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# Mineral bone disorders and kidney disease in hospitalized children with sickle cell anemia

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**Background:** Mineral bone disorders (MBD) are common in sickle cell anemia (SCA). Frequent vaso-occlusive crises (VOC) further impact MBD in children with SCA. We evaluated the prevalence of markers of SCA-related MBD (sMBD) in hospitalized children and assessed the relationship between sMBD and individual mineral abnormalities with kidney disease.

**Methods:** We prospectively recruited 185 children with SCA hospitalized with a VOC. Serum measures of mineral bone metabolism (calcium, phosphate, parathyroid hormone, 25-hydroxy vitamin D, FGF23, osteopontin) were measured at enrollment. The primary outcome was markers of sMBD defined as a composite of hypocalcemia, hyperphosphatemia, hyperparathyroidism, or deficiency in 25-OH vitamin D. Secondary outcomes included individual abnormalities in mineral metabolism. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines were used to define SCA-associated acute kidney injury (AKI). AKI was further assessed using urine NGAL as a marker of tubular injury. Acute kidney disease (AKD) was defined as a composite of AKI, an eGFR < 90 ml/min per 1.73 m<sup>2</sup> using the Cystatin C GFR equation, or evidence of structural injury (positive biomarker test or albuminuria).

**Results:** The mean age of children was 8.9 years and 41.6% were female. The prevalence of sMBD was 47.6%, with hypocalcemia the most frequent abnormality (29.9%, 55/184) followed by hyperphosphatemia (20.7%, 38/184), hyperparathyroidism (8.7%, 16/185), and vitamin D deficiency (5.4%, 10/185). There was no association between sMBD and sKDIGO-defined AKI using serial changes in creatinine or when incorporating biomarkers to define AKI. However, the presence of AKD was associated with a 2.01-fold increased odds of sMBD (95% CI 1.05 to 3.83) and was driven by a decrease in eGFR (OR, 2.90 95% CI: 1.59 to 5.29). When evaluating individual mineral abnormalities, hypocalcemia was associated with AKD and low eGFR while hyperparathyroidism was associated with low eGFR, AKI and structural injury. Vitamin D deficiency was associated with structural kidney injury. Vitamin D deficiency, hyperparathyroidism, and increases in FGF23 and osteopontin predicted mortality ( $p < 0.05$  for all).

**Conclusion:** MBD is common among children with SCA hospitalized with VOC. Biomarkers of kidney injury and bone health may help risk stratify children at risk of sMBD. Routine evaluation of sMBD in children with SCA may improve long-term bone health.

#### KEYWORDS

mineral bone disease, acute kidney injury, sickle cell anemia (SCA), mortality, pediatrics, acute kidney disease (AKD)

## Background

Sickle cell anemia (SCA) is among the commonest inherited hemoglobinopathies and results from a mutation in the  $\beta$ -globin gene (*HBB*) (1). The disease is autosomal recessive and characterized by Hemoglobin S polymerization, vaso-occlusion, and hemolysis which in turn leads to ischemia-reperfusion injury, endothelial dysfunction, oxidative stress, and immune activation (1, 2). SCA is a multi-system disease that causes repeated injury and progressive damage to various organs including the brain, kidneys and bones (2). Vaso-occlusive crises are one of the most common complications among children with SCA and are characterized by microvascular occlusions in the bone marrow leading to intense pain in one or more areas of the skeleton (3). Childhood and adolescence is a critical period of bone growth and bone mineral accrual with 90% of bone mass accumulated by 18 years of age (4, 5). Bone mass attained during childhood and adolescence is one of the most important determinants of skeletal health across the lifespan (6).

