Metabolic acidosis is common and associates with disease progression in children with chronic kidney disease

Jérôme Harambat[1], Kevin Kunzmann[2], Karolis Azukaitis[3,4], Aysun K. Bayazit[5], Nur Canpolat[6], Anke Doyon[3], Ali Duzova[7], Anna Niemirska[8], Betul Sözeri[9], Daniela Thurn-Valsassina[10,11], Ali Anarat[5], Lucie Bessenay[12], Cengiz Candan[13], Amira Peco-Antic[14], Alev Yilmaz[15], Sibylle Tschumi[16], Sara Testa[17], Augustina Jankauskiene[4], Hakan Erdogan[18], Alejandra Rosales[19], Harika Alpay[20], Francesca Lugani[21], Klaus Arbeiter[11], Francesca Mencarelli[22], Aysel Kiyak[23], Osman Dönmez[24], Dorota Drozdz[25], Anette Melk[10], Uwe Querfeld[26] and Franz Schaefer[3]; for the 4C Study Consortium

[1]Pediatric Nephrology Unit, Department of Pediatrics, Bordeaux University Hospital, and Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, team LEHA, UMR 1219, Bordeaux, France; [2]Institute for Medical Biometry and Informatics, Heidelberg University Hospital, Heidelberg, Germany; [3]Division of Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, Heidelberg University Hospital, Heidelberg, Germany; [4]Pediatric Center, Vilnius University, Vilnius, Lithuania; [5]Division of Pediatric Nephrology, Cukurova University Faculty of Medicine, Adana, Turkey; [6]Department of Pediatric Nephrology, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; [7]Division of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey; [8]Department of Nephrology, Kidney Transplantation and Hypertension, The Children's Memorial Health Institute, Warsaw, Poland; [9]Division of Pediatric Nephrology, Ege University Faculty of Medicine, Izmir, Turkey; [10]Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany; [11]Division of Pediatric Nephrology and Gastroenterology, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; [12]Department of Pediatrics, University Hospital Estaing, Clermont Ferrand, France; [13]Division of Pediatric Nephrology, Istanbul Medeniyet University, Göztepe Hospital, Istanbul, Turkey; [14]Department of Nephrology, University Children's Hospital, Faculty of Medicine, Belgrade, Serbia; [15]Department of Pediatric Nephrology, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkey; [16]Inselspital, University of Berne, Berne, Switzerland; [17]Department of Pediatric Nephrology, Fondazione IRCCS Ca' Granda Osp Maggiore Policlinico, Milano, Italy; [18]Bursa Yuksek Ihtisas Teaching and Researching Hospital, Bursa, Turkey; [19]University Children's Hospital, Innsbruck Medical University, Innsbruck, Austria; [20]Department of Pediatric Nephrology, Marmara University Faculty of Medicine, Istanbul, Turkey; [21]Division of Nephrology, Dialysis, Transplantation, University of Genoa, G. Gaslini Institute, Genoa, Italy; [22]Pediatric Nephrology Unit, Department of Pediatrics, S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy; [23]Division of Pediatric Nephrology, Department of Pediatrics, Bakirkoy Children's Hospital, Istanbul, Turkey; [24]Division of Pediatric Nephrology, Uludağ University Faculty of Medicine, Bursa, Turkey; [25]Department of Pediatric Nephrology, Jagiellonian University Medical College, Krakow, Poland; and [26]Department of Pediatric Nephrology, Charité University, Berlin, Germany

Recent studies in adult chronic kidney disease (CKD) suggest that metabolic acidosis is associated with faster decline in estimated glomerular filtration rate (eGFR). Alkali therapies improve the course of kidney disease. Here we investigated the prevalence and determinants of abnormal serum bicarbonate values and whether metabolic acidosis may be deleterious to children with CKD. Associations between follow-up serum bicarbonate levels categorized as under 18, 18 to under 22, and 22 or more mmol/l and CKD outcomes in 704 children in the Cardiovascular Comorbidity in Children with CKD Study, a prospective cohort of pediatric patients with CKD stages 3-5, were studied. The eGFR and serum bicarbonate were measured every six months. At baseline, the median eGFR was 27 ml/min/1.73m^2 and median serum bicarbonate level 21 mmol/l. During a median follow-up of 3.3 years, the prevalence of metabolic acidosis (serum bicarbonate under 22 mmol/l) was 43%, 60%, and 45% in CKD stages 3, 4, and 5, respectively. In multivariable analysis, the presence of metabolic acidosis as a time-varying covariate was significantly associated with log serum parathyroid hormone through the entire follow-up, but no association with longitudinal growth was found. A total of 211 patients reached the composite endpoint (ESRD or 50% decline in eGFR). In a multivariable Cox model, children with time-varying serum bicarbonate under 18 mmol/l had a significantly higher risk of CKD progression compared to those with a serum bicarbonate of 22 or more mmol/l (adjusted hazard ratio 2.44; 95% confidence interval 1.43-4.15). Thus, metabolic acidosis is a common complication in pediatric patients with CKD and may be a risk factor for secondary hyperparathyroidism and kidney disease progression.

