Albuminuria in Pediatric Neurogenic Bladder: Identifying an Earlier Marker of Renal Disease

Rachel O. Millner, Janae Preece, Ann Salvator, Daryl J. McLeod, Christina B. Ching, and Brian Becknell

OBJECTIVE To investigate the role of albuminuria as a clinical marker of early renal disease in children with neurogenic bladder (NGB) in association with commonly used predictors of renal risk.

METHODS Catheterized urine was obtained from 40 patients with NGB at a tertiary pediatric hospital. Albumin-to-creatinine ratio (ACR) was analyzed for associations with estimated glomerular filtration rate, vesicoureteral reflux, hydronephrosis, bladder dynamics, and renal scarring.

RESULTS About 32% (13/40) of NGB patients had elevated ACR (≥30 mg/g. Elevated ACR was associated with Caucasian race, clean intermittent catheterization, hydronephrosis, and vesicoureteral reflux on univariate analysis. In multivariable analysis, presence of vesicoureteral reflux and use of anticholinergic medication were significant predictors of ACR elevation.

CONCLUSION Albuminuria is an established clinical predictor of renal disease and risk for progression to renal failure. The presence of albuminuria in NGB patients with urinary tract abnormalities suggests these patients may be at increased risk for progressive renal disease. This supports the clinical utility of adding ACR to the evaluation of renal risk in pediatric NGB. UROLOGY 133: 199–203, 2019. © 2019 Elsevier Inc.

Neurogenic bladder (NGB) is caused by abnormal innervation to the bladder due to a congenital or acquired neurologic lesion. Neural tube defects, such as myelomeningocele (MMC), are the most common pediatric cause of NGB.[1] Patients with NGB experience kidney injury due to impaired bladder storage and emptying, which leads to increased intravesical pressure, secondary vesicoureteral reflux (VUR), hydronephrosis (HN), and increased susceptibility to urinary tract infection. Consequently, patients with NGB experience greater risk of chronic kidney disease (CKD) and end-stage renal disease than the general population.[2,3] Renal failure is a leading cause of morbidity and mortality in adult and pediatric patients with MMC,[4,5] with up to 30% of MMC children having renal scarring, poor renal growth, or reduced renal function.[5]

Due to the increasing risk of renal dysfunction with age, medical, and surgical management of pediatric NGB primarily focuses on preservation of renal function.[6] Current screening modalities consist of imaging tests, such as voiding cystourethrogram and renal ultrasound (RUS); and bladder function tests, such as urodynamic study (UDS). UDS can be imprecise and difficult to interpret in the pediatric population.[7-9] RUS is capable of identifying upper urinary tract abnormalities, but nonspecific for bladder dysfunction or cause of HN. Moreover, the available methods to determine the need for intervention and potential risk of developing renal damage are currently limited. Renal scarring and elevated serum creatinine are late markers of disease and are often irreversible at the time of discovery. Noninvasive, early markers of renal disease would allow for increased surveillance and earlier intervention to preserve long-term renal function.

Elevated urinary albumin-to-creatinine ratio (ACR >30 mg/g) is an established marker of glomerular injury in patients with CKD,[10-12] but its role in the pediatric population of patients with NGB is unknown. We therefore performed a pilot study to evaluate ACR in children with NGB. We hypothesized that elevated ACR would

Financial Disclosure: *Funding for this study was provided by the Intramural Funding of Nationwide Children's Hospital Center for Clinical and Translational Research (R.O.M). CBC is supported by K08-DK122119 from the US National Institutes of Health (NIH). BB is supported by K08-DK102594 from NIH and a Norman Siegel Research Scholar Grant from the ASN Foundation for Kidney Research. Urinary biomarker analysis was completed by the Biomarker Lab at Cincinnati Children's Research Foundation, directed by Michael Bennett, Ph.D.*

From the Nephrology and Urology Research Affinity Group, The Research Institute at Nationwide Children's Hospital, Columbus, OH; the Nephrology Division, Nationwide Children's Hospital, Columbus, OH; the Division of Pediatric Urology, Children's Hospital of Michigan, Detroit, MI; the Biostatistics Resources at NCH, Columbus, OH, USA; the Division of Pediatric Urology, Department of Surgery, Nationwide Children's Hospital, Columbus, OH; the Center for Surgical Outcomes Research and Center for Innovation in Pediatric Practice, The Research Institute at Nationwide Children's Hospital, Columbus, OH; and the Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH

Address correspondence to: Brian Becknell, M.D., Ph.D. and Christina B. Ching, M.D., Center for Clinical and Translational Research, The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205. E-mail: brian.becknell2@nationwidechildrens.org

Submitted: May 2, 2019, accepted (with revisions): August 12, 2019

associate with clinical findings known to increase the risk of renal disease, such as structural renal abnormalities (scarring, HN, or VUR), abnormal bladder dynamics, and alterations in glomerular filtration rate (GFR).