SCA is associated with growth disturbances and an increased risk of chronic bone problems including chronic pain, avascular necrosis, and vertebral collapse that lead to significant long-term morbidity (3). Mineral bone disorders (MBD) are common in the context of SCA with a number of mineral bone disease abnormalities reported, including hypocalcemia, hyperphosphatemia, hyperparathyroidism, and vitamin D deficiency (7–9). Additional biomarkers that modulate bone mineralization include osteopontin, a phosphorylated glycoprotein that can inhibit bone mineral deposition (10, 11), and fibroblast growth factor-23 (FGF23), a hormone that modulates phosphate handling of the kidneys (12). Acute kidney injury (AKI) develops in up to 36.2% of children with SCA hospitalized with a vaso-occlusive crisis (VOC) (13), and chronic kidney disease (CKD) is reported in up to 26.5% of children with SCA (14). Although MBD is well characterized in CKD (15), there is emerging evidence that MBD occurs during episodes of AKI (16) and may be associated with increased mortality in AKI (17). There are limited data on MBD in children with AKI and SCA thus highlighting the need for more studies.

In this prospective observational study, we evaluated the prevalence of markers of sickle-cell anemia related MBD

(sMBD) in children with SCA hospitalized for a painful VOC and assessed the relationship between sMBD and the presence of AKI and other kidney abnormalities.

## Methods

### Study population

Between January and July in 2019, children with sickle cell anemia admitted with a VOC were screened and consecutively enrolled (18). Children were recruited from Mulago National Referral and Teaching Hospital, which is located in Central Uganda and has a high outpatient and inpatient volume. Most children admitted with a VOC are referred from the hospital's dedicated sickle cell clinic that attends to about 1,400 children per year. Routine care includes daily folic acid supplementation, three-monthly malaria prophylaxis with sulfadoxine-pyrimethamine, and penicillin V for children <5 years of age. Hydroxyurea is approved for use and is available in a limited capacity. Care for children with VOC involves hydration using oral or intravenous fluids and analgesic medications based on pain severity and typically includes paracetamol, non-steroidal anti-inflammatory drugs, and opioids (morphine, tramadol).

Eligibility criteria included an age between 2 and 18 years, documented SCA by hemoglobin electrophoresis (hemoglobin SS), hospitalization for a VOC with a pain score  $\geq 2$  using an age-specific pain scale. Pain was assessed using the face, legs, activity, cry, and consolability (FLACC) scale (1) in children aged 2–3 years, the Wong-Baker Faces pain scale in children 3–7 years and the numeric pain scale in children  $\geq 8$  years (2). Children were only eligible to be enrolled once during the recruitment period. The primary outcome was the presence of sMBD defined as a composite of one or more of the following mineral metabolism abnormalities; hypocalcemia, hyperphosphatemia, hyperparathyroidism, or vitamin D deficiency. The individual mineral metabolism abnormalities were secondary outcomes. A sample size of 185 children was generated assuming a prevalence of hypocalcemia of 14% (9). The sample size formula used was  $n = (z^2 p(1-p)/d^2)$ , where;  $z$  = standard normal variate corresponding to the 95% confidence interval and is 1.96,  $d$  = the required precision of the estimate (0.05),  $p$  = prevalence rate (19).

## Study procedures

On enrollment all children had a complete history and physical exam to assess medication use, signs of infection, and the site and severity of pain. On admission children had venous blood drawn with serum collected in an additive free tube and allowed to clot at room temperature for 30–60 min before centrifugation at 1200 g for 20 min and storage at  $-80^{\circ}\text{C}$  for subsequent biomarker testing. A spot urine sample was collected using a urine bag or urine container for older children for urinalysis, processing, and storage. Urine samples were spun at room temperature for 5 min at 400 g to remove urine sediment. Urine samples were stored at  $-80^{\circ}\text{C}$  until ELISA testing.

Laboratory tests included complete blood count and serum studies to evaluate biochemical abnormalities in mineral metabolism [ $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , phosphorous, intact parathyroid hormone (iPTH)]. The complete blood counts were measured using the Sysmex automated haematology analyser, version XN 450. Serum laboratory tests were measured by the Johns Hopkins University Infectious Disease Institute Laboratory on serum samples using a Cobas machine (Roche Diagnostics, Indianapolis, USA). Intact PTH was measured in serum by the Ebenezer Clinical Laboratory Ltd. using an electrochemiluminescence immunoassay using a Cobas e411 analyzer. Urine albumin and creatinine were tested by the Mulago Hospital Chemistry Laboratory using a Cobas analyzer (Roche Diagnostics, Indianapolis, USA).