Correspondence: *Franz Schaefer, Pediatric Nephrology Division, Center for Pediatrics and Adolescent Medicine, Heidelberg University Hospital, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany. E-mail: franz.schaefer@med.uniheidelberg.de*

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Metabolic acidosis, usually defined as serum bicarbonate <22 mmol/l, is a common complication of chronic kidney disease (CKD), occurring in approximately 20% of adults patients with advanced CKD.[1] It is thought to appear primarily from CKD stages 4 to 5 as a consequence of reduced ability of the kidney to synthesize ammonia, regenerate bicarbonate, and excrete hydrogen ions, and to a lesser extent reabsorb bicarbonate.[2,3] High dietary protein intake resulting in increased net endogenous acid production seems to also be involved in the pathogenesis of metabolic acidosis in CKD.[3] Metabolic acidosis can be observed in earlier stages of CKD, especially in cases of primary renal diseases with tubular function impairment or anatomical damage to the collecting duct such as congenital anomalies of the kidney and urinary tract (CAKUT) or chronic tubulointerstitial nephritis.[3]

The degree of metabolic acidosis is usually mild to moderate in CKD (serum bicarbonate concentration of 15–22 mmol/l). However, animal experiments as well as studies performed in adult patients with CKD suggest that metabolic acidosis may have various deleterious clinical and biological consequences including increased muscle wasting, bone disease, stimulation of inflammation, reduced albumin synthesis, and insulin resistance.[1,3–8] Observational studies have also shown associations between serum bicarbonate and adverse outcomes such as progression to end-stage renal disease (ESRD) and mortality.[9–11] Moreover, 2 randomized clinical trials demonstrated that oral bicarbonate supplementation in acidotic and in nonacidotic adult patients slows the progression of CKD.[12,13]

Indirect evidence derived from rat models and from children with renal tubular acidosis suggests that metabolic acidosis may be a contributory factor of poor growth in children with CKD.[14,15] However, the role of metabolic acidosis in the progression of CKD in children has not been studied to date, and no pediatric longitudinal data have assessed the relationship between low serum bicarbonate and clinical adverse effects in this population.

The aims of this study were to investigate the following: (i) the prevalence and determinants of metabolic acidosis in children with CKD; (ii) its relationship with markers of bone disease, height SD score (HSDS), body mass index, reduced albumin synthesis, and inflammation; and (iii) whether metabolic acidosis is associated with CKD progression in children and adolescents with stage 3 to 5 CKD.

RESULTS

Population characteristics

Data from a cohort of 704 children and adolescents (458 boys) with CKD stages 3 to 5 participating in the Cardiovascular Comorbidity in Children with CKD (4C) Study were analyzed. The patients were enrolled at 55 pediatric nephrology centers in 12 European countries (Turkey 48%, Germany 15%, France 9%, Italy 7%, Poland 6%, UK 5%, Austria 2%, Serbia 2%, Switzerland 2%, Lithuania 1%, Portugal 1%, and Czech Republic 1%). At enrollment, median age was 12.3 (interquartile range [IQR] 9.4–14.9) years, and median estimated glomerular filtration rate (eGFR) was 27 (IQR 20–35) ml/min/1.73 m^2. Underlying renal diseases were CAKUT obstruction (17.8%), CAKUT other (46.3%), cystic kidney diseases (9.8%), glomerulopathies (10.6%), metabolic disorders (4.7%), and other causes (10.8%). Baseline clinical and biochemical characteristics of the population are provided in Table 1. Median follow-up time was 3.3 (1.5–5.0) years.

Prevalence and determinants of metabolic acidosis

Median serum bicarbonate level at baseline was 21.3 mmol/l (IQR 19.0–23.6), and 386 patients (54.8%) were receiving any type of alkali therapy at a median dosage of 54 (IQR 33–88) mg/kg per day bicarbonate equivalent. During follow-up, the estimated average prevalence of metabolic acidosis (<22 mmol/l) according to CKD stage was 43%, 61%, and 45% in CKD stages 3, 4, and 5 (including dialysis), respectively. The probability of having metabolic acidosis increased with CKD stage but decreased significantly with dialysis therapy as compared with predialysis CKD stages (Table 2 and Figure 1). Patients on peritoneal dialysis had a significantly lower likelihood of having metabolic acidosis than those treated with hemodialysis (Table 2). The impact of dialysis on

Table 1 | Population characteristics at baseline (n = 704)

