hereditary disease genetic testing. JAMA Netw Open 2023;6:e2339571.
46. Burke W, Parens E, Chung WK, Berger SM, Appelbaum PS. The challenge of genetic variants of uncertain clinical significance : a narrative review. Ann Intern Med 2022;175:994-1000.
47. Johnson B, Ouyang K, Frank L, Truty R, Rojahn S, Morales A, et al. Systematic use of phenotype evidence in clinical genetic testing reduces the frequency of variants of uncertain significance. Am J Med Genet A 2022;188:2642-51.
48. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho YY, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med 2017;19:1105-17.
49. Kingdom R, Wright CF. Incomplete penetrance and variable expressivity: from clinical studies to population cohorts. Front Genet 2022;13:920390.
50. Rossetti S, Kubly VJ, Consugar MB, Hopp K, Roy S, Horsley SW, et al. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. Kidney Int 2009;75:848-55.
51. Benson PF, Barbarik A, Brown SP, Mann TP. GM1-generalized gangliosidosis variant with cardiomegaly. Postgrad Med J 1976;52:159-65.
52. Arbisser AI, Donnelly KA, Scott CI Jr, DiFerrante N, Singh J, Stevenson RE, et al. Morquio-like syndrome with beta galactosidase deficiency and normal hexosamine sulfatase activity: mucopolysaccharidosis IVB. Am J Med Genet 1977;1:195-205.
53. Vytopil M, Ricci E, Dello Russo A, Hanisch F, Neudecker S, Zierz S, et al. Frequent low penetrance mutations in the Lamin A/C gene, causing Emery Dreifuss muscular dystrophy. Neuromuscul Disord 2002;12:958-63.
54. Burdon KP, Wirth MG, Mackey DA, Russell-Eggitt IM, Craig JE, Elder JE, et al. A novel mutation in the Connexin 46 gene causes autosomal dominant congenital cataract with incomplete penetrance. J Med Genet 2004;41:e106.
55. Shawky RM. Reduced penetrance in human inherited disease. Egypt J Medl Hum Genet 2014;15:103-11.
56. Pereira R, Halford K, Sokolov BP, Khillan JS, Prockop DJ. Phenotypic variability and incomplete penetrance of spontaneous fractures in an inbred strain of transgenic mice expressing a mutated collagen gene (COL1A1). J Clin Invest 1994;93:1765-9.
57. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A 2014;164A:1470-81.
58. Ellingford JM, Hufnagel RB, Arno G. Phenotype and genotype correlations in inherited retinal diseases: population-guided variant interpretation, variable expressivity and incomplete penetrance.

https://doi.org/10.3339/ckd.24.005

Genes (Basel) 2020;11:1274.
59. Vujic M, Heyer CM, Ars E, Hopp K, Markoff A, Orndal C, et al. Incompletely penetrant PKD1 alleles mimic the renal manifestations of ARPKD. J Am Soc Nephrol 2010;21:1097-102.
60. Stein Q, Westemeyer M, Darwish T, Pitman T, Hager M, Tabriziani H, et al. Genetic counseling in kidney disease: a perspective. Kidney Med 2023;5:100668.
61. Burger J, Fonknechten N, Hoeltzenbein M, Neumann L, Bratanoff E, Hazan J, et al. Hereditary spastic paraplegia caused by mutations in the SPG4 gene. Eur J Hum Genet 2000;8:771-6.

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Review Article

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Advances in the use of dried blood spots on filter paper to monitor kidney disease

Carla Nicola[1], Vandréa de Souza[1]

[1]Graduate Health Sciences Program, Universidade de Caxias do Sul, Caxias do Sul, Brazil

Patients with kidney disease require frequent blood tests to monitor their kidney function, which is particularly difficult for young children and the elderly. For these people, the standard method is to evaluate serum creatinine or cystatin C or drug levels through venous sampling, but more recently, evaluation using dried blood spots has been used. This narrative review reports information from the literature on the use of dried blood spots to quantify the main markers used to detect kidney diseases. The ScienceDirect and PubMed databases were searched using the keywords: "dried blood on filter paper," "markers of renal function," "renal function," "creatinine," "cystatin C," "urea," "iohexol," and "iotalamate." Studies using animal samples were excluded, and only relevant articles in English or Spanish were considered. Creatinine was the most assessed biomarker in studies using dried blood spots to monitor kidney function, showing good performance in samples whose hematocrit levels were within normal reference values. According to the included studies, dried blood spots are a practical monitoring alternative for kidney disease. Validation parameters, such as sample and card type, volume, storage, internal patterns, and the effects of hematocrit are crucial to improving the reliability of these results.

Keywords: Creatinine; Cystatin C; Dried blood spot testing; Iohexol; Urea

Introduction

Kidney disease is a public health problem, with more than 750 million people diagnosed worldwide. In 2019, 1.3 million people lost their lives due to kidney failure, and nearly 1.7 million die from acute kidney injury every year [1-3]. Chronic kidney disease (CKD) is a challenge because it manifests with unspecific or no clinical symptoms; symptoms are detected only at more advanced stages [4,5]. The most frequent complications of this disease are cardiovascular disorders, mineral and bone imbalance, and progression of CKD [6].

Kidney diseases can be recognized by identifying an imbalance in markers such as amino acids, lipids, and nucleotides. These compounds can suggest that there is a problem, expediting proper treatment and thus reducing complications [7-9]. The main indicators of kidney injury are albuminuria (albumin to creatinine ratio ≥30 mg/g), urinary sediment abnormalities characteristic of tubular disease, electrolytic disorders, and reduced renal function (glomerular filtration rate [GFR] <60 mL/min/1.73 m^2) [10-12].

Indicators of kidney injury are mainly detected through urine and venous blood samples. These samples must be refrigerated due to the instability of the compounds, which can undergo enzymatic degradation [13,14]. However, dried blood spots (DBS) have gained relevance and may especially benefit populations at risk of CKD [13,15]. DBS is advantageous for infants and el-

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Correspondence to
Carla Nicola
Graduate Health Sciences Program, Universidade de Caxias do Sul, Francisco Getúlio Vargas, 1130 Caxias do Sul 95070-560, Brazil
E-mail: carla1982n@hotmail.com

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derly patients, especially because it requires less blood volume than conventional tests [16,17]. Despite the observed practical advantages, these assays involve methodological concerns that should be discussed, such as homogeneity of the sample point, hematocrit, and sample recovery [18,19].

Hence, this narrative review covers potential applications of DBS including GFR estimation, drug level monitoring, and its advantages and limitations, as well as precautions when applying it in clinical practice.

Markers of kidney function

The GFR, which describes the volume of plasma filtered from the glomerular capillaries into Bowman's capsules per unit of time [20], is considered a sensitive and specific indicator of abnormal kidney function [21]. The gold standard for GFR measurement is determining the clearance of compounds filtered exclusively by the glomeruli. Exogenous markers, such as iohexol, inulin, and iothalamate, meet this criterion, but they are used only in specific situations (e.g., drug adjustment or kidney protocols) due to their cost and complexity [10].

In most circumstances, GFR is estimated using compounds eliminated by the kidneys (creatinine and cystatin C) based on mathematical equations to correct for biological variations [22,23]. Creatinine levels vary according to age, sex, metabolism, muscle mass, and nutritional status. Cystatin C seems to be less dependent on biological factors, but its levels may increase with glucocorticoid use and show poor agreement during pregnancy due to placental production [8,24,25].

Principles and applications of DBS methods

The first officially established tests to use dried whole blood samples on filter paper in the pre-analytical phase of laboratory testing were performed in 1963, with the discovery of an effective low-cost neonatal screening test to identify phenylketonuria [26]. The successful screening of this and other inborn errors of metabolism using DBS has led to its adaptation for a myriad of analytical parameters, such as drug monitoring, protein studies, and infectious disease management [14,27,28]. Table 1 summarizes the main applications of DBS in kidney diseases.

The filter paper method has advantages over conventional venipuncture, since blood collection is easy to perform, less invasive, and relatively painless [29,30]. The paper filter method minimizes the volume of blood taken from patients and may be performed without specialized structures [29]. Furthermore, it is better suited for clinical research and patients who must undergo numerous blood tests or who have damaged veins, as well as for infants and older people [29,31,32].

Table 1. Potential applications of dried blood spots in kidney disease

- Screening and monitoring GFR decline in high-risk patients for CKD progression.
- Drug monitoring or adjustment in patients using nephrotoxic drugs or having underlying kidney disease.
- Patients at high risk for CKD who need multiple blood sampling at home (e.g., underlying diabetes mellitus and infants or elderly patients).

GFR, glomerular filtration rate; CKD, chronic kidney disease.

Determining biochemical parameters from blood samples requires a well-established quality control system [33]. Factors such as sample collection procedure, sample volume, spot quality, filter paper type, drying and storage methods, hematocrit, and the incorporation of internal standards are important parameters for good DBS performance and vary depending on the analyte [34-37].

Relevant factors in DBS methods

Sample collection

In the classic filter paper system, a few drops of whole blood (5–50 μL) are collected on a card by finger prick with a lancet [29]. At this stage, certain precautions are essential, such as thorough disinfection, discarding the first drop of blood, which may contain tissue fluid, completely filling in the card's outlined circle, and drying the sample at room temperature [14,38]. In viability testing of home-collected DBS samples for creatinine analysis, blood adherence to the cards was high, but only 80% of the spots showed accurate saturation and were suitable for analysis [39].

Capillary blood collected by finger prick is a mixture of arterial blood, venous blood, and interstitial fluids. Biomarker concentrations in capillary blood collected in DBS should be different from those found in venous blood [35]. Lower concentrations of cystatin C were found in blood collected by finger prick than in venous blood [40]. GFR measured by iohexol clearance has proven reliable in venous samples and capillary blood spots, although the capillary method overestimated venous GFR by 7.2% [41]. Conversely, both venous sampling and finger stick sampling at 2-time points after iohexol infusion resulted

in an acceptably accurate GFR measurement [42]. Variability in creatinine levels between capillary and venous blood samples was compared using the gold standard method, isotope dilution mass spectrometry, which reinforced the importance of using correction factors derived from validation studies to align the values obtained through each method [43].

Filter paper

The filter paper type may affect the homogeneity and behavior of blood spreading, as well as compound stability and recovery [35,44]. The main types of filter paper are made of cellulose (Whatman, GE Healthcare and Ahlstrom, Perkin-Elmer) or glass microfiber (Agilent Bond Elut DMS and Sartorius) [29,38].

Cellulose-based cards may contain additives, such as enzyme inhibitors or denaturing agents [35,38]. Whatman FTA DMPK-A cards are impregnated with radical inhibitors [sodium dodecyl sulfate, tris(hydroxymethyl) aminomethane] and can promote cell lysis and denature proteins on contact. Similarly, Whatman FTA DMPK-B cards are impregnated with chaotropic agents (guanidinium thiocyanate). Cotton-based cards, such as Whatman FTA DMPK-C, are not impregnated with stabilizing materials and are suitable for protein analysis, as are Whatman 903 and Ahlstrom 226 [33].

Due to the range of available filter cards, the European Bioanalysis Forum recommends fully validating DBS sampling methods for specific paper types [45,46]. Recommended validation parameters include drying conditions, storage stability, the effects of sample recovery, linearity, accuracy, and precision [46].

Hematocrit

Hematocrit variability is the main factor affecting the quality of DBS results [47]. Hematocrit reflects the relative volume of red blood cells and affects blood viscosity. High hematocrit results in low absorption into the card [31]. Human reference values vary according to biological parameters such as age, sex, nutritional status, race, pathological conditions, and pregnancy, in addition to extrinsic factors, such as altitude and smoking [47]. Mathematical equations to correct these variations have been determined based on the patient's baseline value or reference values for men and women [14]. Using computer systems to apply specific correction factors based on demographic data may help correct the impact of hematocrit on DBS measurements and achieve accurate analytical results. However, for precision, many sources of random errors (pipettes, volumetric flasks, detector, extraction procedure) must be accounted for [47].

The effect of hematocrit depends on the analyte of interest, and different results may be obtained according to its physical and chemical properties [48,49]. This effect can be measured either directly or indirectly through endogenous compounds such as sphingomyelin and potassium [47,50]. Incorporating internal standards, in association with accurate volume sampling, whole-spot extraction, and automated direct elution techniques has been shown to minimize the effect of hematocrit and thus improve reliability [51,52].

In studies involving individuals with abnormal hematocrit levels, DBS sampling proved unsuitable for iothalamate analysis [53]. Low hematocrit also significantly influenced creatinine analysis (deviation of 15%), and correction with endogenous compounds (potassium) was suggested [50]. Conversely, some studies reported that hematocrit's effects on precision were within acceptable limits [32,54,55].

Applicability of the DBS technique in nephrology

Measurement of endogenous markers

Using DBS to quantify endogenous markers of kidney function has mainly occurred in the last decade (Table 2) [13,34,40,43,56-63]. A strong correlation was found between conventionally obtained venous blood samples and those collected through DBS [43,57,58]. Using the reference method, creatinine quantification in DBS samples showed good accuracy [58]. Nevertheless, only Dalton et al. [43] compared creatinine levels in whole capillary DBS samples (n=66) using isotope dilution mass spectrometry.

One observed advantage of DBS is the stability of compounds. Creatinine showed 7-day stability at 32 °C in blood collected on Whatman FTA DMPK-C cards [32]. Quraishi et al. [56] also reported that creatine is stable for up to 90 days between 4 °C and 37 °C in serum samples stored on filter discs. Similarly, DBS urea concentrations were stable for up to 120 days at 4 °C and for 90 days at 37 °C [63]. However, cystatin C values decreased when shipping times exceeded 8 days (n=3,149) [34].

Measurement of exogenous markers

To determine the GFR through the clearance of exogenous compounds, blood must be collected several times over specific periods [64]. The filter paper method could simplify this process and be more tolerable in special populations, such as children [42]. Table 3 shows the main studies that have as-

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Table 2. Studies involving measurement of endogenous biomarkers of kidney function through DBS samples

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Sample (range or mean±SD)	Assessment of agreement/performance
Creatinine						
Quraishi [56]	VB Whatman	60	Colorimetric assay	37 °C and 4 °C for 15–90 day	Creatinine range: 0.5–3.3 mg/dL Serum creatinine: 1.99±0.64 mg/dL DBS creatinine: 1.92±0.55 mg/dL	R=0.94, ICC=0.93
Abraham [57]	VB Whatman n3	15	Enzymatic assay	4 °C for 7 day Matrix effect	DBS: 1.39±0.46 mg/dL Serum: 1.35±0.50 mg/dL	R=0.91, ICC=0.92
Silva [13]	VB, CB	106	Colorimetric (Jaffé) assay	Not reported	Adult: 57±12 yr	R=0.48 Mean difference BA (LA): 0 (0.68 to −0.55) Diagnostic cutoff GFR <60 mL/min/1.73 m^2 CKD-EPI: DBS sensitivity 94%, DBS specificity 55%, precision 90%
Nakano [58]	VB	100	MS/MS	Not reported	Pediatric: 7.9 yr Serum creatinine: 0.4 mg/dL Creatinine range: 0.12–1.2 mg/dL Calibration curve: linearity (0.039–5.0 mg/dL) Accuracy: 81.6%–104.9% CV: 0.1%–5.8%	Creatinine: 0.12–1.2 mg/dL R=0.86 Mean difference BA (LA): 0 (−0.087 to +0.09) Creatinine: 0.12–0.8 mg/dL R=0.72 / DBS=0.565×creatinine BA (LA): 0 (−0.081 to 0.091)
Bachini [59]	CB Whatman 903	9	FIA-MS	Not reported	Olympic athletes Serum creatinine: 813.6±102.4 µmol/L (9.20±1.16 mg/dL) DBS creatinine: 812.4±108.1 µmol/L (9.19±1.22 mg/dL)	CV=10.7%, ICC=0.57
Dalton [43]	VB, CB Whatman 903	66	ID-LCMS Colorimetric enzymatic assay	−80 °C Standard 914a	Adult: 24–88 yr Venous DBS creatinine: 0.85±1.10 mg/dL Capillary DBS creatinine: 0.83±1.19 mg/dL	Sensitivity: 100% Specificity: 62.7%–94.9%
Sham [60]	VB	3	LC-MS/MS PSI-MS/MS	2–8 °C	Creatinine: 2.5–20 µg/mL	Precision ≤6.3%, recovery 88%–94%, R^2>0.99
Cystatin C						
Vogl [40]	VB, CB Whatman 903	141	ELISA Nephelometry	−70 °C Hematocrit[a)]	ELISA Intra-assay CV: 5.4%, Inter-assay CV: 7.4% Nephelometry Intra-assay CV: 4.2%, Inter-assay CV: 6.9%	R=0.94 Cystatin C: 0.51–1.02 mg/L DBS sensitivity 94%, DBS specificity 55% Misclassified CKD stage: 31% Correlation venous vs. capillary blood: R=0.97
Crimmins [61]	VB Whatman 903	82	ELISA	−70 °C	Adult: >50 yr Mean cystatin C: 0.75 (0.41–1.39)	R=0.78 Regression: DBS=0.355+0.7×cystatin C Mean difference BA (LA): −0.2 (−0.45 to 0.1)
Crimmins [34]	VB Whatman 903	3,149	ELISA	≥32.2 °C, time before freezing (0–2, 3, 4–5, 6–7, and >8 day) Volume[b)]	Adult: >50 yr Mean cystatin C: 1.2 (0.5–9.2)	R^2=0.78 Regression: DBS=0.43+0.84×cystatin C

(Continued to the next page)

Table 2. Continued

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Sample (range or mean±SD)	Assessment of agreement/performance
Urea						
Plumbe [62]	VB, CB	20	Enzymatic assay	Analysis: <7 day Hematocrit[a)]	CV: 6%	Venipuncture: R=0.99 Regression: DBS=1.07×urea−0.6 Capillary sample: R=0.99 Regression: DBS=1.07×urea+0.1
Quraishi [63]	VB Whatman	75	Enzymatic assay	120 day (4 °C) or 90 day (37 °C) Hematocrit[c)]	Intra-assay CV=4.2%, Inter-assay CV=6.3%	R=0.97, ICC=0.96

DBS, dried blood spots; SD, standard deviation; VB, venous blood; R, Pearson correlation coefficient; ICC, intraclass correlation coefficient; CB, capillary blood; BA (LA), Bland-Altman and limits of agreement; GFR, glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; MS/MS, tandem mass spectrometry; CV, coefficient of variation; FIA-MS, flow injection analysis-mass spectrometry; ID-LCMS, isotope dilution-liquid chromatography/mass spectrometry; LC, liquid chromatography; PSI, paper spray ionization; ELISA, enzyme-linked immunosorbent assay.
[a)]Lowest influence or undefined variations in the assessed parameters. [b)]Presence or [c)]absence of statistical differences in biomarker concentrations according to variations in the assessed parameters.

sessed methods of measuring exogenous markers of kidney function through DBS [41,42,53,65-69]. As found in a previous study, there was strong agreement between DBS and venous GFR, with acceptable bias, precision, and accuracy, especially in patients with GFR <60 mL/min/1.73 m^2 [41]. Linear regression analyses also found good agreement between 82 serum and DBS samples regarding iohexol concentration [65].

Serum medication levels

Simultaneous assessment of kidney function indicators and medications in filter paper collection systems is a promising method for controlling the clearance or toxicity of drugs or their metabolites [70]. Forms of nephrotoxicity include tubular epithelial cell injury (antimicrobials, chemotherapeutic drugs, and venous contrast agents), interstitial nephritis (antibiotics, anti-inflammatory drugs, proton pump inhibitors, and immune-checkpoint inhibitors), and the formation of intratubular crystals (acyclovir, indinavir, antimicrobials, methotrexate, and sulfadiazine) [71].