## Immunoassays

Additional markers related to bone mineral metabolism and kidney function were measured on stored serum and urine samples by enzyme linked immunoassays (ELISA). Serum cystatin C was measured using the R&D Systems Quantikine ELISA (R&D Systems, Minneapolis, MN) at 1:40 dilution with a reported range of 0.06 to 16 mg/L with pooled serum controls and commercial controls on every plate. The assay has been correlated against the Cystatin C reference standard provided by the Joint Research Centre Institute for Reference Material and Measurements (Catalog # ERM-DA471/ IFCC) with a slope of 1.07 and a  $R^2$  value of 0.998. Serum FGF23 was measured using a DuoSet by R&D Systems at a 1:5 dilution with a reported range of 200 to 50,000 pg/ml (R&D Systems, Minneapolis, MN). Serum and urine Osteopontin (OPN) were measured using a DuoSet by R&D Systems at a dilution of 1:100 and 1:1,000 respectively (R&D Systems, Minneapolis, MN). Urine NGAL was measured using an ELISA by BioPorto (Kit 036, BioPorto Diagnostics Inc., Hellerup, Denmark) at a 1:1,000 dilution with a reported range of 5–2000 ng/ml according to the manufacturer's protocol. Vitamin D was measured using a commercial assay (Eagle Biosciences Inc, NH, USA) that recognizes total 25-OH vitamin D (including

vitamin D3 and D2) in serum with a reported range of 5–600 nmol/L. All sample testing was conducted by trained technicians blinded to patient details. Samples below the limit of detection were assigned the lower limit of the assay.

## Assessment of kidney function

Acute kidney injury (AKI) was defined based on the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines as an increase in serum creatinine of  $\geq 0.3$  mg/dl within 48 h or a 50% increase in baseline creatinine within 7 days (3). Kidney function was assessed on enrollment, at 48 h and on day 7 or discharge (whichever happened earlier) using the i-STAT handheld blood analyzer (Abbott Point of Care Inc., Princeton, NJ) with assays traceable to the U.S. National Institute of Standards and Technology (NIST) standard reference material SRM909 with reportable range is 0.20–20.0 mg/dl. For the purposes of defining AKI a creatinine value below the reportable range ( $<0.20$  mg/dl) was assigned a value of 0.19 mg/dl. The participants' lowest creatinine measure was taken as the baseline creatinine. In instances where only a single creatinine measure was available ( $n = 7$ ), we used the Pottel-age based GFR equation assuming a normal GFR of 120 ml/min per  $1.73\text{ m}^2$  (20, 21). As previously described, children with an increase in creatinine from 0.2 to 0.3 mg/dl were not considered to have AKI (13).

Defining AKI using creatinine in children with SCA has limitations as the children are likely to have hyperfiltration, increased tubular creatinine excretion (22) and creatinine levels may be affected by reduced muscle mass (23, 24). We thus used the 23rd Acute Disease Quality Initiative (ADQI-23) consensus guidelines which integrate the use of other functional biomarkers of kidney injury in addition to creatinine in defining and staging AKI (25). Based on the ADOQI-23 guidelines, AKI is staged based on presence of a positive or negative kidney injury biomarker on addition to the fold increase in creatinine as defined by the KDIGO guidelines. In our study, a uNGAL level  $\geq 150$  ng/ml was considered biomarker positive for tubular injury (BM+) (26). Admission eGFR was calculated using the CKiD cystatin C-based equation,  $\text{eGFR} = 70.69 * (\text{cystatin C})^{-0.931}$  (27). Acute kidney disease was defined as one or more of the following: AKI or an eGFR  $< 90$  ml/min per  $1.73\text{ m}^2$  or signs of structural injury (macroalbuminuria or BM+) (25, 28).