Variable	n (%)	Median (IQR)
Male sex	458 (65.1)	
Age, years		12.3 (9.4–14.9)
Family history of kidney disease	155 (22.0)	
Cause of CKD		
CAKUT other	326 (46.3)	
CAKUT obstruction	125 (17.8)	
Cystic kidney disease	69 (9.8)	
Glomerulopathy	75 (10.6)	
Metabolic disorder	33 (4.7)	
Other or unknown	76 (10.8)	
Any comorbidity	375 (53.3)	
Time since CKD stage 2, years (n = 689)		5.3 (1.9–9.0)
Height, SDS		−1.2 (−2.1 to −0.4)
BMI, SDS		0.2 (−0.6 to 0.9)
Tanner stage (n = 690)		
1	232 (33.0)	
2	154 (21.9)	
3	99 (14.1)	
4-5	205 (29.1)	
rhGH treatment	60 (8.5)	
Systolic blood pressure, SDS		0.7 (−0.1 to 1.5)
Diastolic blood pressure, SDS		0.6 (0.0–1.3)
Furosemide treatment	43 (6.1)	
Biochemical parameters		
eGFR, ml/min/1.73 m^2 (n = 681)		27.0 (20.2–28.8)
Urine albumin-to-creatinine, mg/g (n = 674)		349 (91–1243)
Serum bicarbonate, mmol/l (n = 678)		21.3 (19.0–23.6)
Serum albumin, g/l (n = 681)		39.9 (36.8–42.6)
Intact parathormone, pg/ml (n = 675)		125 (71–213)
C-reactive protein, mg/l (n = 681)		0.6 (0.2–3.7)

BMI, body mass index; CAKUT, congenital anomaly of kidney and urinary tract; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; rhGH, recombinant human growth hormone; SDS, SD score.

Table 2 | Adjusted odds ratios for prevalence of metabolic acidosis according to chronic kidney disease stage and dialysis modality

Variable	Adjusted OR	95% CI	*P* value
CKD stage			<0.001
CKD 3	Reference		
CKD 4	2.95	2.03–4.30	
CKD 5	3.12	1.72–5.67	
Dialysis modality			<0.001
CKD 3–5	Reference		
Hemodialysis	0.51	0.32–0.81	
Peritoneal dialysis	0.18	0.11–0.29	

CI, confidence interval; CKD, chronic kidney disease; OR, odds ratio.

metabolic acidosis prevalence was not explained by increased oral alkali prescription because patients on dialysis tended to receive less alkali supplementation than those with CKD (data not shown).

In multivariable analysis, the main determinants of serum bicarbonate were eGFR (increased serum bicarbonate with higher eGFR), age at study entry (lower bicarbonate with older age), CKD duration at baseline (increased bicarbonate with longer time since CKD stage 2), furosemide use (higher bicarbonate in children receiving furosemide), and cause of CKD (cystic kidney diseases, glomerulopathies, and other causes were associated with higher bicarbonate level compared with CAKUT other) (Table 3). Alkali therapy in mg/kg/d was not associated with change in the estimated mean serum bicarbonate level.

After further adjustment for the country of residence, it appeared that the country was a major determinant of serum bicarbonate (significantly lower bicarbonate level in Turkey than in the rest of Europe). In this model, cause of CKD, age at baseline, CKD duration, and furosemide use were no longer significantly associated with serum bicarbonate (Table 3). Indeed, Turkish children tended to be older, to receive furosemide less frequently, and to have a different disease distribution (less cystic kidney disease and less glomerulopathy).

Association of metabolic acidosis with CKD outcomes

In both the univariable and multivariable analysis adjusted for sex, age at baseline, country of residence, cause of CKD, eGFR, and time, metabolic acidosis was positively and independently associated with log intact parathyroid hormone (iPTH) through the follow-up: $\beta = 0.19$ (95% confidence interval [CI] 0.10–0.28, $P < 0.001$) for serum bicarbonate <18 versus ≥22 mmol/l; and $\beta = 0.13$ (95% CI 0.07–0.18, $P < 0.001$) for serum bicarbonate 18–21 versus ≥22 mmol/l.

There was a trend in the association between acidosis and HSDS in univariable analysis, although it did not reach statistical significance ($P = 0.06$ for severe acidosis with bicarbonate <18 mmol/l; $P = 0.12$ for mild acidosis with bicarbonate 18–21 mmol/l). After adjustment for sex, age at baseline, country of residence, cause of CKD, eGFR, use of recombinant human growth hormone, and time, the presence of metabolic acidosis as a time-varying covariate was negatively but not significantly associated with HSDS ($\beta = -0.05$, $P = 0.18$ for serum bicarbonate <18 vs. ≥22 mmol/l; $\beta = -0.03$, $P = 0.21$ for serum bicarbonate <18 vs. ≥22 mmol/l) through the entire follow-up period.