Risk factors, such as advanced age, cardiovascular disease, diabetes, and liver disease, contribute to the development of kidney dysfunction after nephrotoxic drug use [72]. Combined therapies with diuretics, non-steroidal anti-inflammatory drugs, and renin angiotensin system inhibitors may potentiate nephrotoxicity in this group of patients [73]. Since drugs can also accumulate when kidney function is reduced (digoxin, metformin, and lithium), periodical kidney function assessment is needed in these patients [74,75].

Good correlations have been observed between serum and DBS samples for creatinine and immunosuppressant quantification by liquid chromatography-tandem mass spectrometry (Table 4) [32,39,54,74,76-80]. Simultaneous analysis of creatinine and diabetes medications (metformin and sitagliptin) has also shown good accuracy and precision in DBS samples [74,76]. Cystatin C-based measures of renal function improved ceftriaxone clearance prediction in 26 elderly patients [81]. Conversely, vancomycin clearance levels could not be accurately predicted through DBS [54].

Kidney transplant patients also require constant kidney function assessment, in addition to effective dose management of immunosuppressant drugs (cyclosporine, tacrolimus, and mycophenolate) [80,82]. The side effects of these drugs can lead to treatment nonadherence, as shown by Almardini et al. [83], who reported 36% nonadherence to mycophenolate in a group of children. The economic cost and social implications of

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Table 3. Studies involving measurement of exogenous markers of kidney function through DBS samples

Author	Collection method	Sample size	Analytical technique	Storage and quality control	Study population	Assessment of agreement/performance
Iohexol						
Niculescu-Duvaz [65]	VB, CB (3 points) Schleicher & Schuell Grade 903	82	HPLC	−20 °C Hematocrit[a] Recovery[b]	Mean age: 41 yr	R^2=0.953
Mafham [66]	VB, CB (3 points) Schleicher & Schuell Grade 903	81	HPLC	Analysis: <4 hr Hematocrit[a]	Mean age: 53±17 yr GFR 15–124 mL/min/1.73 m^2	Bias ±1.96×SD (mL/min/1.73 m^2) 3-spot iohexol clearance: 1.1±15.1 2-spot iohexol clearance: 0.6±14.9 1-spot iohexol clearance: 4.5±21.2
Maahs [67]	VB, CB (5 points) Whatman 903 Protein Saver	15	HPLC	Analysis: <4 hr Hematocrit[a]	Patients with type 1 diabetes Mean age: 29±12 yr Iohexol IV (1,500 mg)	5-point blood spot GFR: 84.1±15.4 mL/min/1.73 m^2 (R=0.89), mean BA difference=0.16 2-point blood spot GFR: 83.4±15.4 mL/min/1.73 m^2 (R=0.89), mean BA difference=0.81
Salvador [41]	VB, CB (7 points) Whatman 903 Protein Saver	32	HPLC	Hematocrit[a]	Age: <6 yr Iohexol IV (647 mg/mL)	Median (range) reference GFR 65 (6–122) mL/min/1.73 m^2; 2, 3, and 4-point blood spot GFR: R=0.947, R=0.945, and R=0.937, respectively Diagnostic accuracy for 2-point blood spot: 87.5% and 96.9±15% (P15) and 96.9±30% (P30) of the reference GFR respectively GFR <60 mL/min/1.73 m^2, P15 and P30 accuracy 100%
Wang [68]	VB, CB (3 points)	45	Not reported	Not reported	Pediatric patients with chronic kidney disease	R=0.958 Bias 4.26±9.06 mL/min/1.73 m^2
Luis-Lima [69]	VB, CB (7 points) Whatman 903	203	HPLC	Volume[c]	Mean age: 57.3±15.3 yr Mean GFR: 63.6±34.8 mL/min	Capillary blood on card: total deviation index=26% Blood pipetted on card: total deviation index=13% *In vivo* studies: deviation index=9.5%
Staples [42]	VB, CB (4 points) Schleicher & Schuell Grade 903	41	HPLC	Analysis: <5 hr Hematocrit[d]	Age: 1–21 yr Iohexol IV (647 mg/mL) Mean creatinine: 1.13±0.45 mg/dL	Correlation between the DBS and 2-point venous GFR: R=0.95 2-point GFR±10% 4-point GFR: 94% DBS GFR±10% 2-point GFR: 80%
Iothalamate						
Hagan [53]	VB (6 points) Whatman 903 Protein Saver	10	HPLC	Analysis: <5 hr Hematocrit[c]	Mean age: 65.2±13.4 yr Mean GFR: 33.4±10.1 mL/min/1.73 m^2	Regression: slope of 0.95 (95% CI, 0.82–1.17) BA: bias (LA) 2 mL/min (−6 to 10 mL/min) Precision (% coefficient of variation): 3.2%–13.3% Accuracy (% error): 1.3%–3.7%

DBS, dried blood spots; VB, venous blood; CB, capillary blood; HPLC, high-performance liquid chromatography; SD, standard deviation; IV, intravenous; GFR, glomerular filtration rate; BA, Bland-Altman; R, Pearson correlation coefficient; CI, confidence interval; LA, limits of agreement. [a]Concentration corrected according to a mathematical equation. [b]Absence or [c]presence of different statistics in marker oncentrations according to variations in the assessed parameters. [d]Lowest influence or undefined variations in the assessed parameters.

organ rejection due to treatment nonadherence among transplant recipients make it essential to search for a simpler and less invasive method of drug therapy monitoring [78].

Final considerations

Although the early detection of kidney disease through simple and accurate identification of biomarkers is essential, it has been explored by few studies. The studies in this review found DBS to be a promising alternative for quantifying the main biomarkers of kidney diseases, but sources of variability should be considered separately for each analyte. Practical applications should follow strict validation protocols that contain information about sample type, card type, volume, temperature,

Table 4. Studies that simultaneously measured creatinine and medication clearance through DBS samples

Author	Assessed medication	Collection method	Sample size	Analytical technique	Storage and quality control	Study population (yr)	Calibration and performance
Scherf-Clavel [74,76]	Metformin and sitagliptin	VB, CB	70	LC-MS/MS, enzymatic assay	Volume[a]	Mean±SD: 67±11	Limit of quantification Cr: 0.15 mg/dL, Cf capillary vs. plasma=0.916±0.088 R=0.944, mean BA deviation=0.001 mg/dL
Mathew [77]	Tacrolimus	VB, CB Whatman 903	131	LC-MS/MS	Time: 5 day Temperature: ambient Hematocrit[b]	Range: 30–49	Imprecision <12% and limits of clinical acceptance within 15% against the venous samples
Koop [78]	Tacrolimus	VB, CB FTA DMPK-A	21	LC-MS/MS	Time: 4 wk Temperature: ambient	Mean±SD: 14±4.6	Limit of quantification Cr 0.01 mg/dL, accuracy 7.94% Intra- and inter-day precision: 3.48%–4.11%
Al-Uzri [39]	Tacrolimus	VB, CB	30 Subjects 216 cards	LC-MS/MS, colorimetric assay, RIA	Time: 4 wk up to 1 mo on a dissected card Temperature: ambient	Mean±SD: 13.6±5.4 Range: 2–21	Correlation between DBS vs. intravenous samples: tacrolimus: R^2=0.81 Cr: R^2=0.95
Francke [79]	Tacrolimus and cyclosporin	VB, CB	176	LC-MS/MS	Hematocrit[c]	Mean: 62	R=0.953
Veenhof [80]	Tacrolimus and cyclosporin	VB, CB Whatman DMPK-C	172 Subjects 210 cards	LC-MS/MS, enzymatic creatinine assay	1–7 day at room temperature after: −20 °C Hematocrit[b]	Mean±SD: 55±14	Correlation between DBS vs. intravenous samples Mean serum Cr: 149 µmol/L (n=199), R^2=0.97, y=0.73x–1.55 BA bias of −2.1 µmol/L (95% CI, −3.7 to −0.5) BA=[Cr serum µmol/L]=[DBS]/0.73 Mean serum tacrolimus 7.1 µg/L (n=106), R^2=0.93, y=1.0x–0.23, BA bias of −0.28 µg/L (95% CI, −0.45 to −0.12) Mean serum cyclosporine A 109 µg/L (n=61), R^2=0.93, y=0.99x–1.86
Koster [32]	Tacrolimus, sirolimus, everolimus, and cyclosporin	VB, FTA DMPK-C	50	LC-MS/MS, enzymatic assay	32 °C for 1 wk, −20 °C for 29 wk Volume[b] Hematocrit[b]	Not available	Range for Cr: 7-point calibration curve (120–480 µmol/L), 1-point calibration curve (116–7,000 µmol/L), 8-point calibration curve (1–400 µmol/L) Precision and accuracy (all validations): maximum CV of 14.0% and maximum bias of −5.9%
Scribel [54]	Vancomycin	VB, CB Whatman 903	29 Subjects 54 Samples	LC-MS/MS	22 °C and 45 °C for 2 wk Hematocrit[b]	Age: >18 yr	Cr validation: accuracy (99.6%–102.6%), intra-assay precision=2.6%–5.6%, inter-assay precision=3.5%–6.1% DBS and serum comparison: accuracy (94.4%–102.6%), intra-assay precision=2.1%–5.6%, inter-assay precision=3.5%–7.0% Cr serum to DBS concentration ratio: 0.8–1.28; R=0.96 Correlation between DBS vs. intravenous samples: Vancomycin: R^2=0.89 (n=54) DBS capillary blood Vancomycin: R^2=0.93 (n=19) DBS venous blood Cr: R^2=0.95 (n=54)

DBS, dried blood spots; VB, venous blood; CB, capillary blood; LC, liquid chromatography; MS/MS, tandem mass spectrometry; SD, standard devation; Cr, creatinine; Cf, correction factor; R, Pearson correlation coefficient; BA, Bland-Altman; RIA, radioimmunoassay; CI, confidence interval; CV, coefficient of variation.
[a]Absence of differences in marker concentrations according to variations in the assessed parameters. [b]Lowest influence on the assessed parameters. [c]Concentration corrected according to a mathematical equation.

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humidity, and hematocrit parameters. Moreover, the assessment should include control subjects to ensure quality. Finally, future research should include expressive samples of patients at different stages of kidney disease and report information on clinical parameters.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References

1. Bikbov B, Perico N, Remuzzi G; on behalf of the GBD Genitourinary Diseases Expert Group. Disparities in chronic kidney disease prevalence among males and females in 195 countries: analysis of the global burden of disease 2016 study. Nephron 2018;139:313-8.
2. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. Bull World Health Organ 2018;96:414-422D.
3. World Health Organization (WHO). The top 10 causes of death [Internet]. WHO; 2020 [cited 2023 Nov 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
4. Byrne C, Cove-Smith A. Clinical assessment of renal disease. Medicine 2019;47:475-81.
5. Mihai S, Codrici E, Popescu ID, Enciu AM, Albulescu L, Necula LG, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. J Immunol Res 2018;2018:2180373.
6. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet 2013;382:260-72.
7. Biljak VR, Honovic L, Matica J, Kresic B, Vojak SS. The role of laboratory testing in detection and classification of chronic kidney disease: national recommendations. Biochem Med (Zagreb) 2017;27:153-76.
8. Wang YN, Ma SX, Chen YY, Chen L, Liu BL, Liu QQ, et al. Chronic kidney disease: biomarker diagnosis to therapeutic targets. Clin Chim Acta 2019;499:54-63.
9. Rysz J, Gluba-Brzozka A, Franczyk B, Jablonowski Z, Cialkowska-Rysz A. Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. Int J Mol Sci 2017;18:1702.
10. Lamb E. Assessment of kidney function in adults. Medicine 2015;43:368-73.
11. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco AL, De Jong PE, et al. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013;3:1-150.
12. Soares AA. Ferramentas para detecção da doença renal: valores de referência da taxa de filtração glomerular e desempenho das equações de estimativa com creatinina e cistatina C séricas em indivíduos saudáveis [doctor's thesis]. Postgraduate Program in Medical Sciences: Endocrinology, Universidade Federal do Rio Grande do Sul (UFRGS); 2013.
13. Silva AC, Gomez JF, Lugon JR, Graciano ML. Creatinine measurement on dry blood spot sample for chronic kidney disease screening. J Bras Nefrol 2016;38:15-21.
14. Enderle Y, Foerster K, Burhenne J. Clinical feasibility of dried blood spots: analytics, validation, and applications. J Pharm Biomed Anal 2016;130:231-43.
15. Rowland M, Emmons GT. Use of dried blood spots in drug development: pharmacokinetic considerations. AAPS J 2010;12:290-3.
16. Lakshmy R. Analysis of the use of dried blood spot measurements in disease screening. J Diabetes Sci Technol 2008;2:242-3.
17. Gupta K, Mahajan R. Applications and diagnostic potential of dried blood spots. Int J Appl Basic Med Res 2018;8:1-2.
18. Sharma A, Jaiswal S, Shukla M, Lal J. Dried blood spots: concepts, present status, and future perspectives in bioanalysis. Drug Test Anal 2014;6:399-414.
19. Velghe S, Delahaye L, Stove CP. Is the hematocrit still an issue in quantitative dried blood spot analysis? J Pharm Biomed Anal 2019;163:188-96.
20. Shahbaz H, Gupta M. Creatinine clearance. In: StatPearls [Internet]. StatPearls Publishing; 2024. Available from: https:// pubmed.ncbi.nlm.nih.gov/31334948
21. Sodre FL, Costa JCB, Lima JCC. Evaluation of renal function and damage: a laboratorial challenge. J Bras Patol Med Lab 2007;43:329-37.
22. Bruck K, Jager KJ, Dounousi E, Kainz A, Nitsch D, Arnlov J, et al. Methodology used in studies reporting chronic kidney disease prevalence: a systematic literature review. Nephrol Dial Transplant 2015;30 Suppl 4(Suppl 4):iv6-16.
23. George JA, Gounden V. Novel glomerular filtration markers. Adv Clin

Chem 2019;88:91-119.
24. Hocher B, Adamski J. Metabolomics for clinical use and research in chronic kidney disease. Nat Rev Nephrol 2017;13:269-84.
25. Bjornstad P, Anderson PL, Maahs DM. Measuring glomerular filtration rate by iohexol clearance on filter paper is feasible in adolescents with type 1 diabetes in the ambulatory setting. Acta Diabetol 2016;53:331-3.
26. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338-43.
27. Meesters RJ, Hooff GP. State-of-the-art dried blood spot analysis: an overview of recent advances and future trends. Bioanalysis 2013;5:2187-208.
28. Castro AC, Borges LG, Souza Rda S, Grudzinski M, D'Azevedo PA. Evaluation of the human immunodeficiency virus type 1 and 2 antibodies detection in dried whole blood spots (DBS) samples. Rev Inst Med Trop Sao Paulo 2008;50:151-6.
29. Lehmann S, Delaby C, Vialaret J, Ducos J, Hirtz C. Current and future use of "dried blood spot" analyses in clinical chemistry. Clin Chem Lab Med 2013;51:1897-909.
30. Edelbroek PM, van der Heijden J, Stolk LM. Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls. Ther Drug Monit 2009;31:327-36.
31. Koster RA, Alffenaar JW, Botma R, Greijdanus B, Touw DJ, Uges DR, et al. What is the right blood hematocrit preparation procedure for standards and quality control samples for dried blood spot analysis? Bioanalysis 2015;7:345-51.
32. Koster RA, Greijdanus B, Alffenaar JW, Touw DJ. Dried blood spot analysis of creatinine with LC-MS/MS in addition to immunosuppressants analysis. Anal Bioanal Chem 2015;407:1585-94.
33. Lim MD. Dried blood spots for global health diagnostics and surveillance: opportunities and challenges. Am J Trop Med Hyg 2018;99:256-65.
34. Crimmins EM, Zhang YS, Kim JK, Frochen S, Kang H, Shim H, et al. Dried blood spots: effects of less than optimal collection, shipping time, heat, and humidity. Am J Hum Biol 2020;32:e23390.
35. Capiau S, Veenhof H, Koster RA, Bergqvist Y, Boettcher M, Halmingh O, et al. Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. Ther Drug Monit 2019;41:409-30.
36. Denniff P, Woodford L, Spooner N. Effect of ambient humidity on the rate at which blood spots dry and the size of the spot produced. Bioanalysis 2013;5:1863-71.
37. Prentice P, Turner C, Wong MC, Dalton RN. Stability of metabolites in dried blood spots stored at different temperatures over a 2-year period. Bioanalysis 2013;5:1507-14.
38. Malsagova K, Kopylov A, Stepanov A, Butkova T, Izotov A, Kaysheva A. Dried blood spot in laboratory: directions and prospects. Diagnostics (Basel) 2020;10:248.
39. Al-Uzri A, Freeman KA, Wade J, Clark K, Bleyle LA, Munar M, et al. Longitudinal study on the use of dried blood spots for home monitoring in children after kidney transplantation. Pediatr Transplant 2017;21:e12983.
40. Vogl PT. Measurement of cystatin C in dried blood spot specimens [master's thesis]. University of Washington; 2013.
41. Salvador CL, Tondel C, Morkrid L, Bjerre A, Brun A, Bolann B, et al. Glomerular filtration rate measured by iohexol clearance: a comparison of venous samples and capillary blood spots. Scand J Clin Lab Invest 2015;75:710-6.
42. Staples A, Wong C, Schwartz GJ. Iohexol-measured glomerular filtration rate in children and adolescents with chronic kidney disease: a pilot study comparing venous and finger stick methods. Pediatr Nephrol 2019;34:459-64.
43. Dalton RN, Isbell TS, Ferguson R, Fiore L, Malic A, DuBois JA. Creatinine standardization: a key consideration in evaluating whole blood creatinine monitoring systems for CKD screening. Anal Bioanal Chem 2022;414:3279-89.
44. Koster RA, Botma R, Greijdanus B, Uges DR, Kosterink JG, Touw DJ, et al. The performance of five different dried blood spot cards for the analysis of six immunosuppressants. Bioanalysis 2015;7:1225-35.
45. Timmerman P, White S, Cobb Z, de Vries R, Thomas E, van Baar B, et al. Update of the EBF recommendation for the use of DBS in regulated bioanalysis integrating the conclusions from the EBF DBS-microsampling consortium. Bioanalysis 2013;5:2129-36.
46. Timmerman P, White S, Globig S, Ludtke S, Brunet L, Smeraglia J. EBF recommendation on the validation of bioanalytical methods for dried blood spots. Bioanalysis 2011;3:1567-75.
47. Daousani C, Karalis V, Malenovic A, Dotsikas Y. Hematocrit effect on dried blood spots in adults: a computational study and theoretical considerations. Scand J Clin Lab Invest 2019;79:325-33.
48. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. Clin Pharmacokinet 2014;53:961-73.
49. Koster RA, Alffenaar JW, Botma R, Greijdanus B, Uges DR, Kosterink JG, et al. The relation of the number of hydrogen-bond acceptors with recoveries of immunosuppressants in DBS analysis. Bioanalysis 2015;7:1717-22.
50. den Burger JC, Wilhelm AJ, Chahbouni AC, Vos RM, Sinjewel A, Swart EL. Haematocrit corrected analysis of creatinine in dried blood spots