## Assessment of mineral bone abnormalities

Bone mineral abnormalities were defined using the following criteria: hypocalcemia ( $< 2.2$  mmol/L following correction for serum albumin) (29), hyperphosphatemia (phosphorous  $>$

upper limit of normal for age and sex) (30), hyperparathyroid hormone (iPTH > 70 pg/ml) (31), vitamin D deficiency (25-OH vitamin D < 30 nmol/L) (32). A child meeting any of the above criteria was considered to have sickle-cell anemia related MBD (sMBD). In addition, children were classified as having elevated FGF23 and elevated osteopontin if the concentrations exceeded the 95th percentile measured using a population of children with SCA in steady state with the cut-offs as follows: FGF23 > 535 pg/ml and osteopontin > 375 ng/ml.

## Statistical analysis

Data were entered into REDCap electronic data capture tools hosted at Indiana University. Data were analyzed using STATA v17.0 (StataCorp) and GraphPad Prism v9. Data are presented descriptively using median and interquartile range (IQR) for continuous variables and the number and frequency for discrete variables. To examine the relationship between continuous variables and dichotomized measures of sMBD, a Wilcoxon rank-sum test was used for continuous variables. For categorical variables, Pearson's Chi-square or Fisher's exact test were used, as appropriate. Spearman's rank correlation was used to evaluate relationships between measures of bone metabolism and kidney function. Logistic regression was used to evaluate the relationship between AKI and AKD with sMBD or individual mineral metabolism abnormalities and models were adjusted for participant age and sex. Bonferroni correction was used to adjust for multiple testing.

## Results

We assessed sMBD in 185 children with sickle cell anemia hospitalized for a painful VOC as outlined in **Figure 1**. The median age of children was 8.9 years [interquartile range (IQR), 5.9 to 11.8] and 41.6% of participants were female (**Table 1**). The median duration of pain prior to presentation was 3 days (IQR, 2 to 4) with a median pain score of 6 (IQR, 4 to 8). Overall, the prevalence of sMBD was 47.6% (**Figure 1**). Hypocalcemia was the most frequent mineral bone abnormality observed at 29.9% (55/184) followed by hyperphosphatemia at 20.7% (38/184), hyperparathyroidism at 8.7% (16/185) and vitamin D deficiency at 5.4% (10/185). Vitamin D insufficiency (30–50 nmol/L) was present in 24.3% (45/185) of the children.