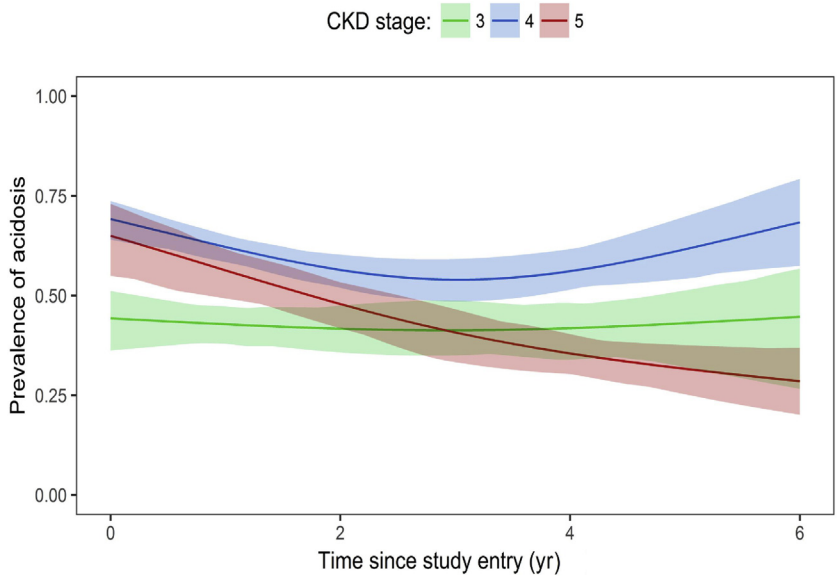

Figure 1 | Prevalence of metabolic acidosis over the follow-up period by time varying chronic kidney disease (CKD) stages. The curves were estimated from a mixed effects model with a nonlinear time effect (cubic spline) and a time-CKD stage interaction; solid lines are estimated prevalences and shaded areas indicate model-based pointwise 95% confidence intervals for the estimated prevalence. The decreasing prevalence over time in CKD stage 5 is due to the fact that this CKD stage includes both patients on conservative treatment and on dialysis which is associated with a reduced likelihood of metabolic acidosis (Table 2).

Table 3 | Factors associated with serum bicarbonate level: results of linear mixed models

Variable	Model 1[a]			Model 2[b]		
	Estimate	95% CI	*P* value	Estimate	95% CI	*P* value
Intercept	20.51	19.53–21.49	<0.001	21.55	20.62–22.47	<0.001
Sex (female vs. male)	−0.24	−0.65 to 0.17	0.25	−0.10	−0.48 to 0.27	0.59
Age at baseline (per year)	−0.09	−0.18 to −0.01	0.04	−0.02	−0.10 to 0.06	0.19
Tanner stage (≥3 vs.1–2)	0.26	−0.31 to 0.83	0.36	0.01	−0.51 to 0.53	0.98
Cause of CKD						
CAKUT other	Reference			Reference		
CAKUT obstruction	0.11	−0.40 to 0.62	0.66	0.18	−0.29 to 0.64	0.46
Cystic kidney disease	0.89	0.24–1.54	<0.01	0.37	−0.23 to 0.98	0.22
Glomerulopathy	0.76	0.05–1.47	0.03	0.31	−0.35 to 0.96	0.36
Metabolic disorder	0.56	−0.34 to 1.46	0.22	0.58	−0.24 to 1.41	0.17
Other	0.72	0.07–1.38	0.03	0.68	0.09–1.28	0.02
eGFR (per 10 ml/min/1.73 m^2)	0.38	0.26–0.50	<0.001	0.37	0.26–0.48	<0.001
Time since CKD stage 2 at baseline (per year)	0.08	0.04–0.12	<0.001	−0.01	−0.05 to 0.03	0.59
Furosemide use (yes vs. no)	0.85	0.17–1.53	0.01	0.43	−0.22 to 1.09	0.19
Alkali therapy (per 10 mg/kg/d)	0.01	−0.01 to 0.04	0.28	0.00	−0.02 to 0.02	0.96
Country of residence (Turkey vs. other European countries)				−2.13	−2.52 to −1.75	<0.001
Time since study entry	0.06	−0.01 to 0.13	0.10	0.05	−0.02 to 0.12	0.14

CAKUT, congenital anomaly of kidney and urinary tract; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.
[a]Not adjusted for country of residence.
[b]Further adjusted for country of residence.

Moreover, no statistically significant associations of metabolic acidosis with body mass index, serum albumin, or log C-reactive protein (CRP) were found.

Metabolic acidosis and CKD progression

During follow-up, 211 patients (30%) reached the primary endpoint defined as ESRD (start of dialysis or preemptive transplantation, or eGFR <10 ml/min/1.73 m^2) or 50% decline in eGFR. The median time to progression to the endpoint was 5.18 years (95% CI 4.76 to unknown).

In a multivariable Cox model including age at baseline, sex, Tanner stage, country of residence, cause of CKD, duration of CKD, baseline eGFR, time-dependent systolic and diastolic blood pressure, and time-dependent albumin-to-protein ratio, a serum bicarbonate level of <18 mmol/l during follow-up was associated with a significantly higher risk of CKD progression to the endpoint compared with a bicarbonate level of ≥22 mmol/l (adjusted hazard ratio [HR] 2.44; 95% CI 1.43–4.15; $P = 0.001$) (Table 4). A moderate metabolic acidosis (18–21 mmol/l) was not associated with an increased hazard of CKD progression (Table 4). The Kaplan-Meier survival curve illustrates the findings of the Cox model by showing a faster CKD progression in children with severe metabolic acidosis: 5-year renal survival was 78% in those without metabolic acidosis, 75% in those with moderate metabolic acidosis and 53% in those with a serum bicarbonate level <18 mmol/l (Figure 2). In addition, we found a significant but extremely small effect of alkali therapy on CKD progression (adjusted HR 0.995 per mg/kg/d bicarbonate equivalent; 95% CI 0.99–1.00; $P = 0.02$) irrespective of serum bicarbonate level (Table 4).