https://doi.org/10.3339/ckd.23.023

through potassium measurement. Anal Bioanal Chem 2015;407:621-7.
51. Abu-Rabie P, Denniff P, Spooner N, Chowdhry BZ, Pullen FS. Investigation of different approaches to incorporating internal standard in DBS quantitative bioanalytical workflows and their effect on nullifying hematocrit-based assay bias. Anal Chem 2015;87:4996-5003.
52. Reyes-Garces N, Alam MN, Pawliszyn J. The effect of hematocrit on solid-phase microextraction. Anal Chim Acta 2018;1001:40-50.
53. Hagan AS, Jones DR, Agarwal R. Use of dried plasma spots for the quantification of iothalamate in clinical studies. Clin J Am Soc Nephrol 2013;8:909-14.
54. Scribel L, Zavascki AP, Matos D, Silveira F, Peralta T, Goncalves Landgraf N, et al. Vancomycin and creatinine determination in dried blood spots: analytical validation and clinical assessment. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1137:121897.
55. Anibaletto Dos Santos AL, Cezimbra da Silva AC, Feltraco Lizot LL, Schneider A, Meireles YF, Hahn RZ, et al. Development and validation of an assay for the measurement of gentamicin concentrations in dried blood spots using UHPLC-MS/MS. J Pharm Biomed Anal 2022;208:114448.
56. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Creatinine measurement and stability in dried serum. J Diabetes Sci Technol 2012;6:988-9.
57. Abraham RA, Kapil U, Aggarwal SK, Pandey RM, Sharma M, Ramakrishnan L. Measurement of creatinine from dried blood spot by enzymatic method. Int J Adv Res Chem Sci 2015;2:42-6.
58. Nakano M, Uemura O, Honda M, Ito T, Nakajima Y, Saitoh S. Development of tandem mass spectrometry-based creatinine measurement using dried blood spot for newborn mass screening. Pediatr Res 2017;82:237-43.
59. Bachini FI, Pereira D, Santos R, Hausen M, Pereira G, Vieira C, et al. Creatine and creatinine quantification in olympic athletes: dried blood spot analysis pilot study. Biol Sport 2022;39:745-9.
60. Sham TT, Badu-Tawiah AK, McWilliam SJ, Maher S. Assessment of creatinine concentration in whole blood spheroids using paper spray ionization-tandem mass spectrometry. Sci Rep 2022;12:14308.
61. Crimmins E, Kim JK, McCreath H, Faul J, Weir D, Seeman T. Validation of blood-based assays using dried blood spots for use in large population studies. Biodemography Soc Biol 2014;60:38-48.
62. Plumbe RM, Worth HG. Dried blood spot test estimation of urea. Ann Clin Biochem 1985;22(Pt 4):408-11.
63. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Analysis of the stability of urea in dried blood spots collected and stored on filter paper. Ann Lab Med 2013;33:190-2.
64. Selistre LS, Cochat P, Rech D, Parant F, Souza VCD, Dubourg L. Associação entre taxa de filtração glomerular (medida por cromatografia liquida de alto desempenho com iohexol) e oxalato plasmático. J Bras Nephrol 2018;40:73-6.
65. Niculescu-Duvaz I, D'Mello L, Maan Z, Barron JL, Newman DJ, Dockrell ME, et al. Development of an outpatient finger-prick glomerular filtration rate procedure suitable for epidemiological studies. Kidney Int 2006;69:1272-5.
66. Mafham MM, Niculescu-Duvaz I, Barron J, Emberson JR, Dockrell ME, Landray MJ, et al. A practical method of measuring glomerular filtration rate by iohexol clearance using dried capillary blood spots. Nephron Clin Pract 2007;106:c104-12.
67. Maahs DM, Bushman L, Kerr B, Ellis SL, Pyle L, McFann K, et al. A practical method to measure GFR in people with type 1 diabetes. J Diabetes Complications 2014;28:667-73.
68. Wang BB, Wu Y, Qin Y, Gong MC, Shi XM, Jing HL, et al. Application of plasma clearance of iohexol in evaluating renal function in Chinese children with chronic kidney disease. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2015;37:171-8.
69. Luis-Lima S, Gaspari F, Negrin-Mena N, Carrara F, Diaz-Martin L, Jimenez-Sosa A, et al. Iohexol plasma clearance simplified by dried blood spot testing. Nephrol Dial Transplant 2018;33:1597-603.
70. Wu H, Huang J. Drug-induced nephrotoxicity: pathogenic mechanisms, biomarkers and prevention strategies. Curr Drug Metab 2018;19:559-67.
71. Perazella MA, Rosner MH. Drug-induced acute kidney injury. Clin J Am Soc Nephrol 2022;17:1220-33.
72. Pinto PS, Carminatti M, Lacet T, Rodrigues DF, Nogueira LO, Bastos MG, et al. Nephrotoxic acute renal failure: prevalence, clinical course and outcome. J Bras Nephrol 2009;31:183-9.
73. Calvo DM, Saiz LC, Leache L, Celaya MC, Gutierrez-Valencia M, Alonso A, et al. Effect of the combination of diuretics, renin-angiotensin-aldosterone system inhibitors, and non-steroidal anti-inflammatory drugs or metamizole (triple whammy) on hospitalisation due to acute kidney injury: a nested case-control study. Pharmacoepidemiol Drug Saf 2023;32:898-909.
74. Scherf-Clavel M, Albert E, Zieher S, Valotis A, Hickethier T, Hogger P. Dried blood spot testing for estimation of renal function and analysis of metformin and sitagliptin concentrations in diabetic patients: a cross-sectional study. Eur J Clin Pharmacol 2019;75:809-16.
75. Lea-Henry TN, Carland JE, Stocker SL, Sevastos J, Roberts DM. Clinical pharmacokinetics in kidney disease: fundamental principles. Clin J Am Soc Nephrol 2018;13:1085-95.
76. Scherf-Clavel M, Hogger P. Analysis of metformin, sitagliptin and creatinine in human dried blood spots. J Chromatogr B Analyt Technol Biomed Life Sci 2015;997:218-28.

77. Mathew BS, Mathew SK, Aruldhas BW, Prabha R, Gangadharan N, David VG, et al. Analytical and clinical validation of dried blood spot and volumetric absorptive microsampling for measurement of tacrolimus and creatinine after renal transplantation. Clin Biochem 2022;105-106:25-34.
78. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2013;926:54-61.
79. Francke MI, van Domburg B, Bouarfa S, van de Velde D, Hellemons ME, Manintveld OC, et al. The clinical validation of a dried blood spot method for simultaneous measurement of cyclosporine A, tacrolimus, creatinine, and hematocrit. Clin Chim Acta 2022;535:131-9.
80. Veenhof H, Koster RA, Alffenaar JC, Berger SP, Bakker SJ, Touw DJ. Clinical validation of simultaneous analysis of tacrolimus, cyclosporine A, and creatinine in dried blood spots in kidney transplant patients. Transplantation 2017;101:1727-33.
81. Tan SJ, Cockcroft M, Page-Sharp M, Arendts G, Davis TM, Moore BR, et al. Population pharmacokinetic study of ceftriaxone in elderly patients, using cystatin C-based estimates of renal function to account for frailty. Antimicrob Agents Chemother 2020;64:e00874-20.
82. Cheung CY, van der Heijden J, Hoogtanders K, Christiaans M, Liu YL, Chan YH, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. Transpl Int 2008;21:140-5.
83. Almardini R, Taybeh EO, Alsous MM, Hawwa AF, McKeever K, Horne R, et al. A multiple methods approach to determine adherence with prescribed mycophenolate in children with kidney transplant. Br J Clin Pharmacol 2019;85:1434-42.

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First-morning urine osmolality changes in children with nocturnal enuresis at the end of treatment

Yun Ha Lee[1,2], Jae Min Chung[1,2,3], Sang Don Lee[1,2,3]

[1]Department of Urology, Pusan National University Yangsan Hospital, Yangsan, Republic of Korea
[2]Department of Urology, Pusan National University School of Medicine, Yangsan, Republic of Korea
[3]Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Republic of Korea

Purpose: The ability to concentrate urine becomes an important index in determining nocturnal enuresis (NE) treatment. The aim of our study was to investigate first-morning urine osmolality (Uosm) changes at the end of treatment compared to before treatment in children with NE.

Methods: A total of 71 children with NE were divided into two groups according to the level of first-morning Uosm before treatment: high group (≥800 mOsm/kg) and low group (<800 mOsm/kg). Baseline parameters were obtained from uroflowmetry, frequency volume charts for at least 2 days, and a questionnaire for lower urinary tract symptoms. All patients were basically treated with standard urotherapy and medication. The first-morning Uosm was measured twice, before treatment and at the end of treatment.

Results: The response rate was higher in the low group after 3 months of treatment than in the high group (P=0.041). However, there was no difference between the two groups at the end of the treatment. In the high group, the first-morning Uosm at the end of treatment did not show a significant change compared to before treatment. In contrast, the first-morning Uosm increased in the low group at the end of treatment (P<0.001). However, it was still lower than that of the high group (P=0.007).

Conclusions: The ability to concentrate nocturnal urine improved at the end of treatment compared to before treatment in the low Uosm NE children. In addition, NE improved faster in the low Uosm group before treatment than in the high group.

Keywords: Child; Nocturnal enuresis; Osmolar concentration; Urinalysis; Urinary bladder

Introduction

Nocturnal enuresis (NE) is identified as intermittent incontinence that occurs exclusively during sleeping periods [1]. The prevalence of NE has been reported to be about 5.6% among children aged 5 to 13 years [2]. Although NE is neither fatal nor life-threatening, it does present a significant risk of psychosocial depression in patients and families, and thus, immediate, adequate treatment is required [3].

The three main mechanisms in the pathophysiology of enuresis are excessive nocturnal urine production, low bladder capacity or increased detrusor activity, and arousal impairment [4]. Recent studies found much higher urine volumes and much lower osmolality values in children with NE compared to

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Correspondence to
Jae Min Chung
Department of Urology, Pusan National University School of Medicine, Pusan National University Yangsan Hospital, 20 Geumo-ro, Mulgeum-eup, Yangsan 50612, Republic of Korea
E-mail: busanuro@hanmail.net

the normal population. The findings were related to the disruption of vasopressin [5,6]. The children with NE with preserved bladder storage function have reduced urine-concentrating ability during the night due to a lack of an arginine vasopressin (AVP) effect [5].

Urine osmolality (Uosm) provides a measure of the number of dissolved molecules in urine per unit of water and urine concentration. Uosm is more accurate than specific gravity and can be used to diagnose a variety of urinary concentration-associated disorders [7]. In several studies, Uosm was used instead of plasma AVP concentrations because Uosm can provide information on AVP [8-10]. Due to methodological difficulties that make urine collection from enuresis episodes a particularly demanding task, nocturnal urine output has previously been approximated through first-morning urine in patients with NE [11]. In addition, measuring Uosm is a simple, non-invasive, routine, and low-cost test that may help guide the optimal treatment of NE [12].

Several previous studies reported a relationship between nocturnal urinary concentrating ability and NE [5,13,14], but no study has investigated how much urinary concentrating ability, especially first-morning Uosm, improves after treating NE patients with low nocturnal urinary concentrating ability. In our previous study, we found that a significantly higher percentage of NE patients who have low first-morning Uosm had a response rate of ≥50% at 1 month and 3 months [6]. With an interesting result, we investigated further the relationship between first-morning Uosm and NE. Finally, the aim of our study was to investigate the response rate of NE treatment according to first-morning Uosm before treatment and first-morning Uosm changes at the end of treatment compared to before treatment in children with NE.

Methods

Data acquisition

After obtaining approval from the Institutional Review Board of Pusan National University Yangsan Hospital (IRB No. 05-2023-032), a retrospective chart review was performed on the prospective cohort data of all children who underwent treatment for NE at our institution from September 2019 to May 2022. Seventy-one children with NE (>3 times/wk) with measurements of the first-morning Uosm before treatment and at the end of treatment were included in this study. Patients diagnosed with organic causes, such as congenital urinary tract anomalies, congenital or acquired neurologic disorders, urinary tract infections, or spinal bifida occulta, were excluded.

Patient evaluation before treatment

All patients completed a questionnaire and a 48-hour frequency/volume (48-h F/V) chart. The questionnaire included items on medical history and urinary symptoms, including frequency, daytime incontinence, urgency, urge incontinence, holding maneuver, and dysfunctional voiding scoring system (DVSS) score. The questionnaire responses and 48-h F/V chart findings were used to confirm the presence of lower urinary tract symptoms (LUTS). Constipation was evaluated using the Leech scores of abdominal X-ray findings for all patients [15].

First-morning urine collection

First-morning urine samples were collected twice from all patients, on the second hospital visit and the final hospital visit at the end of treatment. Patients and parents were instructed to collect first-morning samples in the plastic cups provided and to keep them refrigerated at 4 °C. Samples were evaluated promptly upon arrival at the hospital.

Patient analysis

The 71 patients were divided into two groups according to first-morning Uosm values before treatment: (1) the high group, with a first-morning Uosm of ≥800 mOsm/kg, and (2) the low group, with a first-morning Uosm of <800 mOsm/kg before treatment. Our previous study, which divided the groups based on 800 mOsm, showed significant results, so we divided the groups as before [6]. Daytime maximum voided volume (VV), first-morning VV, and total urine volume were obtained from 48-h F/V charts. Uroflowmetry (UFM) and post-void residual volume (PVR) findings, maximum flow rates (Qmax), VV, average flow rate (Qave), and PVRs were also analyzed.

Treatment and response rate

Following the before-treatment evaluations of patient characteristics, 48-h F/V charts, UFM and PVR, standard urotherapy, and pharmacological therapy were provided in accordance with International Children's Continence Society (ICCS) recommendations. Standard urotherapy included an introduction to LUTS treatment and lifestyle modifications (balanced fluid intake, restriction of nighttime fluid intake, timed bladder and bowel emptying, and optimal posture during voiding). In this study, the alarm treatment, also known as first-line treatment,

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was applied in cases that did not respond to pharmacological therapy.

Primary pharmacological therapy included desmopressin (1-desamino-8-D-arginine vasopressin, dDAVP), propiverine, and/or imipramine. All patients were treated with desmopressin 120 μg at first. The desmopressin dose was increased or decreased (60 μg or 240 μg) depending on the patient's response to the agent. If there was no response (NR) to desmopressin, propiverine or imipramine was added short-term as needed. These drugs were used based on consideration of symptom severity, the presence of any other LUTS, a history of bladder dysfunction.

The response rates were assessed at 3 months and at the end of treatment. The response rate was calculated as a percentage of the reduced rate of current enuresis events compared to the initial enuresis event [response rate=100×(number of initial enuresis event per week–number of current enuresis event per week)/number of initial enuresis event per week]. The patients were categorized into three groups according to ICCS recommendations: complete response (CR), partial response (PR), or NR groups. CR was defined as a 100% reduction in enuresis. PR was defined as a 50% to 99% reduction in enuresis, and NR was defined as a <50% reduction in enuresis (Fig. 1).

End of treatment

Pharmacological therapy was terminated when the child showed consistent findings of CR. In PR, treatment was terminated when the patients felt satisfied. In NR, treatment was continued as long as the patient reported subjective improvement. And treatment was terminated when there was no further benefit. At the end of treatment, the patients were maintained on urotherapy alone without pharmacological therapy. The

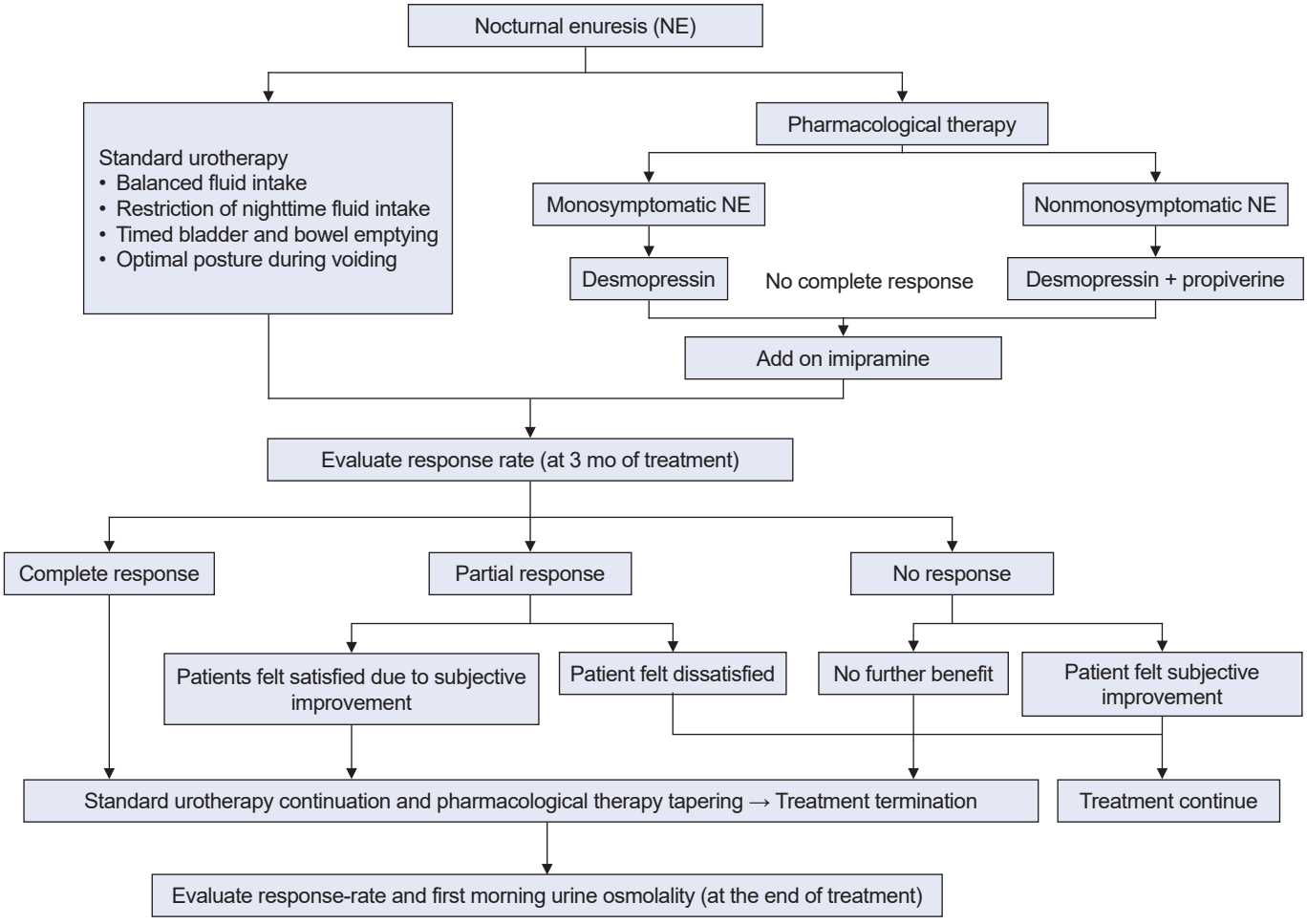

Fig. 1. NE treatment algorithm.

first-morning Uosm at the last visit was re-examined and compared with the first-morning Uosm before treatment (Fig. 1).

Statistical analysis

SPSS version 27 (IBM Corp.) was used for the statistical analyses. *P*-values of <0.05 were considered statistically significant. Continuous variables were analyzed using the Student *t*-test and the Mann-Whitney *U* test. Categorical variables were analyzed using the Pearson chi-square test and Fisher exact test.

Results

Patient characteristics at the first visit

There were no differences in patient characteristics, including age, height, weight, body mass index, follow-up period, constipation, and enuresis frequency, at the first visit between the two groups except for sex (*P*=0.025) (Table 1). There were also no differences in LUTS, such as urine frequency, urgency, daytime incontinence, urge incontinence, holding maneuver, and DVSS scores, between the two groups (Table 1). In the 48-h F/V chart, the first-morning VV and total urine volume in the low group were significantly higher than in the high group (*P*=0.049 and *P*=0.024, respectively). In the UFM test, there was no difference between the two groups in Qmax, VV, delay time, flow time, voiding time, and flow index, except for Qave (*P*=0.015). There was also no difference between the two groups in PVR (Table 2).