## Demographic, clinical, and laboratory characteristics associated with markers of sMBD

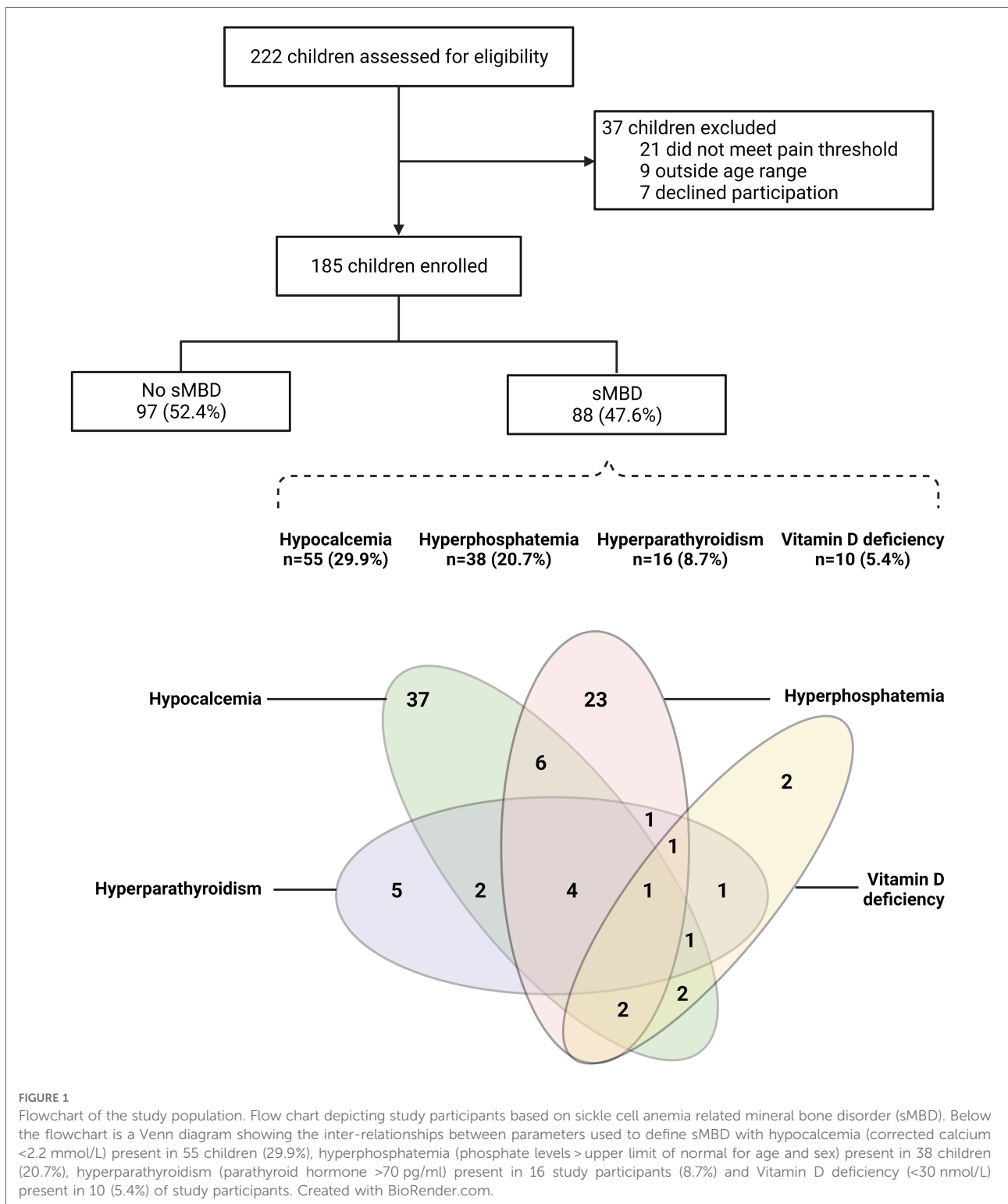
Demographic, clinical, and laboratory characteristics are presented in **Table 1**. Children with sMBD were significantly

older than children without sMBD ( $p=0.008$ ) but were similar in nutritional status and clinical characteristics on admission. There were no differences in the history of pain duration or the severity of pain on admission based on sMBD ( $p>0.05$ ). In addition to differences in the measures used to define sMBD ( $\text{Ca}^{2+}$ ,  $\text{PO}_4^-$ , PTH, 25-OH vitamin D), children with sMBD had higher levels of  $\text{Mg}^{2+}$  and FGF23 compared to children without sMBD ( $p<0.05$  for both). There were differences in kidney function (BUN, Cystatin C) in children with sMBD compared to children without sMBD.

We conducted additional analyses evaluating differences in the same measures in children with and without hypocalcemia and hyperphosphatemia as the most frequent findings in children with sMBD (**Table 2**) and vitamin D deficiency and hyperparathyroidism as the least frequent findings (**Table 2**). Participants with hypocalcemia, hyperphosphatemia, and hyperparathyroidism all had increased levels of Cystatin C and BUN compared to children with normal values for the same marker of mineral metabolism. Further, children with both vitamin D deficiency or with hyperparathyroidism had higher levels of urine NGAL as a measure of proximal tubular injury, higher potassium, osteopontin, and FGF23 compared to children without vitamin D deficiency or hyperparathyroidism. When evaluating mineral abnormalities by age, children with hyperparathyroidism and vitamin D deficiency were older (**Table 2**) and this was further supported by an increase in the frequency of mineral abnormalities across age categories (**Figure 2**). The earliest abnormality to present was hypocalcemia, present in 22% of children <5 years of age followed by hyperphosphatemia that emerged in children between 5 and 10 years of age, while both hyperparathyroidism and vitamin D deficiency were relatively infrequent before 10 years of age (**Figure 2**).