METHODS

Subject recruitment: This study was approved by the Institutional Review Board of Nationwide Children's Hospital (NCH), and patients/families were recruited with informed consent and assent.

The study group consisted of children with NGB 3-21 years of age undergoing UDS or RUS at a tertiary care pediatric hospital. Samples were collected from 12/2015 to 1/2018. Patients were recruited at the time of UDS for a diagnosis of NGB of any cause. Additional patients were recruited at the time of RUS obtained for routine monitoring in a multispecialty MMC Clinic. All children with NGB were catheterization-dependent. Urine samples were collected by catheterization at time of UDS or RUS. Patients were *excluded* if they required chronic dialysis, had a renal transplant, bladder augmentation, or urinary tract infection; or if cystatin C, UDS, or RUS were not performed within a year of urine collection. Urine was collected as a random sample in a sterile container and then processed as discussed below. Information on medications, renal function testing, and results of most recent imaging studies and UDS was collected via review of electronic medical record.

One hundred and forty-seven patients were approached to participate in this study. Thirty-two patients were removed from analysis due to age >21 years or <3 years. Forty-two were removed due to lack of cystatin C measurement. Sixteen were removed due to incomplete data (no RUS, VUDS, voiding cystourethrogram, or cystatin C within 1 year of urine collection). Thirteen patients were removed due to augmented bladder. One patient was removed due to no radiographic evidence of underlying neurologic lesion. Three samples were removed from final analysis due to undetectable urine creatinine levels, as this prevented the calculation of ACR.

Urine processing and analysis for ACR: Urine for albumin measurement was placed in Assay Assure medium (Sierra Molecular Corporation, Incline Village, NV) prior to processing. Samples were centrifuged at 4°C at 750 *g* for 8 minutes, and supernatant was centrifuged again at 3000 rpm for 5 minutes to remove cellular debris. The supernatant was aliquoted and stored at −80°C until analysis. Urine albumin and creatinine was measured by the Cincinnati Children's Hospital Biomarker Laboratory.

Analysis of clinical parameters within the NGB cohort: Estimated GFR was calculated using the Chronic Kidney Disease in Children Study Cystatin C-based GFR equation $(70.69 \times (\text{cysC})^{-0.931})$ for children ≤18 years old and CKD-EPI cystatin C equation $(133 \times \min(\text{cysC}/0.8,1)^{-0.499} \times \max(\text{cysC}/0.8,1)^{-1.328} \times 0.996^{\text{Age}}$ [if female] × 0.932) for patients >18 years old.[13-16] These Cystatin C only equations were used due to more accurate estimate of GFR independent of height and muscle mass.[13]

Urodynamic results were classified as Normal, Safe, Intermediate, or Hostile using parameters defined by the Center for Disease Control Urologic and Renal Protocol for Newborn and Young Child with Spina Bifida[17] as interpreted by the clinical urologist at our institution. RUSs were reviewed for presence and grade of HN and renal scarring as interpreted by the clinical radiologist.

Statistical analysis: Univariate associations between independent variables and the outcome measures (ACR and GFR) were assessed using Mann-Whitney tests for categorical data and Spearman correlations for continuous data. Separate regression analysis was assessed for the 2 outcome measures. Stepwise forward regression was applied to select independent variables that were used to generate the final model. At each step, Akaike's information criterion was calculated to decide whether the variable with the smallest effect should be dropped from the model. ACR was log transformed due to skewness of the data. All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC). Significance was determined by a P value of <.05.

RESULTS

Our study population consisted of 40 children with NGB, mostly as a consequence of MMC (Table 1). A minority of patients had acquired or other congenital causes of NGB with a confirmed CNS lesion. Six patients (15%) had mild to moderate renal insufficiency (GFR 30-90 mL/min/1.73 m^2). None had severe renal insufficiency (GFR <30 mL/min/1.73 m^2). Six patients (15%) had any element of HN: 2 with bilateral Society for Fetal Urology (SFU) grade 3, 2 with unilateral SFU grade 2; 1 with bilateral SFU grade 1; and 1 with unilateral SFU grade 1. Three patients (7%) had both HN and VUR.