Treatment outcomes

Enuresis frequency improved with treatment in both groups. Enuresis frequency in the low group was relatively lower at 3 months of treatment than in the high group (0.73±1.10 times/wk vs. 1.33±1.47 times/wk, *P*=0.027) (Fig. 2). However, these enuresis frequencies were not different between the groups at the end of treatment (0.89±1.93 times/wk vs. 0.49±0.75 times/wk, *P*=0.815) (Fig. 2). In the low group, enuresis frequency was not different between 3 months and the end of treatment (Fig. 2). The response rate was higher in the low group at 3 months of treatment than in the high group (82.2%±22.0% vs. 66.0% ±34.0%, *P*=0.041) (Table 3). However, there was no difference between the two groups at the end of the treatment. Moreover, at the end of treatment, there was no significant difference between the two groups in the number of patients with CR, PR, and NR (Table 3).

Changes in first-morning Uosm at the end of treatment

In the high group, the first-morning Uosm at the end of treatment did not show a significant change compared to before treatment. In contrast, the first-morning Uosm at the end of treatment was increased in the low group compared to before treatment (586.8±147.2 mOsm/kg vs. 780.2±249.1 mOsm/kg, *P*<0.001). However, the first-morning Uosm at the end of

Table 1. Patients characteristics at first visit

Characteristic	High group	Low group	Total	*P*-value
No. of patients	36 (50.7)	35 (49.3)	71 (100)	
Male sex	14 (38.9)	24 (68.6)	38 (53.5)	0.025
Age (mo)	81.9±18.8	93.4±28.0	87.6±24.3	0.092
Range	52–122	57–168		
Height (cm)	120.2±12.1	127.0±15.7	123.6±14.3	0.064
Weight (kg)	26.3±8.35	31.9±16.7	29.0±13.3	0.238
Body mass index (kg/m^2)	17.8±2.72	18.8±5.58	18.3±4.37	0.899
Follow-up period (mo)	12.9±5.44	11.7±6.15	12.3±5.79	0.348
Random urine specific gravity	1.021±0.008	1.018±0.008	1.019±0.008	0.095
First-morning Uosm (mOsm/kg)	1,003±124	587±147	798±249	<0.001
Constipation on KUB	11 (30.6)	12 (34.3)	23 (32.4)	0.739
Enuresis frequency (times/wk)	4.46±2.05	3.97±2.18	4.22±2.11	0.392
Frequency	6 (16.7)	5 (14.3)	11 (15.5)	0.703
Daytime incontinence	12 (33.3)	11 (31.4)	23 (32.4)	0.853
Urgency	16 (44.4)	16 (45.7)	32 (45.1)	0.908
Urge incontinence	7 (19.4)	6 (17.1)	13 (18.3)	0.950
Holding maneuver	10 (27.8)	14 (40.0)	24 (33.8)	0.357
DVSS score sum	3.36±3.46	4.13±4.23	3.74±3.85	0.464

Values are presented as number (%) or mean±standard deviation.
Uosm, urine osmolality; KUB, kidney, ureter, and bladder X-ray; DVSS, dysfunctional voiding scoring system.

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Table 2. Frequency volume chart and uroflowmetry

Characteristic	High group	low group	Total	*P*-value
No. of patients	36 (50.7)	35 (49.3)	71 (100)	
Frequency volume chart				
Daytime maximum VV (mL/time)	150.1±59.3	185.0±117.9	167.0±93.5	0.168
First-morning VV (mL/time)	130.5±78.9	180.2±110.4	154.6±97.9	0.049
Total urine volume (mL)	558.0±290.0	742.7±385.0	646.4±348.8	0.024
Urgency	11 (30.6)	9 (25.7)	20 (28.2)	
Uroflowmetry				
Qave (mL/sec)	10.5±3.95	13.0±4.79	11.7±4.54	0.015
Qmax (mL/sec)	16.9±5.77	20.2±7.88	18.5±7.03	0.058
VV (mL)	137.0±52.6	164.5±111.1	150.5±87.0	0.791
Delay time (sec)	11.4±7.53	14.3±14.0	12.8±11.2	0.730
Flow time (sec)	14.3±8.84	12.2±6.30	13.3±7.71	0.269
Voiding time (sec)	15.3±9.01	13.1±7.14	14.2±8.16	0.138
Flow index	0.84±0.27	0.92±0.23	0.88±0.25	0.080
Post-void residual volume (mL)	10.8±12.8	16.7±42.1	13.7±30.9	0.669

Values are presented as number (%) or mean±standard deviation.
VV, voided volume; Qave, average flow rate; Qmax, maximum flow rates.

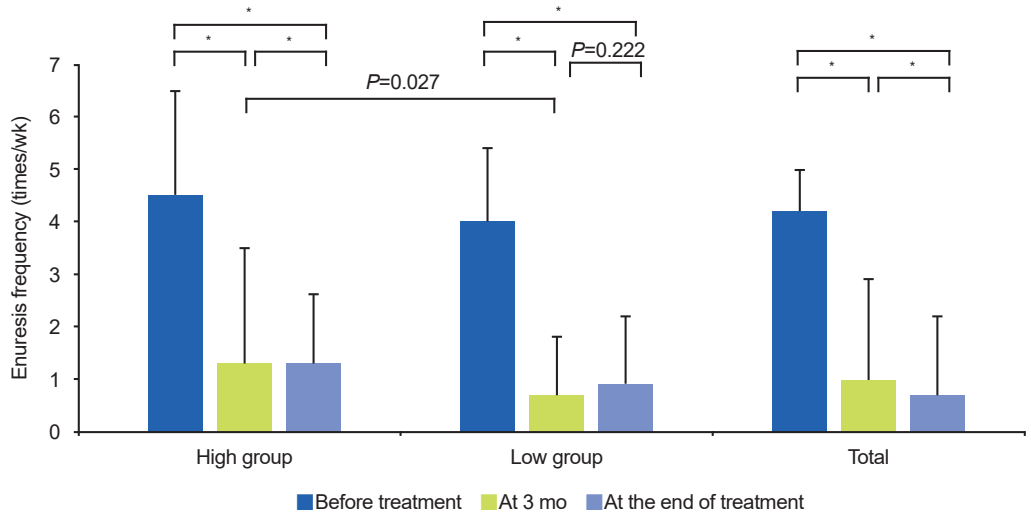

Fig. 2. Improvement of enuresis frequency at 3 months of treatment and at the end of treatment. **P*<0.05.

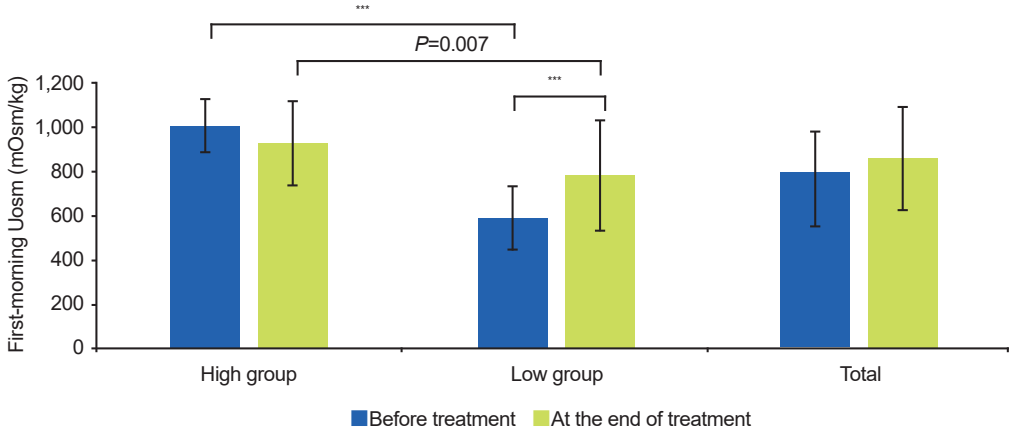

Fig. 3. Change of first-morning urine osmolality (Uosm) before treatment and at the end of treatment. ****P*<0.001.

Table 3. Treatment outcome

Outcome	High group	Low group	Total	*P*-value
No. of patients	36 (50.7)	35 (49.3)	71 (100)	
Response rate (%)				
At 3 mo/before frequency	66.0±34.0	82.2±22.0	73.2±30.0	0.041
At the end/before frequency	86.0±16.7	82.3±28.8	84.0±24.4	0.751
Difference of response rate between 3 mo and the end, *P*-value	0.003	0.452	0.008	
Response				0.557
CR	21 (58.3)	16 (45.7)	37 (52.1)	
PR	12 (33.3)	15 (42.9)	27 (38.0)	
CR+PR	33 (91.7)	31 (88.6)	64 (90.1)	
NR	3 (8.33)	4 (11.4)	7 (9.86)	

Values are presented as number (%) or mean±standard deviation.
CR, complete response; PR, partial response; NR, no response.

treatment in the low group was lower than in the high group (780.2±249.1 vs. 926.9±190.4, *P*=0.007) (Fig. 3). Therefore, Uosm in the low group did not reach the Uosm level in the high group at the end of NE treatment.

Discussion

In our study, Uosm increased after treatment. The first-morning Uosm in the low group increased at the end of treatment (586.8±147.2 mOsm/kg vs. 780.2±249.1 mOsm/kg, *P*<0.001). This study was the first to investigate changes by measuring first-morning Uosm before treatment and at the end of treatment. In our study, the response rate of the low group was better at 3 months of treatment than that of the high group. Also, enuresis frequency in the low group improved more at 3 months of treatment than in the high group. At the end of treatment, the first-morning Uosm increased in the low group. This means that improvements in excessive nocturnal urine production, one of the causes of NE, can be predicted through first-morning Uosm before treatment. Predicting and evaluating treatment response at 3 months of treatment is important because treatment adherence is better when short-term treatment effects are good.

It is well known that a low ability to concentrate nocturnal urine is one of the main mechanisms of NE. Several studies have investigated Uosm as a predictor of NE treatment, but the results have been contradictory [7,8]. Dehoorne et al. [13] described 42 children with monosymptomatic NE (MNE) and night polyuria with high Uosm (>850 mmol/L) not responding to intranasal dDAVP. Thus, nocturnal polyuria with high urinary osmolality with desmopressin-resistant MNE is related to abnormally increased osmotic excretion. In a study of 67 children with enuresis, Sozubir et al. [14] reported a significantly higher number of responders to dDAVP treatment when the Uosm value was <800 mOsm/kg. In their study, lower spot Uosm was the only statistically significant predictor of the desmopressin response. A study by Neveus et al. [5] that included 12 children with enuresis reported a significantly lower baseline Uosm (553±134 mOsm/kg) in dDAVP responders compared to non-responders (920±226 mOsm/kg).

However, Unuvar and Sonmez [16] in a study of 55 NE children and 15 healthy children between the ages of 5 and 15 years investigating Uosm in both daytime and nighttime urine, reported that pretreatment urine volume osmolality values were not predictive factors of response to desmopressin or conditioning therapy. A study of 35 children with enuresis by Eller et al. [8] reported that 27 children demonstrated a CR to desmopressin treatment at doses of 10–30 μg. However, spot Uosm values were not predictive of the desmopressin response. Urine samples were collected at home at times that would best reflect fluctuations in plasma vasopressin levels (8:00, 16:00, and 22:00) [8]. In a study by Folwell et al. in 31 NE patients [17], the mean and peak Uosm of the morning urine samples showed no difference while on treatment with dDAVP compared to placebo. They suggested that early morning Uosm, as a reflection of changes in nocturnal osmolality, was not useful in selecting patients who would respond to treatment. Medel et al. [18] investigated seven healthy children, six primary NE children who were desmopressin responders, and five primary NE children who were desmopressin non-responders. They found no significant difference in mean Uosm at night (from midnight to 8:00 AM). Therefore, they suggested that baseline Uosm was not a significant predictor of response to dDAVP therapy.

In our study, baseline Uosm was a significant predictor of re-

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sponse to treatment. Especially at 3 months of treatment, children with low Uosm showed low enuresis frequency (1.33±1.47 times/wk vs. 0.73±1.10 times/wk, *P*=0.027) and a high response rate (66.0%±34.0% vs. 82.2%±22.0%, *P*=0.041). However, there was no difference at the end of the treatment. The interesting result was that the Uosm range was very wide for each person. In our study, the children's first-morning Uosm range was 160–1,261 mOsm/kg (mean, 797.7 mOsm/kg). Despite treatment with dDAVP, not all children showed an increase in Uosm, and some children had decreased Uosm at the end of treatment. Several reasons can be considered as the causes of this variation. First, the first-morning urine in our study did not reflect all urine that occurred at night. Unlike other studies that collected all urine from midnight to 8:00 AM, our study did not collect all urine that occurred at night. Since night leaks were not collected, the time at which the first-morning urine was produced may have varied from child to child. Second, the collection time of the first-morning urine also varied from child to child. The time to collect the first-morning urine could be different, depending on the waking time of the child. These issues may have caused unexpected biases. Nevertheless, there is a lot of information available in first-morning urine, and the advantages of a simple, non-invasive, and low-cost test are clear. Therefore, it is meaningful to predict the treatment outcome of NE through the first-morning urine in clinical practice.

The guidelines published by the ICCS in 2011 recommend the use of an enuresis alarm or desmopressin, a vasopressin analog, as the standard treatment for MNE and are particularly recommended for patients with nocturnal polyuria [19]. Desmopressin is an efficient and safe treatment for primary MNE, with a reported success rate of 70% to 75% [16]. One of the major actions of desmopressin is to reduce the volume of urine produced overnight to within normal limits [20]. Desmopressin acts on the V2 receptors of the distal tubules and collecting tubules of the kidney, leading to urine concentration and decreased urine volume through the water channel aquaporin 2 [21]. Moreover, desmopressin positively influences the abundance of key sodium transporters in the thick ascending limb and collecting duct. Such an effect appears reasonable as part of the mechanism responsible for the buildup of the medullary osmotic gradient, the driving force for water reabsorption. [22].

Few studies have observed changes in Uosm after treatment with dDAVP. Kamperis et al. [22] reported that Uosm increased significantly at night only after the administration of dDAVP in a group with nocturnal polyuria (from 559±70 mOsm/kg to 876±39 mOsm/kg, *P*<0.001), whereas no significant changes were observed in the controls. This study showed changes in Uosm during one night. In one Korean study, Uosm in the dDAVP-complete responder group was lower than that of the non-responder group before treatment (461.2±192.7 mmol/L vs. 773.5±235.8 mmol/L). Moreover, 2 weeks after starting treatment, Uosm in the dDAVP-complete responder group was significantly increased (from 461.2±192.7 to 591.2±159.8 mmol/L) [23]. Since these two studies investigated Uosm during treatment, there is a limitation that the changes in Uosm at the end of treatment are not known.

A limitation of this study is that it was a single-center, retrospective study, and the number of patients was limited. In the future, a large-scale prospective study should be performed.

In conclusion, the ability to concentrate nocturnal urine improved at the end of treatment compared to before treatment in the low Uosm NE children. NE improved faster in the low Uosm group before treatment than in the high group. However, there was no difference in the treatment effect between the two groups at the end of treatment. In the low group, first-morning Uosm increased after treatment, but it did not reach the level in the high group.

Ethical statements

After obtaining approval from the Institutional Review Board of Pusan National University Yangsan Hospital (IRB No. 05-2023-032), a retrospective chart review was performed on the prospective cohort data of all children who underwent treatment for nocturnal enuresis at our institution.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: JMC
Data curation: YHL, JMC

Formal analysis: YHL
Funding acquisition: JMC
Investigation: YHL, JMC
Methodology: JMC
Project administration: JMC
Visualization: YHL
Writing-original draft: YHL
Writing-review & editing: JMC, SDL
All authors read and approved the final manuscript.

References

1. Austin PF, Bauer SB, Bower W, Chase J, Franco I, Hoebeke P, et al. The standardization of terminology of lower urinary tract function in children and adolescents: update report from the standardization committee of the International Children's Continence Society. Neurourol Urodyn 2016;35:471-81.
2. Chung JM, Lee SD, Kang DI, Kwon DD, Kim KS, Kim SY, et al. An epidemiologic study of voiding and bowel habits in Korean children: a nationwide multicenter study. Urology 2010;76:215-9.
3. Van Herzeele C, Walle JV, Dhondt K, Juul KV. Recent advances in managing and understanding enuresis. F1000Res 2017;6:1881.
4. Arda E, Cakiroglu B, Thomas DT. Primary nocturnal enuresis: a review. Nephrourol Mon 2016;8:e35809.
5. Neveus T, Lackgren G, Tuvemo T, Stenberg A. Osmoregulation and desmopressin pharmacokinetics in enuretic children. Pediatrics 1999;103:65-70.
6. Lee GK, Chung JM, Lee SD. First-morning urine osmolality and nocturnal enuresis in children: a single-center prospective cohort study. Investig Clin Urol 2023;64:501-9.
7. Pagana KD, Pagana TJ. Mosby's manual of diagnostic and laboratory tests. 7th ed. Elsevier Health Sciences; 2017.
8. Eller DA, Homsy YL, Austin PF, Tanguay S, Cantor A. Spot urine osmolality, age and bladder capacity as predictors of response to desmopressin in nocturnal enuresis. Scand J Urol Nephrol Suppl 1997;183:41-5.
9. Kawauchi A, Watanabe H, Miyoshi K. Early morning urine osmolality in nonenuretic and enuretic children. Pediatr Nephrol 1996;10:696-8.
10. Steffens J, Netzer M, Isenberg E, Alloussi S, Ziegler M. Vasopressin deficiency in primary nocturnal enuresis: results of a controlled prospective study. Eur Urol 1993;24:366-70.
11. Eggert P, Kuhn B. Antidiuretic hormone regulation in patients with primary nocturnal enuresis. Arch Dis Child 1995;73:508-11.
12. Abdovic S, Cuk M, Hizar I, Milosevic M, Jerkovic A, Saraga M. Pretreatment morning urine osmolality and oral desmopressin lyophilisate treatment outcome in patients with primary monosymptomatic enuresis. Int Urol Nephrol 2021;53:1529-34.
13. Dehoorne JL, Raes AM, van Laecke E, Hoebeke P, Vande Walle JG. Desmopressin resistant nocturnal polyuria secondary to increased nocturnal osmotic excretion. J Urol 2006;176:749-53.
14. Sozubir S, Ergun G, Celik A, Ulman I, Avanoglu A. The influence of urine osmolality and other easily detected parameters on the response to desmopressin in the management of monosymptomatic nocturnal enuresis in children. Minerva Urol Nefrol 2006;58:207-12.
15. Leech SC, McHugh K, Sullivan PB. Evaluation of a method of assessing faecal loading on plain abdominal radiographs in children. Pediatr Radiol 1999;29:255-8.
16. Unuvar T, Sonmez F. The role of urine osmolality and ions in the pathogenesis of primary enuresis nocturna and in the prediction of responses to desmopressin and conditioning therapies. Int Urol Nephrol 2005;37:751-7.
17. Folwell AJ, Macdiarmid SA, Crowder HJ, Lord AD, Arnold EP. Desmopressin for nocturnal enuresis: urinary osmolality and response. Br J Urol 1997;80:480-4.
18. Medel R, Dieguez S, Brindo M, Ayuso S, Canepa C, Ruarte A, et al. Monosymptomatic primary enuresis: differences between patients responding or not responding to oral desmopressin. Br J Urol 1998;81 Suppl 3:46-9.
19. Neveus T, Eggert P, Evans J, Macedo A, Rittig S, Tekgul S, et al. Evaluation of and treatment for monosymptomatic enuresis: a standardization document from the International Children's Continence Society. J Urol 2010;183:441-7.
20. Vande Walle J, Rittig S, Bauer S, Eggert P, Marschall-Kehrel D, Tekgul S, et al. Practical consensus guidelines for the management of enuresis. Eur J Pediatr 2012;171:971-83.
21. Akagawa S, Tsuji S, Akagawa Y, Yamanouchi S, Kimata T, Kaneko K. Desmopressin response in nocturnal enuresis showing concentrated urine. Pediatr Int 2020;62:701-4.
22. Kamperis K, Rittig S, Radvanska E, Jorgensen KA, Djurhuus JC. The effect of desmopressin on renal water and solute handling in desmopressin resistant monosymptomatic nocturnal enuresis. J Urol 2008;180:707-14.
23. Lee KS, Chang JB, Jang JY, Ko YH, Park YH, Song PH. Role of urine osmolality as a predictor of the effectiveness of combined imipramine and desmopressin in the treatment of monosymptomatic nocturnal enuresis. Yeungnam Univ J Med 2015;32:85-9.