DISCUSSION

The main findings of our study suggest (i) a high prevalence of metabolic acidosis in children with CKD stages 3 to 5 with a decreased serum bicarbonate of <22 mmol/l in about half

Table 4 | Factors associated with CKD progression outcome: multivariable analysis using Cox regression model with time-dependent covariates

Characteristics	HR	95% CI	*P* value
Sex (female vs. male)	0.67	0.43–1.04	0.08
Age at baseline (per year)	1.01	0.92–1.11	0.91
Tanner stage			
1–2	Reference		
3–5	0.88	0.48–1.64	0.70
Country of residence (Turkey vs. other European countries)	0.98	0.62–1.56	0.94
Cause of CKD			
CAKUT other	Reference		
CAKUT obstruction	0.73	0.38–1.41	0.35
Cystic kidney disease	3.19	1.62–6.25	<0.01
Glomerulopathy	1.34	0.65–2.74	0.43
Metabolic disorder	1.94	0.88–4.27	0.10
Other	2.06	1.08–3.90	0.03
Time since CKD stage 2 (per year)	0.99	0.94–1.04	0.66
Baseline eGFR (per ml/min/1.73 m^2)	0.99	0.98–1.02	0.83
Systolic BP (SDS)	1.03	0.84–1.26	0.77
Diastolic BP (SDS)	1.38	1.09–1.73	<0.01
Log albumin-to-creatinine ratio (log mg/g)	1.50	1.24–1.82	<0.01
Serum bicarbonate level			
≥22 mmol/l	Reference		
18–21 mmol/l	1.04	0.63–1.70	0.88
<18 mmol/l	2.41	1.40–4.17	<0.01
Alkali therapy (per mg/kg/d)	0.99	0.99–1.00	0.02

BP, blood pressure; CAKUT, congenital anomaly of kidney and urinary tract; CI, confidence interval; CKD: chronic kidney disease; HR, hazard ratio; eGFR, estimated glomerular filtration rate; SDS, SD score.