Among the study subjects, 11 (27%) had moderately increased ACR (30-300 mg/g) and 2 (5%) had severely increased ACR (>300 mg/g). None of the study participants had been prescribed renin-angiotensin system (RAS) blockade (ie, angiotensin converting enzyme inhibitor or angiotensin AT(1)-receptor antagonist). Of the NGB patients with moderate to severely increased ACR, only 3 had reduced GFR (43-87 mL/min/1.73 m^2), and 2 had increased GFR (>120 mL/min/1.73 m^2).

We next sought to identify associations between demographic factors, known markers of renal risk in NGB patients, and alterations in ACR. ACR was significantly associated with HN, active VUR, MMC, Caucasian race, and requirement for clean, intermittent catheterization (CIC) on univariate analysis (Table 2). The association of ACR with VUR and MMC remained significant in the multivariable model but the association with HN, CIC, and Caucasian race was lost (Table 3). On multivariable regression analysis, increased ACR was associated with the presence of VUR and use of anticholinergic medication (ACH). MMC diagnosis was independently associated with decreased ACR compared to other causes of NGB (Table 3). We did evaluate for the interaction of presence of active VUR and HN on determining ACR, but did not find this was significant in either univariate or multivariate analysis. There were only 3 subjects to which this applied and thus the power to detect such an interaction may help explain our findings.

We also evaluated associations between demographic and clinical predictor variables and GFR in NGB patients. In this analysis, only HN was found to be independently predictive of decreased GFR (Tables 2 and 4).

DISCUSSION

This pilot study is provocative in that we found that a third of NGB patients being managed at our institution had an abnormal ACR. Additionally, NGB patients with elevated ACR were more likely to have HN and VUR on univariate analysis. The association between elevated ACR and VUR was preserved on multivariable analysis. HN and VUR are frequently used as clinical markers to

Table 1. Subject demographic data

	Study Population
Sample size (*n*)	40
Age (y)	
Mean	10.8
Median (IQR)	8 (5.8, 13.4)
Sex (%)	
Male	9 (22)
Female	31 (78)
Cause of NGB	
Myelodysplasia	28
Tethered cord	4
Spinal cord injury	3
Cerebral palsy	2
Transverse myelitis	2
Sacrococcygeal teratoma	1
Urologic surgery (%)	
Mitrofanoff	4 (10)
Ureteral reimplantation (bilateral)	3 (8)
Intradetrusor botulinum toxin	2 (5)
Sacral nerve neuromodulation	1 (3)
CKD Stage (GFR = mL/min/1.73m^2) (%)	
1 Normal GFR (GFR ≥90)	34 (85)
2 Mild (GFR 60-89)	5 (13)
3 Moderate (GFR 30-59)	1 (2)
CIC (%)	
+	36 (90)
−	4 (10)
Hydronephrosis (%)	
Present	6 (15)
SFU grade 1 (unilateral)	*1*
SFU grade 1 (bilateral)	*1*
SFU grade 2 (unilateral)	*2*
SFU grade 3 (bilateral)	*2*
Absent	34 (85)
Video urodynamic study (VUDS) (%)	
Safe	19 (47)
Intermediate	14 (35)
Hostile	7 (18)
Renal scarring (%)	
Present	2 (5)
Absent	38 (95)
Vesicoureteral reflux (%)	
Present	11 (28)
Grade 1	*4*
Grade 2	*2*
Grade 3	*5*
Absent	29 (72)
Anticholinergic use (%)	
Yes	13 (32)
No	27 (68)

assess for risk of renal deterioration. Presence of HN triggers further clinical investigation or even prompts a change in management.

Our data also suggest that presence of HN is significantly associated with reduced GFR, which has not been previously described in patients with NGB. These findings suggest that despite normal GFR in the majority of pediatric NGB patients, they may be at elevated risk for progressive renal disease based on increased ACR. Thus measuring GFR alone may provide a false sense of security, and addition of routine albuminuria screening may prove useful in early identification of patients with unrecognized CKD. Early recognition of CKD allows for prompt evaluation and treatment of sequelae of CKD such as hypertension, anemia, bone disease, and growth failure.