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Risk factors for recurrent urinary tract infections in young infants under the age of 24 months

Min Hwa Son[1], Hyung Eun Yim[1]

[1]Department of Pediatrics, Korea University Ansan Hospital, Korea University College of Medicine, Ansan, Republic of Korea

Purpose: Recurrent urinary tract infections (UTIs) in children is a major challenge for pediatricians. This study was designed to investigate the risk factors for recurrent UTIs and determine the association between recurrent UTIs and clinical findings, including growth patterns in infants and children younger than 24 months of age.

Methods: We retrospectively reviewed the medical records of 147 patients <24 months of age with UTIs who were hospitalized between August 2018 and October 2021. The patients were divided into recurrent and single UTI episode groups. Clinical findings and anthropometric and laboratory data were compared between the two groups.

Results: In the recurrent UTI group, the weight-for-length (WFL) percentile at the first UTI diagnosis was lower compared to the single UTI episode group, and the weight-for-age percentile at 3-month and 6-month follow-ups after the first UTI decreased (all $P<0.05$). In univariable logistic regression analysis, higher birth weight, lower WFL percentile, the presence of hydronephrosis, acute pyelonephritis or vesicoureteral reflux, the use of prophylactic antibiotics, and non-*Escherichia coli* infections were associated with the development of recurrent UTIs (all $P<0.05$). However, in the multivariable analysis, only the presence of hydronephrosis and prophylactic antibiotic use were independently related to UTI recurrence ($P<0.05$).

Conclusions: The presence of hydronephrosis at the first UTI can be helpful for predicting UTI recurrence in young children aged <24 months. Antibiotic prophylaxis may be associated with UTI recurrence. Potential growth delay should be carefully monitored in infants with recurrent UTI.

Keywords: Body-weight trajectory; Growth; Hydronephrosis; Recurrence; Urinary tract infections

Introduction

Urinary tract infections (UTIs) are the most common serious bacterial infection in children, with 8.4% of girls and 1.7% of boys experiencing a UTI before age 6 [1]. UTIs account for 5% to 10% of febrile diseases in children younger than 24 months, and the recurrence rate is known to be 10% to 30% [2]. Recurrent UTIs increase the risk of kidney scarring, which is associated with hypertension in about 10% of pediatric patients and with dialysis and transplantation in approximately 20% [3]. Given that the symptoms and signs of UTI, except for fever, do not appear well in children under 2 years of age [4], many studies have tried to identify the risk factors for UTI, as it is important to quickly recognize these factors and prevent UTI recurrence.

In the pediatric population, young age, sex, vesicoureteral reflux (VUR), and bladder bowel dysfunction have received attention as important risk factors for recurrent UTIs [5-9]. In a multicenter prospective cohort study of 500 pediatric patients

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Correspondence to
Hyung Eun Yim
Department of Pediatrics, Korea University Ansan Hospital, Korea University College of Medicine, 123 Jeokgeum-ro, Danwon-gu, Ansan 15355, Republic of Korea
E-mail: ped7427@korea.ac.kr

aged 2 months to less than 6 years, the rate of recurrent UTIs was 25.4% in those with VUR and 17.3% in those without VUR. The probability of UTI recurrence was the highest if the VUR stage was 3 or higher, at 28.9% [5]. According to a study by Park et al. [6], younger age at the first UTI, bilateral VUR, grade 4–5 VUR, and hydronephrosis on the initial ultrasonography significantly increased the risk of UTI recurrence. Although the use of prophylactic antibiotics is recommended for children with VUR [7], whether these prophylactic antibiotics prevent UTI is unclear [8]. Craig et al [9]. reported that long-term, low-dose trimethoprim-sulfamethoxazole was associated with a decrease in the number of UTIs in pediatric patients. However, other studies reported that it was not related and only increased the risk of resistant bacteria [10]. Obesity has recently become a more serious and global public health problem [11], but many studies have shown that being underweight also increases the risk of infection. An analysis of 1,747 patients who visited the emergency room at the University of Oklahoma Children's Hospital found that underweight patients had more hospital visits, experienced respiratory diseases more often, and had a higher incidence of fractures than normal and overweight patients [12]. Also, in the United States, underweight children aged 2 to 19 years reported increased rates of surgical site infections following orthopedic surgery [13]. Host resistance to a UTI, especially in the acute phase, is highly dependent on innate immunity [14]. The innate immune system plays an essential role in the prevention of recurrent and invasive UTIs, and irreversible parenchymal tissue damage can occur if this system does not work properly [15]. An investigation of 48 types of serum cytokines and growth factors in female patients aged 18 to 49 without underlying diseases at a single center in the United States reported that factors required for the development and differentiation of monocytes, macrophages, and neutrophils were increased in patients with recurrent UTIs [16]. In another study of patients aged 6 months to less than 21 years, the median urinary neutrophil gelatinase-associated lipocalin (NGAL) level in patients with recurrent UTIs was 15 ng/mL, which was significantly lower than that in the control group of healthy children, at 30 ng/mL [17].

In this study, we hypothesized that abnormal growth trajectories in young infants might be associated with suppressed immune responses and enhanced bacterial vulnerability, namely, UTI recurrence. Therefore, we aimed to identify risk factors for recurrent UTIs, determine whether abnormal growth patterns were related to recurrent UTIs, and compare host inflammatory responses between patients with recurrent UTIs and those with a single UTI episode.

Subjects and Methods

Patient characteristics and inclusion criteria

This was a retrospective observational study of patients who were hospitalized in the Department of Pediatrics and Adolescents at Korea University Ansan Hospital from August 21, 2018, to October 20, 2021, for UTIs. Among them, patients aged 0 to 24 months who were first diagnosed with a UTI and followed up for more than 6 months in an outpatient setting were included. The diagnosis of a first UTI was as follows: (1) hospitalized for febrile UTI, fever (≥38 °C); (2) positive results on urinalysis for pyuria (≥5 white blood cells [WBCs] per high-power field) or nitrate; and (3) a positive urine culture collected from a catheterized specimen (defined as the growth of a single bacterial type to ≥50,000 colony-forming units/mL), and (4) no previous history of UTI. A single UTI was defined as no reinfection within 12 months after the onset of the first UTI. Patients who had 2 or more UTIs within 12 months were included in the recurrent UTI group. Reinfection was defined as infection with either the same bacteria as earlier or a different type of bacteria following a negative bacterial culture after treatment for a previous UTI. Patients were excluded if they satisfied any of the following conditions: (1) acute kidney injury, (2) chronic kidney disease, (3) underlying systemic disease, or (4) congenital anomalies of the kidney and urinary tract except for VUR and/or hydronephrosis (Fig. 1). Patients with systemic diseases, including pulmonary

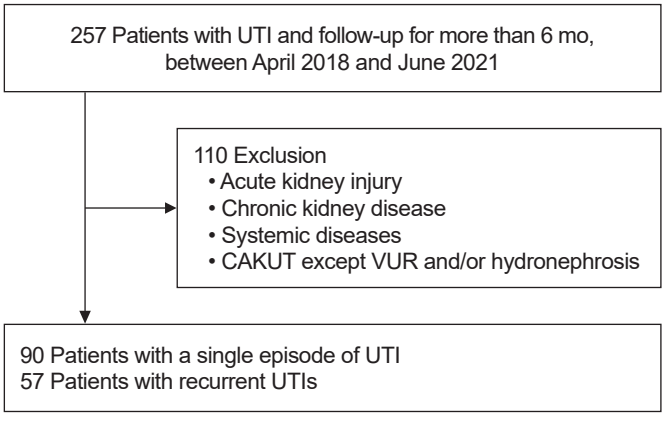

Fig. 1. Study flow diagram. UTI, urinary tract infection; CAKUT, congenital anomalies of the kidney and the urinary tract; VUR, vesicoureteral reflux.

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atresia with ventricular septal defect, metabolic disease, chronic respiratory failure, viral infections, and others, were excluded. Grades 1 to 3 of VUR were assigned to the low grade category, and grades 4 and 5 were assigned to the high grade. Prophylactic antibiotics were used in cases with high grade VUR and acute pyelonephritis (APN) after the initial UTI. Patients were defined to have APN if the uptake of ^{99m}Tc-dimercaptosuccinic acid (DMSA) at 1–5 mCi was decreased in focal, multifocal, or diffuse areas due to defects in the kidney cortex [18,19]. Hydronephrosis was defined as dilatation and distension of the kidney collecting system of one or both kidneys, and the radiology department at our hospital uses the Society for Fetal Urology system to define it [20]. All data were obtained based on the ethical principles for medical research in human subjects established in the Declaration of Helsinki in 1975 and revised in 2000.

Measurements and laboratory assessments

The growth chart for children and adolescents in 2017 [21] was used to determine weight-for-length (WFL) percentiles. Clinical data, including host and immunological factors, imaging data, and growth patterns, were investigated in the single UTI group and the recurrent group. Clinical information on birth weight, prenatal ultrasonography abnormalities, and previous UTI episodes was obtained by questionnaires and medical chart review. WFL was calculated by examining body weight and height at the time of hospitalization for the first UTI. The same was done in the case of recurrence. The weight percentiles of the single UTI group and the recurrent UTI group were compared at the time of admission and after 3 months and 6 months. In addition, to compare the innate immune system, WBCs, the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelets (PLTs), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP), urinary, and plasma NGAL levels of the two groups were compared at admission and discharge. Urine samples were obtained through urethral catheterization. Kidney ultrasonography and initial DMSA scans were performed at the time of the UTI diagnosis. Voiding cystourethrography was performed if APN or hydronephrosis was present on kidney ultrasonography or if APN was diagnosed on renal DMSA scan.

Statistical analysis

All analyses were conducted using R statistical software (version 4.3.0; The R Foundation). For comparison between the two groups, the Mann-Whitney *U* test or two-sample *t*-test was used for continuous variables, and the Pearson chi-square test or Fisher exact test was used for categorical variables. Continuous variables are presented as the mean ± standard deviation or standard error of the mean. Categorical variables are presented as numbers (%). A paired *t*-test or signed rank test was used to compare weight and weight percentiles in each group, and a two-sample test or the Mann-Whitney *U* test was used to compare weight and weight percentiles between the two groups. Inflammatory responses at admission and discharge were compared in the single and recurrent UTI groups using the Mann-Whitney *U* test. Univariable logistic regression analysis was performed to analyze variables associated with UTI recurrence. Parameters associated with UTI recurrence in univariable logistic regression analysis ($P<0.05$) were included in a multivariable logistic regression analysis. However, among them, VUR was excluded from the multivariable analysis because it was identified as a multicollinearity suspected variable when checking the variance inflation factor. In all statistical analyses, a *P*-value of <0.05 was considered significant.

Results

Patient demographics

Out of 257 patients who were diagnosed with a first UTI and underwent outpatient treatment for more than 6 months, 110 patients were excluded, 90 patients were included in the single UTI group, and 57 patients were included in the recurrent group (Fig. 1). In the recurrent group, the average WFL percentile was lower, and the frequency of prophylactic antibiotic use was higher than those in the single UTI group (all $P<0.05$). There were differences in uropathogens between the two groups ($P<0.05$). Analysis with Pearson chi-square test with Yates continuity correction showed a difference between the *Escherichia coli* and non-*E. coli* groups. Both extended-spectrum β-lactamase-positive and -negative *E. coli* were included in the *E. coli* group. *Klebsiella aerogenes*, *Enterococcus faecalis*, and *Enterobacter cloacae* complex were identified in cases other than *E. coli*. Patients who visited our hospital after taking antibiotics because the bacteria were confirmed at another hospital were excluded from uropathogen variable because no bacteria were found in our hospital. Hydronephrosis, VUR, and hydronephrosis with VUR were significantly higher in the recurrent group than in the single UTI group ($P<0.05$). There were eight patients in the single UTI group and one patient in the recurrent UTI group who did not undergo voiding cystoure-

throgrаphy, so the total number of patients in these categories was 82 and 56, respectively. There were no differences between hemoglobin, WBC, PLT, CRP, urinary, or plasma NGAL levels in the two groups. Since our hospital is a tertiary hospital, patients usually come after a referral from a local hospital. Therefore, we included some cases where bacteria were not confirmed here because the patient took antibiotics for a UTI before coming to our hospital (Table 1).

Comparison of growth pattern changes in single and recurrent UTI groups

The mean weight percentile tended to decrease in both groups from the time of initial diagnosis to the 3-month and 6-month assessments in each group. Weight percentiles at 3 or 6 months after the initial UTI were not different between the two groups. However, weight percentile declines for 6 months after the first UTI diagnosis seemed to be more prominent in the recurrent UTI group compared to the single UTI group. Weight percentiles at 3 and 6 months were significantly reduced in the recurrent UTI group compared to the initial UTI (Table 2, Fig. 2A). However, the WFL percentiles at the first and second UTI admissions were not different in the recurrent UTI group (Fig. 2B).

Comparison of inflammatory responses in patients with single and recurrent UTIs

Inflammatory responses were compared at admission and discharge in the single and recurrent UTI groups. There were no differences in the WBC, NLR, MLR, PLT, PLR, CRP, urinary NGAL/creatinine (Cr), or plasma NGAL levels at admission and discharge between the two groups. Some statistical data were obtained by excluding people with missing values. Urinary NGAL/Cr was measured in 73 single UTI patients, and in 29 recurrent UTI patients at admission and in 83 and 46 patients in each group at discharge. Plasma NGAL concentrations were determined in 88 and 46 patients, respectively, in each group at admission and in 83 and 47 patients in each group at discharge (Table 3).

Univariable and multivariable logistic regression analyses of recurrent UTIs

Univariable and multivariable logistic regression analyses were performed to test each parameter as an independent predictor of UTI recurrence in the recurrent UTI group. Univariable logistic regression analysis showed that higher birth weight, lower WFL percentile, prophylactic antibiotic use, the presence of hydronephrosis, VUR, APN, and non-*E. coli* infections were associated with UTI recurrence (all $P<0.05$). Multivariable logistic regression analysis showed that the prophylactic antibiotic use (odds ratio [OR], 18.8; 95% confidence interval [CI], 3.04–116) and presence of hydronephrosis (OR, 16.8; 95% CI, 4.04–70.2)

Table 1. characteristics and clinical findings

Characteristic	Single UTI (n=90)	Recurrent UTI (n=57)	*P*-value
Age at first UTI diagnosis (mo)	3.79±3.15	3.25±3.52	0.094[a]
Male sex	64 (71.1)	47 (82.5)	0.173[b]
Prematurity	78 (86.7)	48 (84.2)	0.863[b]
Birth weight (g)	3,134±562	3,350±645	0.116[a]
Abnormal prenatal US	4 (4.44)	6 (10.5)	0.187[c]
Weight (kg)	7.21±1.71	6.86±1.62	0.251[a]
Weight percentile	78.3±24.8	80.5±22.8	0.507[a]
Height (cm)	63.4±6.48	62.9±6.41	0.614[a]
Height percentile	73.2±29.3	79.9±27.2	0.050[a]
Weight-for-length percentile	72.7±22.0	63.9±26.1	0.043[a]
Prophylactic antibiotic use	7 (7.78)	31 (54.4)	<0.001[b]
Uropathogens[d]			0.021[b]
E. coli	80 (95.2)	46 (83.6)	
Non-*E. coli*	4 (4.76)	9 (16.4)	
Hydronephrosis	5 (5.56)	19 (33.3)	<0.001[b]
VUR[e]	15 (18.3)	32 (57.1)	<0.001[b]
Low grade[f]	4	6	
High grade[g]	11	26	
Hydronephrosis+VUR[e]	0	9 (15.8)	<0.001[c]
Acute pyelonephritis	51 (56.7)	42 (73.7)	0.056[b]
Hb (g/dL)	10.8±1.08	10.9±1.27	0.572[a]
WBC (/mm^3)	15,485±6,548	15,725±5,264	0.383[a]
PLT (10^3/mm^3)	421±119	401±122	0.409[a]
PLR	92.5±46.4	84.7±40.4	0.354[a]
CRP (mg/L)	4.44±3.87	4.66±3.42	0.513[a]
uNGAL (ng/mL)	562±820	610±877	0.996[a]
uNGAL/Cr	52.9±131	43.4±55.6	0.838[a]
pNGAL (mg/L)	134±126	171±165	0.218[a]
BUN (mg/dL)	9.04±3.54	8.98±3.46	0.590[a]
Cr (mg/dL)	0.54±2.71	0.26±0.06	0.321[a]

Values are presented as mean±standard deviation or number (%).
UTI, urinary tract infection; US, ultrasonography; *E. coli, Escherichia coli;* VUR, vesicoureteral reflux; Hb, hemoglobin; WBC, white blood cell; PLT, platelet; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; uNGAL, urine neutrophil gelatinase-associated lipocalin; Cr, creatinine; pNGAL, plasma neutrophil gelatinase-associated lipocalin; BUN, blood urea nitrogen.
[a]Mann-Whitney *U* test. [b]Pearson chi-square test with Yates' continuity correction. [c]Fisher exact test for count data. [d]The total number of people for this variable is 84 in the single UTI group and 55 in the recurrent UTI group. [e]The total number of people for these variables is 82 in the single UTI group and 56 in the recurrent UTI group. [f]Low grade: 1 to 3 grade of VUR. [g]High grade: 4 and 5 grade of VUR.

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were significant predictors of UTI recurrence (P<0.05, respectively). VUR was identified as a multicollinearity variable, so it was excluded from the multivariable analysis when checking the variance inflation factor (Table 4).

Discussion

This study aimed to investigate the risk factors for recurrent UTIs in young children and determine if there were differences in growth trajectories between patients with single and recurrent UTIs. When the single UTI group and the recurrent

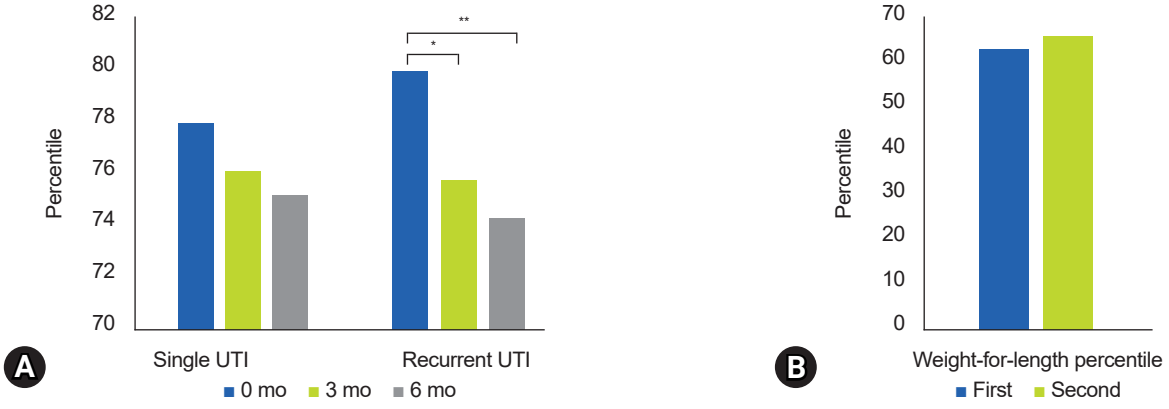

Fig. 2. (A) Comparison of weight percentiles at 0, 3, and 6 months after the first UTI in the single and recurrent UTI groups. (B) Comparison of weight-for-length percentiles in the first and second episodes in the recurrent UTI group. UTI, urinary tract infection. *P<0.05, **P<0.01.