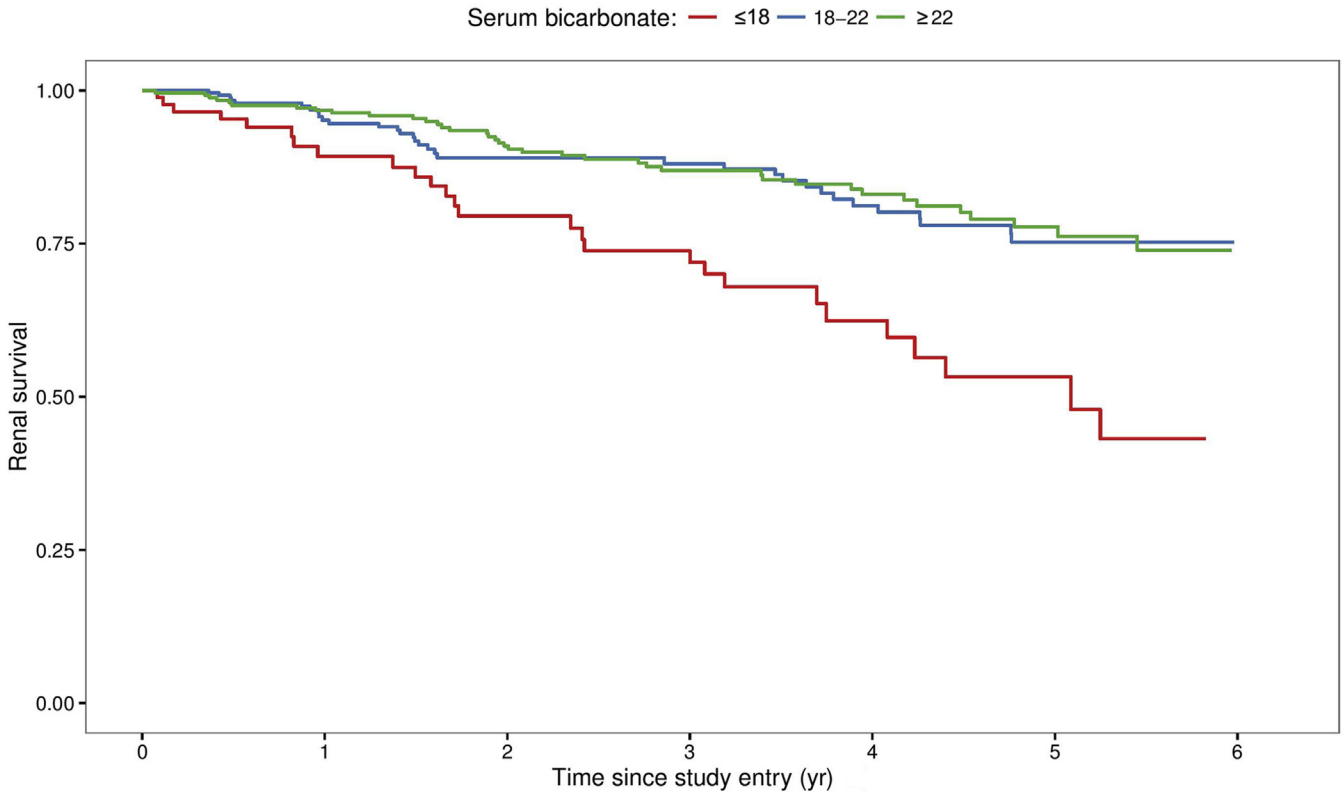

Figure 2| Survival-free from chronic kidney disease progression (defined as occurrence of end-stage renal disease or 50% decline in estimated glomerular filtration rate according to follow-up serum bicarbonate levels).

of patients, (ii) a significant association of metabolic acidosis with high PTH levels, and (iii) a strong association between severe metabolic acidosis during follow-up and increased risk of CKD progression and ESRD.

Although being a long-standing therapeutic target for both adult and pediatric nephrologists, metabolic acidosis has been poorly studied in children with CKD and evidence is lacking on its prevalence and clinical consequences. In a previous single-center pediatric study of CKD complications, only 8% of children with CKD stage 3 ($n = 38$) but 55% of those with CKD stages 4 to 5 ($n = 15$) had metabolic acidosis defined as either bicarbonate supplementation or serum bicarbonate <20 mmol/l.[16] Among 586 CKD children with a median GFR of 44 ml/min/1.73 m^2 participating in the Chronic Kidney Disease in Children (CKiD) study, mean serum bicarbonate at baseline was 22 mmol/l; the risk of acidosis was 3-fold higher in those with a GFR of <30 ml/min/1.73 m^2 versus >50 ml/min/1.73 m^2, and 29% of children received alkali therapy.[17] In the present 4C Study, median serum bicarbonate at baseline was slightly lower (21 mmol/l), most likely due to a lower eGFR of the cohort at study entry (27 ml/min/1.73 m^2), and the likelihood of having metabolic acidosis was also 3 times higher in CKD stages 4 and 5 than in CKD stage 3. This confirms that more severe metabolic acidosis predominantly appears from CKD stages 4 to 5, although many children, especially those with CAKUT, show mildly decreased serum bicarbonate in CKD stage 3. The proposed mechanism in CKD patients is a decreased ammonia excretion with GFR decline in the presence of preserved endogenous acid production, leading to a positive acid balance as CKD worsens.[2,18] We also found a highly significant impact of dialysis initiation, resulting in a decreased prevalence of metabolic acidosis among dialyzed patients as a likely consequence of the additional base load delivered in the dialysate. This decreased acidosis prevalence was not due to increased alkali therapy prescription after start of dialysis because dialyzed children actually received less alkali therapy than those with CKD. However, dialysis does not reverse metabolic acidosis in a proportion of children who may have higher net endogenous acid production, typically adolescents who often consume high amounts of animal proteins.

In our study, 43% to 60% of children with CKD stages 3 to 5 had serum bicarbonate levels of <22 mmol/l, and approximately 20% had serum bicarbonate levels of <20 mmol/l, a prevalence of acidosis that is much higher than in the adult CKD population.[1] For example, only 8% of subjects exhibited overt metabolic acidosis in the NephroTest cohort of adults with moderate CKD (median GFR: 37 ml/min/1.73 m^2).[18] Moreover, children seem to have low serum bicarbonate values at eGFR levels at which adults might be expected to still have normal serum bicarbonate levels. The difference is likely explained by the high prevalence of renal dysplasia in children. Indeed, dysplastic kidneys predispose to more

severe acidosis due to early tubular dysfunction, even when GFR is only moderately reduced. We found that children with CAKUT had lower serum bicarbonate levels than did all other disease groups. In addition to eGFR decline and cause of CKD, other determinants of low serum bicarbonate included older age and lesser use of furosemide. After including country of residence in the model, it became apparent that Turkish children more frequently had metabolic acidosis than other European children, a finding partly mediated by differences in kidney disease distribution and treatment.