The overall prevalence of end-stage renal disease due to NGB in childhood has been reduced by modern advances in medical and surgical management, as well as institution of CIC.[18,19] CIC was associated with increased ACR on univariate analysis, and multivariable analysis identified an association between ACH use and ACR. Small patient numbers limit the interpretation of these findings in this pilot study. CIC and ACH are often prescribed in NGB patients in an effort to limit renal and bladder injury, and we speculate that increased ACR is an early reflection of occult renal injury in such patients.

Renal failure should be preventable in children with NGB with aggressive bladder management and detection of early CKD. Standard, creatinine-based pediatric GFR equations overestimate true renal function in nonambulatory children due to their low muscle mass and atypical anthropometric measurements.[16,20] To combat this issue in our study, we use an estimated GFR calculation using cystatin C alone. Stage 1 CKD is defined in standard practice guidelines as a normal GFR with any structural abnormality of the urinary tract, known renal insult, or abnormality of the urinary sediment.[12] Based on this definition, 40% of children in our cohort would classify as stage 1 CKD based on the presence of albuminuria and/or structural urinary tract abnormality. Any degree of CKD places patients at risk for progression of renal insufficiency, particularly in the presence of albuminuria.[21] Albuminuria is often present in the setting of increased or normal GFR before apparent reductions in GFR, making the ACR an ideal screening tool for identification of occult renal injury.

Albuminuria is an established prognostic indicator for progression of CKD, cardiovascular, and all-cause mortality in patients with CKD.[21] The etiology of albuminuria in NGB patients likely varies. Potential causes include glomerular hyperfiltration as a consequence of reduced nephron mass; direct glomerular injury; or proximal tubular injury.[22] Patients with NGB may have reduced nephron mass as a consequence of acquired renal scars due to pyelonephritis or high-grade VUR, which increases their risk for glomerular hyperfiltration and secondary focal and segmental glomerulosclerosis.[23]

Our study adds to the current literature by specifically analyzing the relationship between albuminuria and commonly used clinical studies in patients with NGB. We found that ACR was elevated in NGB patients, despite the majority showing normal GFR. Thus elevated ACR may predict patients with occult renal damage before apparent reduction in GFR and identify children at higher risk for progression of CKD. RAS blockade is known to reduce albumin excretion in the urine and is recommended in the treatment of albuminuria.[24] Current guidelines suggest checking urinary ACR yearly in all patients with CKD and recommend treatment with RAS blockade

Table 2. Univariate associations of ACR and GFR with NGB patient characteristics

	GFR mL/min/1.73^2: Median (IQR)	*P* Value	ACR mg/g: Median (IQR)	*P* Value
Male (*n* = 9)	113.74 (98.53, 134.78)		22.07 (19.61, 114.85)	
Female (*n* = 31)	98.53 (98.53, 113.74)	.37	19.62 (9.81, 37.45)	.16
Caucasian (*n* = 32)	98.54 (98.54, 113.74)		**23.29 (16.42, 67.65)**	
Other Race (*n* = 8)	113.74 (98.54, 124.26)	.36	**9.06 (7.99, 18.75)**	**.003**
MMC (*n* = 28)	113.74 (98.54, 113.74)	.19	19.58 (10.71, 28.92)	.05
Other NGB (*n* = 12)	98.54 (87.02, 133.39)		**34.85 (18.55, 139.50)**	
HN + (*n* = 6)	**92.77 (87.02, 98.54)**		**45.17 (32.26, 172.86)**	
HN − (*n* = 34)	**113.74 (98.54, 113.74)**	**.004**	**19.61 (11.98, 29.66)**	**.02**
Scarring + (*n* = 38)	106.14 (98.54, 113.74)		20.28 (12.65, 38.06)	
Scarring − (*n* = 2)	106.14 (98.54, 113.74)	.94	15.05 (9.82, 20.28)	.46
VUDS				
Safe (*n* = 19)	98.54 (98.54, 113.74)		20.77 (15.36, 38.06)	
Intermediate (*n* = 14)	113.74 (98.54, 113.74)		18.97 (9.82, 28.19)	
Hostile (*n* = 7)	98.54 (87.02, 134.78)	.70	22.92 (15.21, 139.53)	.56
VUR				
Present (*n* = 11)	98.54 (98.54, 113.74)	.67	**38.06 (19.63, 172.86)**	**.02**
Absent (*n* = 29)	113.74 (98.54, 113.74)		**19.36 (11.98, 28.19)**	
ACH + (*n* = 26)	113.74 (98.54, 113.74)	.11	21.42 (15.21, 114.85)	.11
ACH − (*n* = 14)	98.54 (87.01, 113.74)		18.32 (9.82, 22.92)	
CIC + (*n* = 36)	113.74 (98.54, 113.74)	.26	**21.42 (15.29, 46.85)**	**.01**
CIC − (*n* = 4)	98.54 (92.78, 106.14)		**8.66 (6.05, 14.24)**	

ACH, anticholinergic use.
Bolded values indicate statistical significance ($p < 0.05$).