Table 2. Comparisons of weight percentiles of 0, 3, and 6 months after first UTI

	First UTI	After 3 mo	After 6 mo	Diff[a]	P-value[a]	Diff[b]	P-value[b]
Single	78.4±24.9	76.4±24.2	75.5±24.8	−1.99±17.7	0.074[c]	−2.84±23.0	0.094[c]
Recurrent	80.5±22.6	76.0±24.8	74.5±23.1	−4.51±14.2	0.010[c]	−5.93±17.4	0.012[d]
P-value				0.244[e]		0.212[e]	

UTI, urinary tract infection; Diff, differences.
[a]First UTI vs. after 3 mo. [b]First UTI vs. after 6 mo. [c]Singed rank test. [d]Paired t-test. [e]Mann-Whitney test.

Table 3. Comparisons of inflammatory responses between the single and recurrent UTI groups

Variable	At admission			At discharge		
	Single UTI (n=90)	Recurrent UTI (n=57)	P-value[a]	Single UTI (n=90)	Recurrent UTI (n=56)	P-value[a]
WBC (/mm^3)	15,485±6,548	15,725±5,264	0.383	8,483±2,214	8,111±2,313	0.374
NLR	1.74±1.21	1.74±1.03	0.706	0.31±0.16	0.35±0.24	0.538
MLR	0.37±0.21	0.32±0.17	0.139	0.15±0.14	0.16±0.08	0.087
PLT (10^3/mm^3)	421±118	401±122	0.409	474±134	440±140	0.054
PLR	92.5±46.4	84.7±40.4	0.354	88.8±31.7	91.1±37.9	0.939
CRP (mg/L)	4.44±3.87	4.66±3.42	0.513	0.63±0.57	0.68±0.61	0.560
uNGAL/Cr	52.9±131	43.4±55.6	0.838	5.52±4.76	5.94±3.98	0.417
pNGAL (mg/L)	134±126	172±165	0.218	57.2±29.6	80.3±101	0.348

Values are presented as mean±standard deviation.
UTI, urinary tract infection; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLT, platelet; PLR, platelet -to-lymphocyte ratio; CRP, C-reactive protein; uNGAL, urine neutrophil gelatinase-associated lipocalin; Cr, creatinine; pNGAL, plasma neutrophil gelatinase-associated lipocalin.
[a]Wilcoxon rank sum test (or Mann-Whitney test).

Table 4. Univariable and multivariable logistic regression analyses for the recurrent UTI

Variable	Univariable		Multivariable	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
First UTI age	0.95 (0.86–1.06)	0.334		
Male sex	1.91 (0.84–4.34)	0.123		
Abnormal prenatal US	2.53 (0.68–9.39)	0.166		
Birth weight	1.00 (1.00–1.01)	0.040	1.00 (0.99–1.01)	0.286
WFL percentile	0.99 (0.97–1.00)	0.031	0.99 (0.97–1.01)	0.371
Prophylactic antibiotic use	14.1 (5.57–35.9)	<0.001	18.8 (3.04–116)	0.002
Hydronephrosis	8.50 (2.95–24.5)	<0.001	16.8 (4.04–70.2)	<0.001
VUR	5.96 (2.76–12.9)	<0.001		
APN	2.14 (1.04–4.41)	0.039	0.98 (0.36–2.69)	0.967
Uropathogens				
Non-*E. coli*	3.91 (1.14–13.4)	0.030	0.88 (0.16–5.00)	0.889

UTI, urinary tract infection; CI, confidence interval; US, ultrasonography; WFL, weight-for-length; VUR, vesicoureteral reflux; APN, acute pyelonephritis; *E. coli, Escherichia coli.*

UTI group were compared, the recurrent UTI group had a lower WFL percentile and a higher proportion of hydronephrosis and/or VUR and prophylactic antibiotic use. While there were no differences in growth patterns for 6 months between the two groups, weight percentiles in the recurrent UTI group were significantly reduced at 3 and 6 months compared to initial UTI percentiles. The presence of hydronephrosis at the time of the first UTI and prophylactic antibiotic use after the initial UTI were found to be independently associated with recurrent UTIs in children aged <24 months.

Many studies have already reported that VUR [5,8,10] and prenatal hydronephrosis [22] are associated with recurrent UTIs. In a study of 290 children under 5 years of age, recurrent UTIs were associated with kidney defects [8], and UTI recurrence could be predicted in infants under 3 months old when they were infected with bacteria other than *E. coli* at the time of their first febrile UTI [23]. In addition, non-*E. coli* UTI in the first febrile UTI may be useful in predicting imaging abnormalities [23,24]. While we also found that the presence of hydronephrosis, APN, VUR, and non-*E. coli* infections were associated with UTI recurrence, of them, only hydronephrosis was an independent risk factor. Since transient, prenatal, refluxing, and non-refluxing hydronephrosis were all included in our category of patients with hydronephrosis, the causal relationship between congenital or refluxing hydronephrosis and UTI recurrence remains unclear. However, the existence of hydronephrosis itself with or without VUR at the first UTI diagnosis might predict UTI recurrence in children aged <24 months old. To the authors' knowledge, this was the first finding that young children with hydronephrosis at the first UTI diagnosis were at a higher risk of developing recurrent UTIs.

A paper reviewing recurrent UTI studies found that, although controversial, the effectiveness of prophylactic antibiotic therapy was not significant and could even increase the risk of recurrence [2]. Conway et al. [10] found that prophylactic antibiotic use was not associated with a reduced risk of recurrent UTIs but was associated with an increased risk of resistant infections. In the present study, the use of prophylactic antibiotics was found to be a factor related to recurrent UTIs, which may be because more patients with reflux were included in the recurrence group (57.1%) compared to the single UTI group (18.3%). Multivariable analysis showed that antibiotic prophylaxis was an independent risk factor for recurrence. Therefore, the advantages of continuous antibiotic prophylaxis to prevent recurrence in children after the first UTI may be limited in young children. However, there is a need for more randomized controlled trials evaluating the benefits and/or harms of using prophylactic antibiotics in children with UTIs with VUR for a longer period of time.

Our previous paper [25] using Korean National Health Screening data and National Health Insurance Service data showed that the risk of UTIs and APN was higher in underweight boys aged 2 to 6 years compared to the normal weight group after adjusting for age, sex, birth weight, and preterm birth. In a prospective observational chart review study [12], children with underweight status (body mass index less than 5%) had an increased risk of being admitted from the emergency room, even after adjusting for age and sex, especially for respiratory infections and fractures. For many years, the World Health Organization Nutrition Department has been monitor-

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ing trends in child malnutrition using anthropometric measurements (e.g., weight and height) [26]. Underweight status is a direct indicator of chronic and acute malnutrition because it reflects both low height-for-age and low weight-for-age [26,27]. UTIs are more common in malnourished children than in well-nourished children, and the risk of UTIs increases with the severity of malnutrition [28]. In the present study, the recurrent UTI group had a lower WFL percentile than the single UTI group at the first UTI diagnosis. Weight-for-age percentile significantly declined for 6 months in the recurrent UTI group, while there were no differences in the single UTI group. More interestingly, higher birth weight and lower WFL percentile were related to UTI recurrence in univariable analysis. These findings suggest that children with abnormal growth patterns have a high risk of recurrent UTIs. However, large-scale prospective, longitudinal cohort studies may be required to address this issue as the length of the observation period in this study was short, and the information on WFL percentiles was limited.

Severe malnutrition is associated with immune deficiency, which is thought to make affected children more susceptible to serious infections [29]. Urinary secretory immunoglobulin A (IgA), an immunoglobulin synthesized locally at mucosal surfaces, is an important immunological defense that prevents bacterial adhesion to the periurethral epithelium and urothelium [30]. Secretory IgA production may be less secreted in malnourished children, which may predispose them to infections due to immune dysregulation. One study found that patients with recurrent UTIs had lower urinary levels of secretory IgA [31]. Additionally, a rat experiment showed that severe protein malnutrition dramatically suppressed the secretory immune component [32], suggesting that dietary protein plays a site-specific role in the developmental expression of the immune system. NGAL is an iron-carrier protein produced from neutrophilic granules that plays a vital role in the innate immune system response to bacterial infection [33,34]. During infection, bacteria require iron for growth and metabolic activity in the host. Thus, the host activates neutrophils to release NGAL to prevent bacteria from absorbing iron [34]. Malnourished children have decreased transferrin and increased levels of free unbound iron, which may lead to a favorable environment for bacterial growth, leading to urosepsis and UTIs [35]. Although there was no statistically significant difference in our study, urinary NGAL/Cr values tended to be lower in the recurrent UTI group compared to the single UTI group. This is consistent with the previously mentioned study that found the urinary NGAL values were reduced in patients with recurrent UTIs compared to the non-UTI group [17]. This suggests that the host defense system in recurrent UTI group may be more vulnerable. In addition, malnourished children have impaired cell-mediated immunity, phagocytic function, complement levels, and inadequate cytokine production, as well as atrophy of the lymph nodes, tonsils, and thymus, which may make them more susceptible to UTI [35]. CRP and total leukocyte counts, including absolute neutrophil count, are important indicators of acute bacterial infection [36]; NLR and MLR are effective in diagnosing bacterial infections in hospitalized patients with fever [37]. Neutrophils from recurrent UTI patients not only had significantly reduced bactericidal function but also reduced activation ability compared to healthy controls. This reduction in neutrophil function results in incomplete bacterial clearance, which predisposes to recurrent infections [38]. In addition to their essential role in killing bacteria, activated neutrophils can cause extensive parenchymal damage in the infected urinary tract. Neutrophil-derived cyclooxygenase-2 is thought to be a factor causing inflammation associated with severe recurrent cystitis [16]. Neutrophils cause severe tubulointerstitial nephritis in interleukin-8 receptor-deficient mice [39]. Monocytes and macrophages are highly suitable for regulating neutrophil function during UTI [40]. However, the host defense mechanisms that prevent invasive bacterial infections are not fully elucidated [15]. In our study, host inflammatory responses related to WBC, NLR, MLR, CRP, and PLR did not differ between the two groups. This might be due to the fact that both innate and adaptive immune systems are relatively immature in early life and could mask the role of immune defenses in the susceptibility of young children to recurrent UTIs. It is also unclear whether other immune responses play a role in increased vulnerability to recurrent infections. Since we had a low number of patients, the results should be confirmed in a larger cohort of pediatric patients.

This study had the following limitations. First, the study was conducted at a single institution, and the sample size was relatively small. Second, this was a retrospective study. We used only general laboratory values, such as WBC, PLT, CRP, urinary NGAL/Cr, and plasma NGAL values, which were performed when children with UTIs were hospitalized. Underlying innate and adaptive immunity was not fully assessed in the present study. Because there was no data on height during the study period, it was not possible to compare WFL percentiles between the single UTI group and the recurrent group. Using only the

weight percentile may be insufficient for assessing growth trajectories in infancy and childhood. Finally, kidney scarring was not assessed because of insufficient data. A better understanding of the risk factors for kidney scarring and worsening kidney function would be helpful for the management of children with recurrent UTIs. Therefore, multicenter prospective studies involving a large number of patients are needed to address these limitations.

In conclusion, UTI recurrence should be monitored in children with hydronephrosis at the first UTI diagnosis or those using prophylactic antibiotics. Also, when UTI is first diagnosed in infants under 2 years of age, an abnormal growth pattern with a higher birth weight, a lower WFL percentile, and reduced weight percentiles at least 6 months after the first UTI, the presence of VUR and APN, and confirmed infections with bacteria other than *E. coli* may partially account for UTI recurrence.

Ethical statements

The IRB and the Research Ethics Committee of Korea University Ansan Hospital approved this study (IRB number: 2022AS0087). The IRB exempted the requirement for informed consent because of the retrospective nature of this study. Personal identifiers were completely removed, and data were analyzed anonymously.

Conflicts of interest

Hyung Eun Yim, an Editor-in-Chief of the Journal, was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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References

1. Hellstrom A, Hanson E, Hansson S, Hjalmas K, Jodal U. Association between urinary symptoms at 7 years old and previous urinary tract infection. Arch Dis Child 1991;66:232-4.
2. Williams G, Craig JC. Prevention of recurrent urinary tract infection in children. Curr Opin Infect Dis 2009;22:72-6.
3. Jodal U. The natural history of bacteriuria in childhood. Infect Dis Clin North Am 1987;1:713-30.
4. Finnell SM, Carroll AE, Downs SM; Subcommittee on Urinary Tract Infection. Technical report: diagnosis and management of an initial UTI in febrile infants and young children. Pediatrics 2011;128:e749-70.
5. Keren R, Shaikh N, Pohl H, Gravens-Mueller L, Ivanova A, Zaoutis L, et al. Risk factors for recurrent urinary tract infection and renal scarring. Pediatrics 2015;136:e13-21.
6. Park S, Han JY, Kim KS. Risk factors for recurrent urinary tract infection in infants with vesicoureteral reflux during prophylactic treatment: effect of delayed contrast passage on voiding cystourethrogram. Urology 2011;78:170-3.
7. Elder JS, Peters CA, Arant BS, Ewalt DH, Hawtrey CE, Hurwitz RS, et al. Pediatric Vesicoureteral Reflux Guidelines Panel summary report on the management of primary vesicoureteral reflux in children. J Urol 1997;157:1846-51.
8. Panaretto K, Craig J, Knight J, Howman-Giles R, Sureshkumar P, Roy L. Risk factors for recurrent urinary tract infection in preschool children. J Paediatr Child Health 1999;35:454-9.
9. Craig JC, Simpson JM, Williams GJ, Lowe A, Reynolds GJ, McTaggart SJ, et al. Antibiotic prophylaxis and recurrent urinary tract infection in children. N Engl J Med 2009;361:1748-59.
10. Conway PH, Cnaan A, Zaoutis T, Henry BV, Grundmeier RW, Keren R. Recurrent urinary tract infections in children: risk factors and association with prophylactic antimicrobials. JAMA 2007;298:179-86.
11. Bessesen DH. Update on obesity. J Clin Endocrinol Metab 2008;93:2027-34.
12. Wyrick S, Hester C, Sparkman A, O'Neill KM, Dupuis G, Anderson M, et al. What role does body mass index play in hospital admission rates from the pediatric emergency department? Pediatr Emerg Care 2013;29:974-8.
13. Cies JJ, Chan S, Hossain J, Brenn BR, Di Pentima MC. Influence of body mass index and antibiotic dose on the risk of surgical site infections in

https://doi.org/10.3339/ckd.24.003

pediatric clean orthopedic surgery. Surg Infect (Larchmt) 2012;13:371-6.
14. Ragnarsdottir B, Lutay N, Gronberg-Hernandez J, Koves B, Svanborg C. Genetics of innate immunity and UTI susceptibility. Nat Rev Urol 2011;8:449-68.
15. Ching C, Schwartz L, Spencer JD, Becknell B. Innate immunity and urinary tract infection. Pediatr Nephrol 2020;35:1183-92.
16. Hannan TJ, Roberts PL, Riehl TE, van der Post S, Binkley JM, Schwartz DJ, et al. Inhibition of cyclooxygenase-2 prevents chronic and recurrent cystitis. EBioMedicine 2014;1:46-57.
17. Forster CS, Johnson K, Patel V, Wax R, Rodig N, Barasch J, et al. Urinary NGAL deficiency in recurrent urinary tract infections. Pediatr Nephrol 2017;32:1077-80.
18. Jaksic E, Bogdanovic R, Artiko V, Saranovic DS, Petrasinovic Z, Petrovic M, et al. Diagnostic role of initial renal cortical scintigraphy in children with the first episode of acute pyelonephritis. Ann Nucl Med 2011;25:37-43.
19. Piepsz A, Colarinha P, Gordon I, Hahn K, Olivier P, Roca I, et al. Guidelines for 99mTc-DMSA scintigraphy in children. Eur J Nucl Med 2001;28:BP37-41.
20. Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. Pediatr Radiol 1993;23:478-80.
21. Kim JH, Yun S, Hwang SS, Shim JO, Chae HW, Lee YJ, et al. The 2017 Korean National Growth Charts for children and adolescents: development, improvement, and prospects. Korean J Pediatr 2018;61:135-49.
22. Braga LH, Mijovic H, Farrokhyar F, Pemberton J, DeMaria J, Lorenzo AJ. Antibiotic prophylaxis for urinary tract infections in antenatal hydronephrosis. Pediatrics 2013;131:e251-61.
23. Chong SL, Leow EH, Yap CJY, Chao SM, Ganesan I, Ng YH. Risk factors for imaging abnormalities after the first febrile urinary tract infection in infants ≤3 months old: a retrospective cohort study. BMJ Paediatr Open 2023;7:e001687.
24. Honkinen O, Jahnukainen T, Mertsola J, Eskola J, Ruuskanen O. Bacteremic urinary tract infection in children. Pediatr Infect Dis J 2000;19:630-4.
25. Yim HE, Han KD, Kim B, Yoo KH. Impact of early-life weight status on urinary tract infections in children: a nationwide population-based study in Korea. Epidemiol Health 2021;43:e2021005.
26. de Onis M, Blossner M. The World Health Organization Global Database on Child Growth and Malnutrition: methodology and applications. Int J Epidemiol 2003;32:518-26.
27. Alemayehu GM, Cherie KT, Chernet AG. Severity of malnutrition among underweight children and family-related factors: a cross-sectional analysis of data from the 2019 Ethiopian Demographic and Health Survey (EDHS). Health Sci Rep 2022;5:e860.
28. Bagga A, Tripathi P, Jatana V, Hari P, Kapil A, Srivastava RN, et al. Bacteriuria and urinary tract infections in malnourished children. Pediatr Nephrol 2003;18:366-70.
29. Babirekere-Iriso E, Musoke P, Kekitiinwa A. Bacteraemia in severely malnourished children in an HIV-endemic setting. Ann Trop Paediatr 2006;26:319-28.
30. Deo SS, Vaidya AK. Elevated levels of secretory immunoglobulin A (sIgA) in urinary tract infections. Indian J Pediatr 2004;71:37-40.
31. Riedasch G, Heck P, Rauterberg E, Ritz E. Does low urinary sIgA predispose to urinary tract infection? Kidney Int 1983;23:759-63.
32. Sullivan DA, Vaerman JP, Soo C. Influence of severe protein malnutrition on rat lacrimal, salivary and gastrointestinal immune expression during development, adulthood and ageing. Immunology 1993;78:308-17.
33. Xu SY, Carlson M, Engstrom A, Garcia R, Peterson CG, Venge P. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. Scand J Clin Lab Invest 1994;54:365-76.
34. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci U S A 2006;103:1834-9.
35. Uwaezuoke SN. The prevalence of urinary tract infection in children with severe acute malnutrition: a narrative review. Pediatric Health Med Ther 2016;7:121-7.
36. Shaikh S, Salim E, Ram PV, Memon SS, Zubairi A, Khawaja SA, et al. Correlation of C-reactive protein and total leukocyte count in acute infections: a single center study. Pak J Surg 2019;35:271-4.
37. Naess A, Nilssen SS, Mo R, Eide GE, Sjursen H. Role of neutrophil to lymphocyte and monocyte to lymphocyte ratios in the diagnosis of bacterial infection in patients with fever. Infection 2017;45:299-307.
38. Condron C, Toomey D, Casey RG, Shaffii M, Creagh T, Bouchier-Hayes D. Neutrophil bactericidal function is defective in patients with recurrent urinary tract infections. Urol Res 2003;31:329-34.
39. Frendeus B, Godaly G, Hang L, Karpman D, Lundstedt AC, Svanborg C. Interleukin 8 receptor deficiency confers susceptibility to acute experimental pyelonephritis and may have a human counterpart. J Exp Med 2000;192:881-90.
40. Schiwon M, Weisheit C, Franken L, Gutweiler S, Dixit A, Meyer-Schwesinger C, et al. Crosstalk between sentinel and helper macrophages permits neutrophil migration into infected uroepithelium. Cell 2014;156:456-68.