We observed a significant association between metabolic acidosis and higher mean PTH level. This association might reflect a causal relationship. Indeed, chronic metabolic acidosis contributes to bone disease in patients with CKD. Animal and human studies have demonstrated that metabolic acidosis can stimulate bone resorption, inhibit bone formation, reduce vitamin D levels, and stimulate PTH secretion.[3,19,20] Data evaluating the effects of alkali therapy or acidosis correction on bone disease in the CKD population remains limited. A trial conducted in adult dialysis patients suggested that secondary hyperparathyroidism seen in patients with high bone turnover could be reversed and bone turnover stimulated in those with low turnover.[5]

Although acidosis is believed to be a substantial contributor to poor growth in CKD, data indicating that metabolic acidosis may impair longitudinal growth in pediatric CKD are scarce. In a cross-sectional analysis of the CKiD cohort, baseline HSDS was significantly lower in children with serum bicarbonate level <18 mmol/l compared with those with a baseline level >22 mmol/l.[21] In a German cohort of pediatric kidney transplant recipients, an association of borderline significance between serum bicarbonate and HSDS (a 0.04 increase in HSDS per 1 mmol/l rise in serum bicarbonate) was reported.[22] There is, however, some evidence that alkali therapy can induce normal growth in children with renal tubular acidosis even after severe stunting.[23,24] Despite the trend toward an association of serum bicarbonate with HSDS in univariable analysis, we failed to show a significant association in multivariable analysis. It is conceivable that serum bicarbonate is actually on the causal pathway relationship between cause of CKD and HSDS (i.e., that metabolic acidosis is a marker for CAKUT, a disease associated with poor statural growth).

To the best of our knowledge, this is the first longitudinal study showing the association of metabolic acidosis and disease progression in a large cohort of children with CKD. We found that, independent of age, baseline eGFR, albuminuria, blood pressure, and other potential confounders, a follow-up serum bicarbonate of <18 mmol/l was associated with a 2-fold higher risk of CKD progression (defined as occurrence of ESRD or 50% decline in eGFR) compared with normal bicarbonate levels of ≥22 mmol/l. Our findings corroborate data from recent adult studies that have shown associations between metabolic acidosis or dietary acid load and worsening kidney function.[9,25–29] The exact mechanism by which metabolic acidosis affects kidney disease progression is unclear. Although the synthesis of ammonium by the kidney decreases, its production by residual nephrons actually rises as CKD progresses, resulting in progressive tubular and interstitial injury.[2] It is believed that this tubular toxicity of elevated ammonium concentrations is mediated by the renin-angiotensin-aldosterone system or the alternative complement pathway.[30,31] Our data suggest a nonlinear effect of metabolic acidosis on CKD progression. Indeed, there seems to be a threshold effect in our study, with a faster eGFR decline when serum bicarbonate decreases below 18 mmol/l. We might speculate that bicarbonate is a buffer system, with capacities being exhausted at a certain serum level, leading to intracellular acidosis and renal tubulointerstitial tissue damage. A recent study in transplant recipients showed a similar threshold effect on graft survival as our study in native kidneys although at a higher serum bicarbonate level.[32] To date, no interventional studies have assessed the benefit of alkali supplementation in the pediatric CKD population. Among adult CKD patients, 2 relatively small clinical trials have shown that the correction of metabolic acidosis in patients with low serum bicarbonate level[12] or daily oral sodium bicarbonate in patients with hypertensive nephropathy but no metabolic acidosis[13] may slow the rate of eGFR decline. Several other well-designed trials are ongoing.[2] A few additional studies have shown that dietary intervention with alkali-inducing fruits and vegetables can reduce acid load and preserve kidney function without producing hyperkalemia.[33] In our study, no effect of alkali therapy on serum bicarbonate concentration was detected. However, there was most likely a treatment-by-indication bias, and the 4C observational cohort is not designed to investigate the benefit of a given therapy.

Our study has several limitations and strengths. First, whereas eGFR was calculated from centrally measured urea, creatinine, and cystatin C, it was not possible to use standardized laboratory testing for serum bicarbonate in this multicenter study. Moreover, in the absence of available data on pH and partial pressure of CO_2, we used low serum bicarbonate as a surrogate of metabolic acidosis. Second, since dietary intake is not recorded in the 4C Study, we were unable to take protein and acid load into account. Finally, as this is an observational cohort, unmeasured confounding factors cannot be excluded, thus limiting causal inference. However, the large sample size and the duration and completeness of follow-up along with the detailed collection of clinical and biochemical parameters support the translation of the growing evidence on the deleterious effects of metabolic acidosis from the adult to the pediatric CKD population. Indeed, the substantially higher prevalence of metabolic acidosis in children with CKD suggests an even greater clinical relevance of the impact of this complication in pediatric kidney disorders.

In conclusion, metabolic acidosis is a common complication in pediatric CKD patients and is associated with secondary hyperparathyroidism and kidney disease progression.

Our observational data suggest that serum bicarbonate level within normal range may be a target to slow the progression of CKD. In order to test this hypothesis, the evaluation of strategies focusing on improving dietary acid load and metabolic acidosis are warranted in children with CKD.

METHODS

Population

The 4C Study is a prospective observational cohort study in pediatric patients with CKD. Inclusion criteria were age 6 to 17 years and eGFR 10–60 ml/min per 1.73 m^2. Exclusion criteria were existing transplants, active systemic vasculitis, renal artery stenosis, coexisting primary cardiovascular anomalies, and anomalies of the limbs preventing diagnostic procedures. The objectives, design, and methods of the 4C Study have been described elsewhere.[34] The study was approved by institutional review boards and local ethics committees in all participating centers, and parents or legal guardians provided informed consent for study participation.