## Kidney function and its relationship with altered mineral metabolism

We further assessed the relationship between kidney function and sMBD based on the presence of AKI and AKD defined during hospitalization. Overall, 36.2% of children had AKI defined based on serial changes in serum creatinine, with 16 children BM+ (8.6%) based on a positive uNGAL test, and 69.7% had AKD (**Figure 3**). There was no difference in sMBD based on the presence of sub-clinical AKI or AKI status (BM– vs. BM+). However, there was an increase in sMBD in children with AKD that encompassed reduced GFR using the Cystatin C based eGFR equation or structural abnormalities including BM+ or macroalbuminuria. A low eGFR defined using Cystatin C was also independently associated with sMBD (**Figure 3**). We further assessed the relationship between individual mineral abnormalities and AKI and AKD. Both BM+ AKI and BM– AKI were



associated with hyperparathyroidism while BM + AKI was associated with vitamin D deficiency. There was no association between AKI and hypocalcemia or hyperphosphatemia. Children with AKD had increased odds for having hypocalcemia while a low Cystatin C defined eGFR

was associated with both hypocalcemia and hyperparathyroidism. Presence of a marker of structural kidney damage was associated with a 9-fold increased odds of having vitamin D deficiency (95% CI 2.37 to 34.17) (Figure 4).

TABLE 1 Demographic, clinical and laboratory characteristics on admission associated with bone mineral disease.

	Combined (n = 185)	No sMBD (n = 97)	sMBD (n = 88)	p value
<b>Demographic characteristics</b>				
Age, years, median (IQR)	8.9 (5.8, 11.8)	7.8 (5.0, 11.1)	10.2 (6.7, 13.0)	0.008
Sex, n (%) Female	77 (41.6)	41 (42.3)	36 (40.9)	0.851
Height-for-age z score	-1.4 (-2.3, -0.4)	-1.2 (-2.0, -0.3)	-1.5 (-2.7, -0.5)	0.054
Weight-for-age z score	-1.5 (-2.1, -0.5)	-1.6 (-2.2, -0.6)	-1.2 (-1.9, -0.2)	0.243
Weight-for-height z score	-1.6 (-2.2, -0.4)	-1.6 (-2.6, -0.6)	-1.3 (-2.1, 0.3)	0.197
BMI-for-age z score	-1.3 (-2.3, -0.4)	-1.3 (-1.9, -0.4)	-1.2 (-2.7, -0.3)	0.485
Mid Upper Arm Circumference, cm	16 (15.0, 17.8)	16 (14.7, 17.4)	16.5 (15.1, 18.0)	0.111
<b>Clinical characteristics</b>				
Temperature, °C	37.1 (36.7, 37.8)	37.1 (36.7, 37.8)	37.1 (36.6, 37.9)	0.830
Heart rate, bpm	108 (98, 121)	108 (98, 121)	110 (95, 124)	0.765
Respiratory rate, bpm	29 (24, 36)	29 (24, 36)	30 (25, 36)	0.764
Blood pressure category, n (%)				
Hypotensive	2 (1.1)	0	2 (2.3)	0.306
Normotensive	149 (80.5)	78 (80.4)	71 (80.7)	
Hypertension	34 (18.4)	19 (19.6)	15 (17.1)	
<b>Pain Assessment</b>				
Pain Score, median (IQR)	6 (4, 8)	6 (4, 8)	6 (4, 8)	0.290
Duration of pain in days	3 (2, 4)	3 (2, 5)	3 (2, 4)	0.548
<b>Complete blood count</b>				
WBC × 10 <sup>3</sup> /μl	22.6 (16.7, 33.8)	22.6 (15.5, 33.8)	22.8 (16.7, 36.1)	0.724
Hemoglobin, g/dl	7.2 (6.3, 8.3)	7.3 (6.5, 8.4)	7.1 (6.0, 7.9)	0.188
Platelet count × 10 <sup>9</sup> /L	418 (306, 529)	427 (295, 514)	403 (307, 534)	0.913
<b>Bone mineral measures</b>				
Ca <sup>2+</sup> , mmol/L	2.2 (2.1, 2.3)	2.3 (2.2, 2.3)	2.2 (2.1, 2.2)	<0.001
PO <sub>4</sub> , mg/dl	5.0 (4.2, 5.7)	4.7 (4.1, 5.4)	5.5 (4.3, 6.5)	<0.001
Mg <sup>2+</sup> , mmol/L	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)	1.0 (0.9, 1.1)	0.023
PTH, pg/ml	30.7 (20.4, 44.7)	24.7 (17.6, 37.1)	35.4 (24.6, 54.7)	<0.001
25-OH Vitamin D, nmol/L	60.3 (46.1, 74.7)	63.6 (51.4, 79.5)	56.5 (40.6, 71.6)	0.003
FGF-23, pg/ml	229 (195, 722)	211 (195, 503)	273 (195, 1139)	0.038
<b>Kidney function</b>				
Creatinine, mg/dl	0.30 (0.19, 0.40)	0.30 (0.19, 0.30)	0.30 (0.19, 0.50)	0.032
BUN, mg/dl	4 (3, 7)	3 (3, 5)	4 (3, 8)	<0.001
Sodium, mmol/L	138 (135, 140)	138 (135, 140)	137 (134, 140)	0.271
Potassium, mmol/L	3.8 (3.5, 4.1)	3.7 (3.5, 4.1)	3.8 (3.6, 4.1)	0.389
Cystatin C, mg/L	0.8 (0.6, 1.0)	0.7 (0.6, 0.9)	0.9 (0.7, 1.2)	<0.001
Enrolment eGFR (CKiD, Cystatin C)	86 (68, 107)	94 (76, 118)	78 (61, 100)	<0.0001
Urine NGAL, ng/ml	9.8 (5.0, 33.8)	10.6 (5.0, 38.2)	9.1 (5.0, 27.4)	0.754