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Case Report

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A rare case of childhood-onset systemic lupus erythematosus associated end-stage renal disease with cerebral abscess and hemorrhage

Jee Hyun Kim[1], Jae Il Shin[1,2,3], Ji Hong Kim[1,4], Keum Hwa Lee[1,2,3]

[1]Department of Pediatrics, Yonsei University College of Medicine, Seoul, Republic of Korea
[2]Division of Pediatric Nephrology, Severance Children's Hospital, Seoul, Republic of Korea
[3]Institute of Kidney Disease Research, Yonsei University College of Medicine, Seoul, Republic of Korea
[4]Department of Pediatrics, Gangnam Severance Hospital, Seoul, Republic of Korea

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that affects multiple organs. More than half of the patients with SLE have kidney involvement, and up to 10% of patients with lupus nephritis develop end-stage renal disease (ESRD). Central nervous system (CNS) involvement in SLE occurs in 21% to 95% of patients. Severe neurological manifestations such as seizures, cerebrovascular disease, meningitis, and cerebrovascular accidents can develop in childhood-onset SLE, but cerebral infections, such as brain abscess and hemorrhage, are seldom reported in lupus nephritis, even in adults. Here, we report a rare case of childhood-onset SLE with ESRD, cerebral abscess, and hemorrhage. A 9-year-old girl diagnosed with lupus nephritis was administered high-dose steroids and immunosuppressant therapy to treat acute kidney injury (AKI) and massive proteinuria. The AKI deteriorated, and after 3 months, she developed ESRD. She received hemodialysis three times a week along with daily peritoneal dialysis to control edema. She developed seizures, and imaging showed a brain abscess. This was complicated by spontaneous cerebral hemorrhage, and she became unstable. She died shortly after the hemorrhage was discovered. In conclusion, CNS complications should always be considered in clinical practice because they increase mortality, especially in those with risk factors for infection.

Keywords: Brain abscess; Case reports; Cerebral hemorrhage; Kidney failure, chronic; Lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that affects multiple organs. It frequently involves various systems such as renal, joints, skin, cardiovascular, nervous, and hematologic systems [1].

Childhood-onset SLE (cSLE) and adult-onset SLE (aSLE) have different phenotypes [2]. Neuropsychiatric, renal, and hematological manifestations are more common in cSLE than in adult-onset disease [2]. Patients with cSLE have common hematological manifestations such as leukopenia, lymphopenia, thrombocytopenia, and hemolytic anemia [3]. In addition, kidney involvement is more frequent and shows more severe manifestations in cSLE patients than in aSLE [1]. About 50% to 75% of cSLE patients have renal involvement, and >90% develop lupus nephritis within 2 years of diagnosis [4]. Moreover,

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Correspondence to
Keum Hwa Lee
Department of Pediatrics, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea
E-mail: azsagm@yuhs.ac

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10% to 30% of cSLE patients with lupus nephritis progress to end-stage renal disease (ESRD) within 15 years of diagnosis [5].

Central nervous system (CNS) involvement in SLE occurs in 21% to 95% of patients [6]. Neuropsychiatric manifestations include psychosis, anxiety, mood disorders, seizures, and hemorrhage [6]. These manifestations are caused by the disease or secondary CNS sequelae of SLE induced by treatment with steroids and immunosuppressants [6]. However, neurological complications such as cerebral infections, brain abscesses, and hemorrhage are seldom reported in lupus nephritis, even in adults. These are extremely rare in cSLE compared to aSLE, and the result is usually fatal. Here, we report a rare case of cSLE with ESRD, brain abscess, and cerebral hemorrhage.

Case report

A 9-year-old girl, previously diagnosed with idiopathic thrombocytopenic purpura (ITP) at another hospital, was referred to our hospital because of severe acute kidney injury (AKI) in June 2021. She was admitted to the pediatric intensive care unit (PICU) for continuous renal replacement therapy. She showed volume overload (uncontrolled hypertension, both pleural effusion, ascites) and decreased kidney function (blood urea nitrogen [BUN] 57.6 mg/dL, serum creatinine 0.76 mg/dL) with anuria, massive hematuria, and proteinuria, decreased platelet counts, a malar rash, joint pain, and immunologic findings of reduced complement levels, including complement components 3 and 4, and positive antinuclear antibody and anti-double-stranded DNA (Tables 1, 2). We finally diagnosed the patient with lupus nephritis, which was cSLE with kidney involvement.

Table 1. Initial laboratory results of patient

Variable	Reference	Result
Hematologic		
WBC (×10^3 μL)	4.0–10.8	5.40
PLT count (×10^3 μL)	150–400	28.0
Mucocutaneous		
Malar rash	Negative	Positive
Chemistry		
BUN (mg/dL)	7–17	57.0
Creatinine (mg/dL)	0.37–0.72	0.76
Complement proteins		
C3 (mg/dL)	90–180	14.2
C4 (mg/dL)	30–200	2.83
Renal		
Urine protein	Negative	4+
Urine protein-to creatinine ratio (g/gCr)	0–0.15	17.7
Urine RBC (/μL)	0–8.8	488.4
SLE-specific antibodies		
Anti-dsDNA antibody	Negative	Positive
Anti-Sm antibody	Negative	Positive
Antiphospholipid antibodies		
Anti-cardiolipin antibody	Negative	Negative
Anti-beta2GP1 antibody	Negative	Negative
Lupus anticoagulant	Negative	Negative

WBC, white blood cell; PLT, platelet; BUN, blood urea nitrogen; C3, complement component 3; C4, complement component 4; RBC, red blood cell; SLE, systemic lupus erythematosus; Anti-dsDNA, anti-double stranded DNA; Anti-Sm, anti-Smith; Anti-beta2GP1, anti-beta2-glycoprotein I.

Table 2. Serological results after diagnosis of systemic lupus erythematosus

Variable	Initial[a]	4 mo[b]	7 mo[b]
Rheumatoid factor (normal range, 0–15.0 IU/mL)	8	11	8
Lupus anticoagulant	Negative	Positive	Positive
Antinuclear antibody	Positive (1:640)[a]	Negative (1:80)[a]	Not done
Anti-dsDNA antibody	Positive (≥320)[a]	Negative (1:10)[a]	Negative (1:10)[a]
Anti-RNP antibody	Positive (16)[a]	Negative	Negative
Anti-Sm antibody	Positive (33)[a]	Negative	Negative
Anti-SS-A/Ro antibody	Positive (23)[a]	Negative	Negative
Anti-SS-B/La antibody	Negative	Negative	Negative
Anti-cardiolipin antibody IgM	Negative	Negative	Not done
Anti-cardiolipin antibody IgG	Negative	Negative	Not done
Anti-beta2GP1 IgG	Negative	Negative	Not done
Anti-beta2GP1 IgM	Negative	Negative	Not done

Anti-dsDNA, anti-double stranded DNA; Anti-RNP, anti-ribonucleoprotein; Anti-Sm, anti-Smith; Anti-SS-A/Ro, anti-Sjögren's-syndrome-related antigen A/Ro; Anti-SS-B/La, anti-Sjögren's-syndrome-related antigen B/La; IgM, immunoglobulin M; IgG, immunoglobulin G; Anti-beta2GP1, anti-beta2-glycoprotein I.
[a]The value in parentheses is the titer of serology. [b]Post initial treatment.

To treat the AKI caused by lupus nephritis, high-dose steroids (methylprednisolone 0.50–0.75 g/day for two doses) and immunosuppressant therapy (cyclophosphamide 500 mg/body surface area once in September 2021; mycophenolate mofetil 1,500 mg/day from August 2021 to January 2022) was administered. The AKI deteriorated, however, and after 3 months, she reached ESRD status. Given this situation, decreasing lupus activity became more important than preserving kidney function. So we had to add tacrolimus while using other immunosuppressants after 1 month (tacrolimus 2 mg/day from September 2021 to January 2022). Peritoneal dialysis (PD) alone was ineffective in resolving fluid retention, such as pericardial effusion, pleural effusions, hypertension, and elevation of BUN and serum creatinine levels. Therefore, we performed hemodialysis (HD) three times a week with daily PD. In December 2021, 6 months after her first admission, she was discharged with all dialysis plans. Unfortunately, she was hospitalized in the PICU again because of seizures January 2022. Brain magnetic resonance imaging was performed, and the patient was diagnosed with a brain abscess (Fig. 1). During surgery, spontaneous cerebral hemorrhage was observed (Fig. 2). Even though we performed a burr-hole operation immediately, her vital signs became unstable, and then shortly after, she died because of disseminated intravascular coagulation (DIC) (Table 3, Fig. 3).

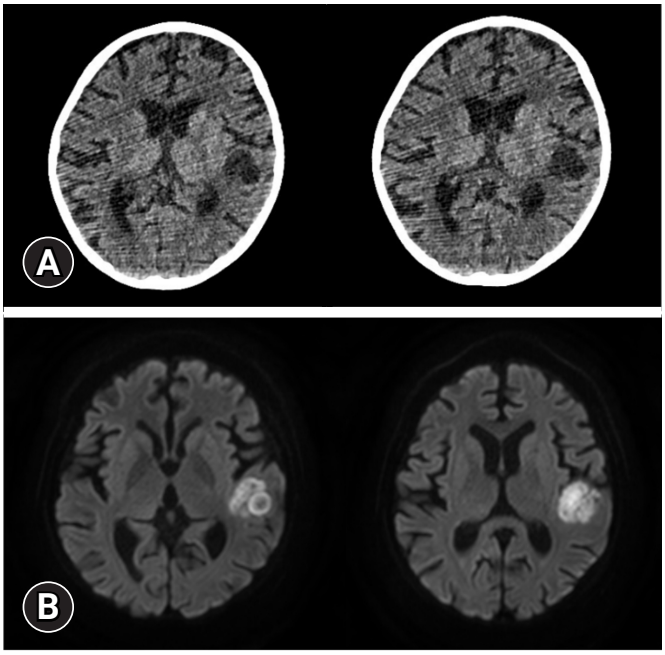

Fig. 1. Radiological imaging of the brain. (A) Initial brain computed tomography (CT) of the patient showed an approximately 2.3 cm low-density lesion in the left temporoparietal lobe. This lesion presented the possibility of a cystic lesion or low-density mass, such as cystic encephalomalacia. (B) Brain magnetic resonance imaging of the patient after Brain CT. It showed an approximately 2.7 cm rim-enhancing mass with diffusion restriction in the left superior temporal gyrus, suggesting a brain abscess.

Discussion

cSLE accounts for 15% to 20% of all SLE cases [2]. Although both cSLE and aSLE involve multiple organs, cSLE has several different clinical manifestations, with hematological, renal, and neuropsychiatric manifestations being more common [2].

Although lymphopenia is more prevalent with aSLE, both thrombocytopenia and hemolytic anemia are significantly more prevalent in the cSLE group [3]. If only hematologic symptoms are present initially, it may be mistaken for a hematologic disease, and SLE may be missed in both age groups. Although there are limited studies in children, some studies reported that some patients developed SLE after ITP [7]. The prevalence of these patients ranges from 3% to 12% [7]. Patients initially have thrombocytopenia as an isolated presentation. Therefore, they could be diagnosed with ITP since they do not have symptoms of SLE other than thrombocytopenia [7]. Another study reported that 2.96% of childhood ITP patients subsequently developed SLE [8]. In our case, thrombocytopenia was an early symptom of SLE, and the patient was diagnosed and treated for

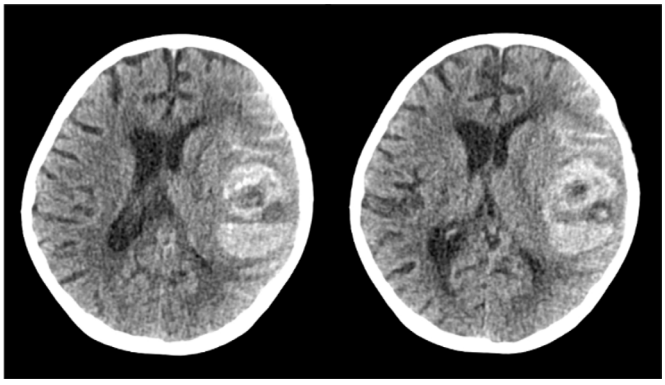

Fig. 2. Secondary brain computed tomography of the patient. Spontaneous intracranial hemorrhage in the left parietal lobe occurred in a previously noted left temporal lobe abscess.

ITP for 2 years. The patient was finally diagnosed with SLE when she was re-examined for symptoms and signs such as malar rash and renal manifestations such as pitting edema, oliguria, BUN, and serum creatinine elevation after transfer to our hospital. Even hematological problems alone may necessitate im-

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Table 3. Laboratory results after cerebral hemorrhage

Variable	Reference	Result[a)]
Hematologic		
WBC (×10^3 μL)	4.0–10.8	4.32 (9.09)
Hemoglobin (g/dL)	14.0–18.0	7.80 (10.1)
Hematocrit (%)	40.0–50.0	23.9 (30.6)
PLT count (×10^3 μL)	150–400	54 (41)
Chemistry		
BUN (mg/dL)	7–17	10.3 (97.7)
Creatinine (mg/dL)	0.37–0.72	0.76 (4.87)
Complement proteins		
C3 (mg/dL)	90–180	59.7 (64.7)
C4 (mg/dL)	30–200	9.92 (18.9)
Renal		
Urine protein-to creatinine ratio (g/gCr)	0–0.2	74.8 (>190.1)
SLE-specific antibody		
Anti-dsDNA antibody	Negative	Negative
Anti-Sm antibody	Negative	Negative
Antiphospholipid antibody		
Anti-cardiolipin antibody	Negative	Negative
Anti-beta2GP1 antibody	Negative	Negative
Lupus anticoagulant	Negative	Positive
Coagulation		
PT (INR)	0.89–1.12	Undetectable (0.97)
aPTT (sec)	26.8–40.6	undetectable (24.4)
Fibrinogen (mg/dL)	200–400	49 (300)
FDP (μg/mL)	0–5	8.83 (7.84)
D-Dimer (ng/mL)	0–243	799 (956)
Echocardiography	No significant finding	
Culture		
Blood	No growth	
CSF	No growth	
Peritoneal fluid	No growth	

WBC, white blood cell; PLT, platelet; BUN, blood urea nitrogen; C3, complement component 3; C4, complement component 4; SLE, systemic lupus erythematosus; Anti-dsDNA, anti-double stranded DNA; Anti-Sm, anti-Smith; Anti-beta2GP1, anti-beta2-glycoprotein I; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; FDP, fibrin degradation products; CSF, cerebrospinal fluid.
[a)]The values in parentheses are the results at the time of seizure.

munochemical tests for SLE. However, it is unclear whether our patient developed SLE after ITP or had an isolated presentation of thrombocytopenia as the initial symptom of SLE.

Kidney involvement is the most common manifestation of SLE and a predictor of poor survival outcomes [7]. Lupus nephritis is more common and severe in cSLE than in aSLE [9]. Although the 5-year renal survival rates in children with cSLE have markedly improved in recent decades, the most recent rate ranges from 52% to 91%, which is still low [9]. Also, the mortality rate of cSLE with lupus nephritis is 19 times greater than that in the same age groups. It is higher than aSLE, which has an eight times higher mortality than the same age group [9]. cSLE with lupus nephritis with frequent and severe symptoms of kidney involvement leads to high-dose corticosteroids and immunosuppressive treatment [10]. Inevitable life-threatening side effects could occur when these drugs are used for remission of the disease. In addition, they could increase the risk of serious infections. A study reported that the poor prognosis of cSLE with ESRD is primarily associated with infectious disease [11]. Infectious diseases such as septicemia, peritonitis, pulmonary infection, and other infections are secondary common causes of mortality during the 5 years from initiation of renal replacement therapy in cSLE with ESRD [11].

cSLE shows more common psychiatric manifestations than aSLE [12]. In our case, the patient was treated with systemic corticosteroids and immunosuppressants for lupus nephritis for 7 months. She had intractable fluid retention even though she was treated with simultaneous PD and HD, and concentrated administration of immunosuppressants was ineffective. There was a risk of infection while undergoing both dialyzes; however, immunosuppressive drugs had to be used to control the SLE activity. Immunosuppressants are not the only cause of the increased risk of infection. Several factors could contribute to the increased risk of infections in patients with SLE [13]. Doria et al. [13] insisted that there are factors that increase the risk of infections, such as organ injury, immunosuppressant use, and T- and B-cell exhaustion. In our study, our patient underwent HD and PD simultaneously. HD and PD catheters could be risk factors for infections, which could lead to prolonged hospitalization, poor morbidity, and mortality [14]. Besides the aforementioned causes, infections can arise from several other factors. Uremia-induced immune dysfunction in ESRD patients increases their vulnerability to infection. Uremia is associated with impaired immune responses from neutrophils and lymphocytes, complement system dysfunction, and reduced antibody production, which leads to immunosuppression [15]. Diabetes is also a major risk factor for nosocomial infections in HD patients [15].