Data collection and definitions

Clinical and biochemical examinations were performed every 6 months. Detailed patient and medication history was recorded at each visit. For the purpose of this study, all patients with at least 1 serum bicarbonate measurement were included, and data from the CKD and dialysis periods but not the transplantation period were used. Renal diagnoses were categorized as CAKUT with predominant obstructive uropathy (CAKUT obstruction), CAKUT without obstruction (CAKUT other), cystic kidney disease, glomerulopathy, metabolic disorder, and other. Height and body mass index were expressed in SD scores according to the Center for Disease Control growth charts.[35] The eGFR was calculated from serum creatinine, cystatin C, urea, and height according to the updated Schwartz formula.[36] CKD stage was defined according to Kidney Disease Outcomes Quality Initiative classification. Urine albumin excretion was assessed using a spot urine albumin-to-creatinine ratio expressed in mg/g. Metabolic acidosis was defined as severe (serum bicarbonate value of <18 mmol/l), moderate (serum bicarbonate value of 18–21 mmol/l), or no acidosis (≥ 22 mmol/l). Alkali therapy dose was expressed in mg/kg/d bicarbonate equivalent.

Laboratory analyses

Biochemical parameters including serum creatinine, urea, cystatin C, iPTH, CRP, and albumin were measured in a central laboratory (Synlab, Heidelberg, Germany) from stored serum samples collected at each 6-month visit. Serum albumin (normal range 38–54 g/l) and CRP (normal <5 mg/l) levels were determined by a turbidimetric assay (Modular P analyzer; Roche Diagnostics, Indianapolis, IN), and iPTH (normal range 14–72 pg/ml) was determined by a chemiluminescent immunoassay (Advia Centaur XP; Siemens Healthcare Diagnostics, Erlangen, Germany). Serum creatinine was measured centrally by an enzymatic method. Bicarbonate measurements were determined locally.

Statistical analyses

Population characteristics were described with median and interquartile range for continuous variables and percentages for categorical variables. A cubic spline mixed-effects model with 3 knots located at 0, 3, and 6 years was used to plot the time-varying prevalence of metabolic acidosis (defined as serum bicarbonate <22 mmol/l) per CKD stage and corresponding model-based 95% CIs (Figure 1). Adjusted odds ratios (ORs) and 95% CIs for occurrence of metabolic acidosis for CKD stage (reference CKD stage 3) and dialysis modality (reference CKD) were calculated. Overall prevalence within the respective CKD stages was estimated from a time-constant model.

A linear mixed model with random intercept and slope was used to assess determinants of the predicted mean serum bicarbonate value over time. The model was adjusted for sex, age at baseline, duration of CKD (i.e., time since CKD stage 2 at baseline), cause of CKD, time-dependent eGFR, Tanner stage, use of furosemide, and time-dependent alkali therapy dose. The same model was then further adjusted for the country of residence (Turkey vs. other European countries). Multiple imputation by chained equations was used for missing data on eGFR (15%) and serum bicarbonate (10%).

Longitudinal associations of metabolic acidosis with outcomes such as HSDS, body mass index, iPTH, CRP, and serum albumin were investigated during the CKD period only by linear mixed-effects models. Variables were assessed for normality, and iPTH, CRP, and urine albumin-to-creatinine ratio were log-transformed to minimize violation of the normality assumption. Models were adjusted for sex, age at baseline, country of residence, cause of CKD, and time-dependent eGFR. The model of longitudinal HSDS determinants was additionally adjusted for the use of growth hormone (yes or no). For the assessment of the association between metabolic acidosis and serum albumin, the model was also adjusted for albumin-to-protein ratio.

To assess whether metabolic acidosis was associated with deterioration of kidney function over time, a Cox proportional hazards model with serum bicarbonate level (in 3 categories) as time-varying covariate was performed. The primary endpoint was a CKD progression event defined as the occurrence of ESRD (start of dialysis, preemptive kidney transplantation performed, or GFR $<$ 10 ml/min/1.73 m^2 at a study visit) or as a 50% decline in eGFR. Patient data were censored at time of death, loss to follow-up, or last available visit by July 1, 2016, whichever occurred first. The Cox model was adjusted for age at baseline, sex, Tanner stage, country of residence, cause of CKD, duration of CKD, baseline eGFR, time-dependent systolic and diastolic blood pressure, and time-dependent albumin-to-protein ratio. The model was not adjusted for renin-angiotensin system inhibitors because there was no association between serum bicarbonate levels and renin-angiotensin system inhibitor use and it was therefore not considered a confounding factor. Survival free from CKD progression was illustrated using a Kaplan-Meier method with time-varying categories of bicarbonate levels (<18, 18–21, and ≥ 22 mmol/l).[37] Adjusted HRs were reported with their 95% CIs. Statistical analyses were performed using R software.

DISCLOSURE

All the authors declared no competing interests.

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