Table 3. Regression analysis of ACR

Variable	Univariate Coeff. (95% CI)	*P* Value	Multivariable Adjusted Coeff. (95% CI)	*P* Value
Male	0.19 (−0.19, 0.58)	.30	0.29 (−0.02, 0.59)	.06
Caucasian	0.47 (0.09, 0.85)	**.01**		
MMC	−0.42 (−0.75, −0.09)	**.01**	−0.47 (−0.75, −0.19)	**.001**
ACH	0.30 (−0.03, 0.63)	.07	0.31 (0.04, 0.59)	**.02**
CIC	0.55 (0.04, 1.07)	**.03**		
Hydro	0.49 (0.06, 0.92)	**.03**		
VUDS	0.03 (−0.19, 0.25)	.79		
VUR	0.48 (0.14, 0.81)	**.006**	0.45 (0.17, 0.74)	**.003**
Hydro*VUR	0.33 (−0.49, 1.17)	.42		

Bolded values indicate statistical significance ($p < 0.05$).

Table 4. Regression analysis of GFR

Variable	Univariate Coeff. (95% CI)	*P* Value	Multivariable Adjusted Coeff. (95% CI)	*P* Value
Male	7.77 (−6.96, 22.51)	.29		
Caucasian	−7.39 (−22.82, 8.03)	.34		
MMC	8.12 (−5.24, 21.49)	.23		
ACH	5.95 (−7.00, 18.89)	.36		
CIC	8.80 (−11.81, 29.43)	.39		
Hydro	−25.79 (−41.09, −10.48)	**.002**	−25.79 (−41.09, −10.48)	**.002**
VUDS	−3.16 (−11.44, 5.12)	.44		
VUR	−5.78 (−19.64, 8.08)	.40		
Hydro*VUR	−11.58 (−43.74, 20.57)	.47		

Bolded values indicate statistical significance ($p < 0.05$).

if ACR is >300 mg/g, or if >30 mg/g in the setting of hypertension.[10] Our study suggests that certain pediatric patients with NGB may benefit from RAS initiation to reduce ACR and prevent CKD progression. Additionally, we identified significant associations between reduced GFR and presence of HN, as well as the presence of VUR and increased albuminuria. Further studies are warranted to evaluate the predictive value of these and other radiologic findings in identifying patients with reduced GFR, albuminuria, and progressive CKD in the NGB population.

Our study is limited by a small sample size and its cross sectional nature. This limited analysis stratified by grade of HN and VUR. Ideally, we would have compared varying SFU grade HN and high-grade vs low-grade VUR. We also acknowledge the poor sensitivity of RUS for renal scarring. As a pilot study, however, we feel the information it lends is important and dictates planning future studies. Prospective data on urine ACR and long term outcomes are needed to truly validate ACR as a marker of renal disease in the NGB population. Additionally, urine

was collected as a random daytime sample and frozen prior to analysis, as opposed to a fresh, first morning collection. However, CKD guidelines do not recommend a timed morning collection to screen patients for albuminuria, making our method generalizable to the typical clinical collection. In further prospective studies, first morning spot urine sampling or 24 hour urine collections could further validate our findings. Furthermore, studies assessing the effect of freezing and thawing urine samples for albumin measurement actually show that albumin may be *under*estimated in frozen samples,[25] further strengthening our results and even implying our results may be an underestimation of albuminuria in children with NGB. Additionally, patients with surgically augmented bladders were excluded to avoid confounding factors in this study, but should be evaluated in the future given their higher risk for renal disease.