The patient had several factors which made her vulnerable to infection; renal failure due to SLE activity, long-term use of systemic corticosteroids, simultaneous and longer use of HD and PD catheters, longer hospital stays, and hyperglycemia due to long-term steroid use. Concerned about those risk of infection,

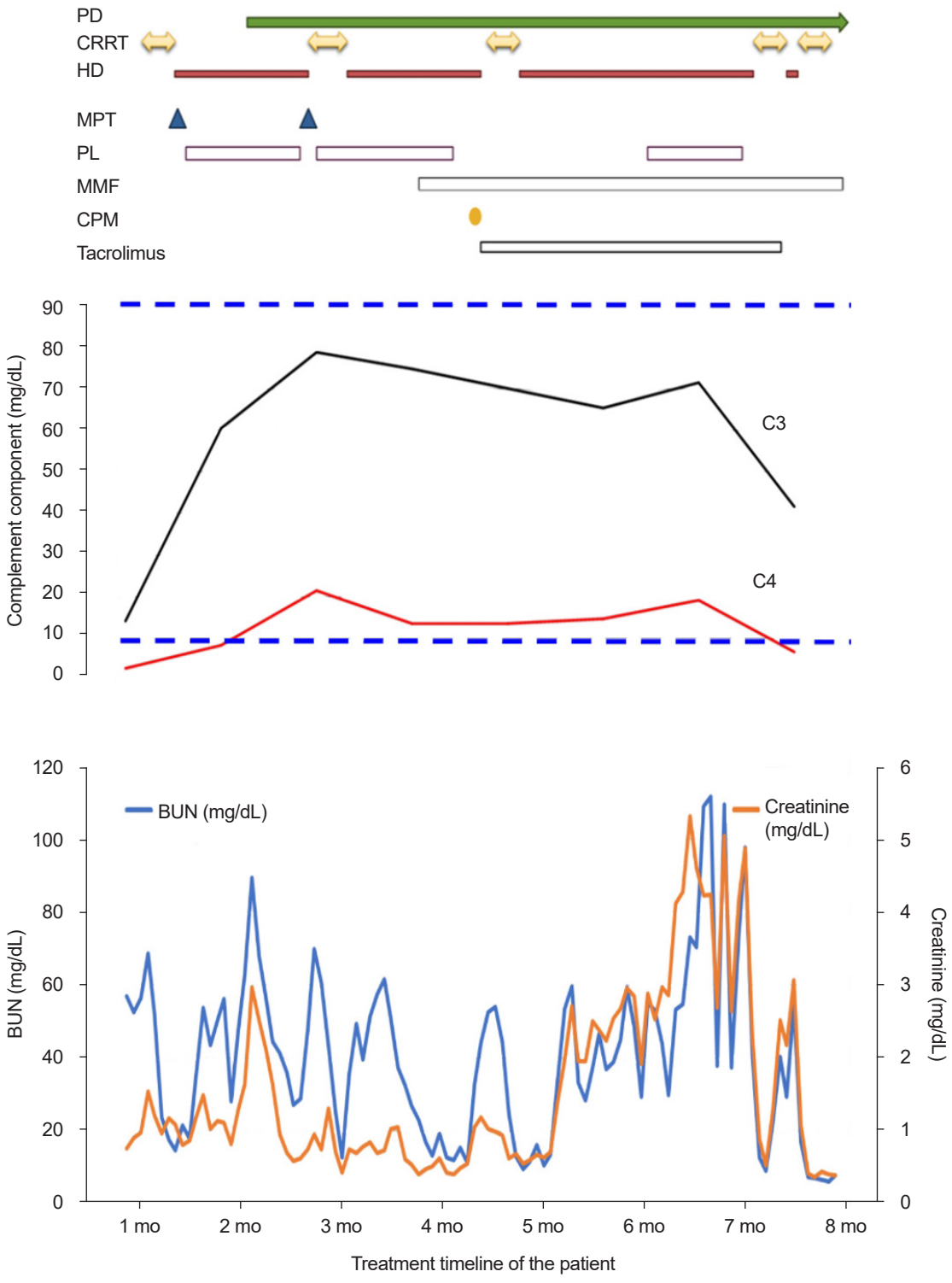

Fig. 3. Trend of blood urea nitrogen (BUN) and creatinine with dialysis and medical treatment. The patient's kidney damage gradually progressed. To resolve the acute kidney injury and fluid retention, she started hemodialysis (HD) and peritoneal dialysis (PD) together and high-dose steroids and immunosuppressants were administered. Despite aggressive treatment for 8 months, she developed a brain abscess and hemorrhage associated with treatment-related infectious side effects. Finally, the patient was unable to tolerate the hemodynamic instability and died. Dotted lines mean lower margin of normal limit. CRRT, continuous renal replacement therapy; MPT, methylprednisolone therapy; PL, prednisolone; MMF, mycophenolate mofetil; CPM, cyclophosphamide.

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we began immunosuppressant after 3 months. Nevertheless, she developed seizures and brain abscesses. She also experienced prolonged thrombocytopenia combined with a high bleeding tendency due to ESRD and uncontrolled DIC, which led to cerebral hemorrhage. In short, she had multiple risk factors for severe infections and more potent treatment-related side effects, such as hematological and neurological complications, than other cSLE patients.

In conclusion, it is essential to recognize that even patients with isolated symptoms, such as ITP, may be exhibiting early manifestations of SLE. When Lupus nephritis is diagnosed, prompt treatment is essential to prevent the progression to ESRD. If resolved as soon as possible, it could prevent treatment-related complications and allow for discontinuation of medications. During treatment, it is important to consider the possibility of complications such as brain abscesses and hemorrhage. Although rare, these complications must always be considered in clinical practice because they increase mortality rates.

Ethical statements

This report was approved by the Institutional Review Board of Severance Children's Hospital (IRB No. 4-2022-1276). Written consent was waived.

Conflicts of interest

Jae Il Shin and Keum Hwa Lee are editorial board members of the journal but were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Conceptualization: JHK, KHL
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Project administration: JHK, JIS, JHK, KHL
Visualization: JHK
Writing-original draft: JHK, KHL
Writing-review & editing: JHK, JIS, JHK, KHL
All authors read and approved the final manuscript.

References

1. Sinha R, Raut S. Pediatric lupus nephritis: management update. World J Nephrol 2014;3:16-23.
2. Fatemi A, Matinfar M, Smiley A. Childhood versus adult-onset systemic lupus erythematosus: long-term outcome and predictors of mortality. Clin Rheumatol 2017;36:343-50.
3. Ambrose N, Morgan TA, Galloway J, Ionnoau Y, Beresford MW, Isenberg DA, et al. Differences in disease phenotype and severity in SLE across age groups. Lupus 2016;25:1542-50.
4. Levy DM, Kamphuis S. Systemic lupus erythematosus in children and adolescents. Pediatr Clin North Am 2012;59:345-64.
5. Bertsias GK, Boumpas DT. Connective tissue diseases: Lupus nephritis-winning a few battles but not the war. Nat Rev Rheumatol 2011;7:441-2.
6. Hanly JG, McCurdy G, Fougere L, Douglas JA, Thompson K. Neuropsychiatric events in systemic lupus erythematosus: attribution and clinical significance. J Rheumatol 2004;31:2156-62.
7. Manger K, Manger B, Repp R, Geisselbrecht M, Geiger A, Pfahlberg A, et al. Definition of risk factors for death, end stage renal disease, and thromboembolic events in a monocentric cohort of 338 patients with systemic lupus erythematosus. Ann Rheum Dis 2002;61:1065-70.
8. Kittivisuit S, Vachvanichsanong P, McNeil E, Chotsampancharoen T. Childhood-onset systemic lupus erythematosus and immune thrombocytopenia: prevalence and risk factors. Pediatr Blood Cancer 2021;68:e29146.
9. Bennett M, Brunner HI. Biomarkers and updates on pediatrics lupus nephritis. Rheum Dis Clin North Am 2013;39:833-53.
10. Pereira T, Abitbol CL, Seeherunvong W, Katsoufis C, Chandar J, Freundlich M, et al. Three decades of progress in treating childhood-onset lupus nephritis. Clin J Am Soc Nephrol 2011;6:2192-9.
11. Hiraki LT, Lu B, Alexander SR, Shaykevich T, Alarcon GS, Solomon DH, et al. End-stage renal disease due to lupus nephritis among children in the US, 1995-2006. Arthritis Rheum 2011;63:1988-97.
12. Hersh AO, von Scheven E, Yazdany J, Panopalis P, Trupin L, Julian L, et al. Differences in long-term disease activity and treatment of adult patients with childhood- and adult-onset systemic lupus erythematosus. Arthritis Rheum 2009;61:13-20.
13. Doria A, Arienti S, Rampudda M, Canova M, Tonon M, Sarzi-Puttini P. Preventive strategies in systemic lupus erythematosus. Autoimmun Rev 2008;7:192-7.

14. Poinen K, Quinn RR, Clarke A, Ravani P, Hiremath S, Miller LM, et al. Complications from tunneled hemodialysis catheters: a Canadian observational cohort study. Am J Kidney Dis 2019;73:467-75.
15. Abbasi SH, Aftab RA, Chua SS. Risk factors associated with nosocomial infections among end stage renal disease patients undergoing hemodialysis: a systematic review. PLoS One 2020;15:e0234376.

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ChiKD CHILDHOOD KIDNEY DISEASES

Instructions For Authors

Revised 2023. 02. 20

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tables, and figures should not exceed 5,000 words. The number of references is limited to 100.

3) **Mini-reviews:** Mini-reviews provide a concise review or critical summary for a specific topic related to pediatric nephrology. Most mini-review articles are invited by the editors; however, unsolicited submissions may also be considered for publication. Mini-review articles are accepted after peer review. They should have the following format: title page, unstructured abstract of no more than 200 words and keywords, main text (introduction, body text, conclusion), references, tables, figures, and figure legends. The length of the text excluding references, tables, and figures should not exceed 3,000 words. A maximum of 2 tables or 2 figures is allowed. The number of references is limited to 50.
4) **Original articles:** These are papers containing the results of clinical or laboratory investigations, which are sufficiently well documented to be acceptable to critical readers. The original articles should be organized in the following order: title page, structured abstract of no more than 250 words and keywords, main text (introduction, methods, results, discussion), references, tables, figures, and figure legends. Maximum length: 4,000 words of text (not including the abstract, tables, figures, and references). A maximum of 6 tables or 6 figures is allowed. The number of references should not exceed 40.
5) **Case reports:** Case reports should be organized in the following order: title page, unstructured abstract of no more than 200 words) and keywords, main text (introduction, case report, discussion), references, tables, figures, and figure legends. The length of the text, excluding references, tables, and figures, should not exceed 2,500 words. A maximum total of 6 tables and figures may be included. The number of references is limited to 15.
6) **Editorials:** Editorials should be commentaries on articles published recently in the journal. Editorial topics could include active areas of research, fresh insights, and debates. The order of the submitted manuscript should include a title page, discussion, conflict of interest, acknowledgments (if applicable) and references. The text should be limited to 1,500 words and 10 references. A maximum total of 2 tables and figures may be included.
7) **Correspondence:** Correspondence (letters to the editor) may be in response to a published article, or a short, free-standing piece expressing an opinion. A brief case report can be published as a letter to the editor. Correspondence should be no longer than 1,000 words of text and 10 references. Letters can be edited by the Editorial Board. Responses by the author of the subject paper may be provided in the same issue or next issue of the journal. Replies by authors should not exceed 500 words of text and 5 references. A maximum total of 2 tables and figures may be included.

Table 1 shows the recommended maximums of manuscripts according to publication type.

Table 1. Recommended maximums for articles submitted to ChiKD

Type of article	Abstract (words)	Text (words)	References	Tables & figures
Review	200	5,000	100	No limits
Mini-review	200	3,000	50	2 Tables, 2 figures
Original article	250	4,000	40	6 Tables, 6 figures
Case report	200	2,500	15	Total 6
Editorial	No	1,500	10	Total 2
Correspondence	No	1,000	10	Total 2
In reply	-	500	5	Total 2

2. General guidelines

- Manuscripts must be written in English. Authors (particularly non-native English speakers) who submit a manuscript should have it checked by a professional editing service prior to submission and must submit proof of English editing. For an extensive revised paper, reviewer or editor can request an English editing certificate again. The editors reserve the right to return a manuscript to the author if the quality of English is poor.
- The manuscript must be submitted in MS Word format (doc or docx).
- The text of the manuscript, including tables and their footnotes and figure legends, must be double-spaced and in standard 12-point font on an A4 size page. All pages should be numbered consecutively starting with the title page.
- Drug and chemical names should be stated in standard chemical or generic nomenclature. For medicine, use generic names. If a brand name should be used, insert it in parentheses after the generic name.
- Units of measure should be presented according to the International System (SI) of units. All units must be preceded by one space except for percentage (%) and degree (°).
- Descriptions of genes or related structures in a manuscript should include the names and official symbols provided by the US National Center for Biotechnology Information

(NCBI) or the HUGO Gene Nomenclature Committee.
- 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 and 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).
- Statistical expression: mean and standard deviation should be described as mean±SD, and mean and standard error as mean±SE. *P*-values should be described as $P<0.05$ or $P=0.003$.

3. Reporting guidelines for specific study designs

For specific study designs, such as randomized controlled studies, studies of diagnostic accuracy, meta-analyses, observational studies, and nonrandomized studies, authors are encouraged to also consult the reporting guidelines relevant to their specific research design. A good source of reporting guidelines is the EQUATOR Network (https://www.equator-network.org/) and the National Library of Medicine (https://www.nlm.nih.gov/services/research_report_guide.html).

FORMAT OF MANUSCRIPTS

We recommend using the template provided at http://www.chikd.org/authors/authors.php to format the manuscript.

1. Title page

The title page should include: (1) the concise and informative title of the article; (2) the full name(s) of the author(s); (3) the institutional affiliation(s) of the author(s); (4) the running title, of 10 words or less; (5) the e-mail address, telephone number of the corresponding author; and (6) notes. If several authors and institutions are listed, it should be made clear with which department and institution each author is affiliated. For a multicenter study, each individual's affiliation should be indicated using a superscript Arabic number 1,2,3.... The corresponding author or first author should be clearly designated. In a separate paragraph, an address for correspondence including the name of the corresponding author address (institutional affiliation, city, zip code, and country), and e-mail address should be given. The running title should not be a declarative or interrogative sentence. Notes (disclaimers) include ethics approval and consent to participate, conflict of interest, funding, authors' contributions, additional contributions, and ORCID of all authors. All contributors who do not meet the criteria for authorship as defined above should be listed in an additional contribution section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

2. Abstract and keywords

1) **Abstract:** Original articles provide a structured abstract of less than 250 words, which should be divided into the following sections:

- **Purpose:** A single sentence describing why the study was done and the type of study carried out. Clinical studies should include the setting (e.g., practice or hospital).
- **Methods:** The total number of species of animals or subjects, with (where relevant) the method of selection. For in vitro studies, specify the cell and tissue used, the assays or assessments carried out, and the statistical tests applied.
- **Results:** The main results obtained, providing means (±SD or SE) or medians (with ranges) and significance levels, where necessary. Clinical data should include any withdrawals.
- **Conclusions:** Implications based on the methods and results presented.

Abbreviations, if needed, should be kept to an absolute minimum, and given with proper identifications.

2) **Keywords:** Authors should provide, and identify as such, up to 5 keywords or short phrases that will assist indexers in cross-indexing the article and can be published with the abstract. Use terms from the Medical Subject Headings (MeSH) list of Index Medicus; if suitable MeSH terms are not yet available for recently introduced terms, present terms may be used. Keywords should be listed in alphabetical order and the first letter of a keyword should be capitalized (e.g., Hematuria; Nephrotic syndrome).

3. Main text

1) **Introduction:** The introduction should contain enough references to the most pertinent papers to inform readers and describe others' relevant findings. It also includes the specific question driving the authors' particular investigation.
2) **Methods:** We endorse the principles articulated in the Declaration of Helsinki and expect that all investigations involving human materials have been performed in accordance with these principles. Animal experiments must be reviewed and approved by an appropriate committee (Institutional Animal Care and Use Committee) for the care and use of animals. Studies involving pathogens requiring a high degree of biosafety should pass the review of a relevant Institutional Biosafety Committee. The approval of the experimental protocol should be described in the Methods section. An explanation of the experimental methods should be concise and sufficient for repetition by other qualified investigators. Procedures that have been published previously should not be described in detail; however, new or significant modifications of previously published procedures need full descriptions. The sources of special chemicals or preparations should be given (i.e., name of company). The methods of statistical analyses and the criteria used to determine statistical significance (i.e., the significance level) should be described. Case reports, case histories, or case descriptions do not contain separate Methods or Results sections.
3) **Results:** This part should be presented logically using text, tables, and illustrations. Excessive textual repetition of table or figure content should be avoided.
4) **Discussion:** The data should be interpreted concisely without repeating materials already presented in the Results section. Speculation is permitted, but it must be supported by the authors' presented data and be well-founded.

4. References

In the text, references should be cited with Arabic numerals in brackets, numbered in the order cited. In the references section, the references should be numbered and listed in order of appearance in the text. Authors are responsible for the accuracy and completeness of their references and correct text citations.

- List all authors up to six in number. If there are more than six authors, list the first six and add "et al." to the last author's name.
- Papers in press may be listed among the references with the journal name and tentative year of publication.
- Unpublished data or personal communications can be listed only with the author's written permission.
- Other types of references not described below should follow the Recommendations of ICMJE (https://www.nlm.nih.gov/bsd/uniform_requirements.html).

Journal article:

1. Jung J, Lee JH, Kim KS, Song SH, Moon DH, Yoon HM, et al. Management strategies for congenital isolated hydronephrosis and the natural course of the disease. Child Kidney Dis 2022;26:1-10.
2. Aier A, Pais P, Raman V. Psychosocial aspects of children with chronic kidney disease and their families. Clin Exp Pediatr 2021 Nov 10 [Epub]. https://doi.org/10.3345/cep.2021.01004

Book or book chapter:

3. Volpe JJ. Neurology of the newborn. 5th ed. Saunders/Elsevier; 2008.
4. Hong CE. Textbook of pediatrics. 9th ed. Korea Textbook Publishing Co.; 2008.
5. Pan ES, Cole FS, Weinttrub PS. Viral infections of the fetus and newborn. In: Taeusch HW, Ballard RA, Gleason CA, editors. Avery's diseases of the newborn. 8th ed. Elsevier Saunders; 2005. p. 495–529.

Website

6. International Committee of Medical Journal Editors. Recommendations for the conduct, reporting, editing, and publication of scholarly work in medical journals [Internet]. International Committee of Medical Journal Editors; 2021 [cited 2022 Jan 10]. Available from: http://www.icmje.org/recommendations/

5. Table(s)

Tables should be typed double-spaced on separate pages within the manuscript, and they should be titled and numbered in Arabic numerals in the order of their first citation in the text. Each column should be given a short heading. Only the first letter of the first word in each row and column should be capital letters. If numerical measurements are given, the unit of measurement should be included in each heading. The sta-

tistical significance of observed differences in the data should be indicated by the appropriate statistical analysis. All abbreviations should be defined in footnotes. For special remarks, superscripts a), b), c)... should be used. No more than 6 tables are needed. Tables should follow the references on separate pages.

6. Figure(s)

The author is responsible for submitting prints that are of sufficient quality to permit accurate reproduction, and for approving the final color galley proof. ChiKD assumes no responsibility for the quality of the photography as it appears in the journal. Symbols, arrows, or letters used in photographs should contrast with the background. A legend for each light microscopic photograph should include the name of the stain and magnification (i.e., H&E, ×400); electron microscopic photography should have an internal scale marker. All kinds of figures may be reduced, enlarged, or trimmed for publication by the editor. No more than 6 figures are needed. All legends for figures should be double-spaced. Figure legends should follow tables on separate pages. Do not use a separate sheet for each legend. Figure legends should describe briefly the data shown and explain any abbreviations or reference points in the photograph. The figures should be numbered in the form Fig. 1, Fig. 2, and Fig. 3. Related figures should be combined into one figure, with each subfigure denoted by the letters, A, B, C, and so on, following the Arabic number of the main figure (i.e., Fig. 1A; Fig. 1B, C; Fig. 1A–C). Figures should be submitted in the TIFF or EPS file formats. If the only possible file format is JPEG, it must be in the highest quality with minimum compression. It is recommended to size original figure widths to 4 inches wide. The minimum requirements for digital resolution are:

- 900 DPI/PPI for black and white images, such as line drawings or graphs.
- 300 DPI/PPI for picture-only photographs.
- 600 DPI/PPI for photographs containing pictures and line elements, i.e., text labels, thin lines, arrows.

MANUSCRIPT PROCESSING AFTER ACCEPTANCE

1. Final version

After a paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of authors should be double-checked, and if the originally submitted image files were of poor resolution, higher resolution image files should be submitted at this time. TIFF and PDF formats are preferred for the submission of digital files of photographic images. Files containing figures must be named according to the figure number (ex: Fig. 1. tiff). Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible on reduction to the journal's column widths. All symbols must be defined in the figure caption. If references, tables, or figures are moved, added, or deleted during the revision process, they should be renumbered to reflect such changes so that all tables, references, and figures are cited in numeric order.

2. Manuscript corrections

Before publication, the manuscript editor will correct the manuscript such that it meets the standard publication format. The author(s) must respond within 2 working days when the manuscript editor contacts the author for revisions. If the response is delayed, the manuscript's publication may be postponed to the next issue.

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4. Confirmation of acceptance

Once the manuscript is at the publisher, confirmation of acceptance by ChiKD will be issued. Upon registering for the board exams, a receipt of confirmation can be ordered for an accepted manuscript.

5. Post-publication discussions

Post-publication discussions can be held through letters to the editor. If any readers have concerns about any articles published, they can submit a letter to the editor related to the arti-

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Questions regarding manuscript submission may be sent to:

Editorial Office
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Checklist

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