CONCLUSION

In this pilot study, elevated levels of urinary albumin were identified in a group of pediatric patients with NGB despite the majority having normal estimated GFR. This suggests that occult renal damage may occur in some patients and may provide an opportunity to identify individuals who may benefit from early RAS initiation or closer clinical surveillance. HN and VUR were associated with increased ACR, strengthening their association with risk for renal deterioration. Additionally, HN correlated with lower GFR in children with NGB, suggesting a combination of albuminuria and radiographic measures may more accurately risk stratify these patients

References

1. Verpoorten C, Buyse GM. The neurogenic bladder: medical treatment. *Pediatr Nephrol*. 2008;23:717–725.
2. Lawrenson R, Wyndaele JJ, Vlachonikolis I, Farmer C, Glickman S. Renal failure in patients with neurogenic lower urinary tract dysfunction. *Neuroepidemiology*. 2001;20:138–143.
3. Sung BM, Oh DJ, Choi MH, Choi HM. Chronic kidney disease in neurogenic bladder. *Nephrology*. 2018;23:231–236.
4. Singhal B, Mathew KM. Factors affecting mortality and morbidity in adult spina bifida. *Eur J Pediatr Surg*. 1999;9(Suppl 1):31–32.
5. Muller T, Arbeiter K, Aufricht C. Renal function in meningomyelocele: risk factors, chronic renal failure, renal replacement therapy and transplantation. *Curr Opin Urol*. 2002;12:479–484.
6. Lewis MA, Webb NJ, Stellman-Ward GR, Bannister CM. Investigative techniques and renal parenchymal damage in children with spina bifida. *Eur J Pediatr Surg*. 1994;4(Suppl 1):29–31.
7. Dudley AG, Adams MC, Brock 3rd JW, et al. Interrater reliability in interpretation of neuropathic pediatric urodynamic tracings: an expanded multicenter study. *J Urol*. 2018;199:1337–1343.
8. Dudley AG, Casella DP, Lauderdale CJ, et al. Interrater reliability in pediatric urodynamic tracings: a pilot study. *J Urol*. 2017;197(3 Pt 2):865–870.
9. Sullivan J, Lewis P, Howell S, Williams T, Shepherd AM, Abrams P. Quality control in urodynamics: a review of urodynamic traces from one centre. *BJU Int*. 2003;91:201–207.
10. Chapter 3: management of progression and complications of CKD. *Kidney Int Suppl*. 2013;3:73–90.
11. Chapter 2: definition, identification, and prediction of CKD progression. *Kidney Int Suppl*. 2013;3:63–72.
12. Chapter 1: definition and classification of CKD. *Kidney Int Suppl*. 2013;3:19–62.
13. Fox JA, Dudley AG, Bates C, Cannon Jr. GM. Cystatin C as a marker of early renal insufficiency in children with congenital neuropathic bladder. *J Urol*. 2014;191(5 Suppl):1602–1607.
14. Schwartz GJ, Schneider MF, Maier PS, et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. *Kidney Int*. 2012;82:445–453.
15. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20–29.
16. Dangle PP, Ayyash O, Kang A, et al. Cystatin C-calculated glomerular filtration rate-a marker of early renal dysfunction in patients with neuropathic bladder. *Urology*. 2017;100:213–217.
17. Jonathan C, Routh EYC. Design and methodological considerations of the centers for disease control and prevention urologic and renal protocol for the newborn and young child with spina bifida. *J Urol*. 2016;196:1728–1734.
18. Filler G, Gharib M, Casier S, Lodige P, Ehrich JH, Dave S. Prevention of chronic kidney disease in spina bifida. *Int Urol Nephrol*. 2012;44:817–827.
19. Oakeshott P, Hunt GM, Poulton A, Reid F. Expectation of life and unexpected death in open spina bifida: a 40-year complete, nonselective, longitudinal cohort study. *Dev Med Child Neurol*. 2010;52:749–753.
20. Zappitelli M, Parvex P, Joseph L, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. *Am J Kidney Dis*. 2006;48:221–230.
21. Peralta CA, Shlipak MG, Judd S, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA*. 2011;305:1545–1552.
22. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens*. 1988;1(4 Pt 1):335–347.
23. Brenner BM. Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int*. 1983;23:647–655.
24. de Jong PE, Curhan GC. Screening, monitoring, and treatment of albuminuria: public health perspectives. *J Am Soc Nephrol*. 2006;17:2120–2126.
25. Brinkman JW, de Zeeuw D, Duker JJ, et al. Falsely low urinary albumin concentrations after prolonged frozen storage of urine samples. *Clin Chem*. 2005;51:2181–2183.