(continued)

TABLE 1 Continued

	Combined ( <i>n</i> = 185)	No sMBD ( <i>n</i> = 97)	sMBD ( <i>n</i> = 88)	<i>p</i> value
<b>Urinalysis</b>				
Albuminuria category (adjusted to uCr), <i>n</i> (%)				
A1, <3 mg/mmol	105 (60.0)	59 (64.1)	46 (55.4)	0.501
A2, 3–30 mg/mmol	55 (31.4)	26 (28.3)	29 (34.9)	
A3, >30 mg/mmol	15 (8.6)	7 (7.6)	8 (9.6)	

Data presented are median (interquartile range) or *n* (%). Differences in continuous variables were assessed using Wilcoxon rank sum test. Differences in categorical variables were assessed using Pearson's Chi-square test or Fisher's exact test, as appropriate.

## Relationship between markers of sMBD and mortality

In this study, 6/185 (3.2%) of participants died in-hospital with a similar frequency of mortality among children with sMBD 3/88 (3.4%) compared to children without sMBD 3/97 (3.1%). While infrequent, the presence of hyperparathyroidism and vitamin D deficiency were associated with increased mortality (Figure 5). There were significant increases in the median level of serum bone biomarkers FGF23 ( $p < 0.0001$ ) and osteopontin ( $p < 0.01$ ) in children who died in-hospital compared to survivors ( $p < 0.001$ ) (Figure 5). Both elevated FGF23 and osteopontin were associated with higher mortality ( $p < 0.001$ ) (Figure 5).

## Relationship between markers of mineral bone metabolism

Finally, we conducted correlation analysis to evaluate relationships between individual measures of mineral bone metabolism and kidney function (Figure 6). Within markers of mineral metabolism, parathyroid hormone was positively correlated with phosphate levels and negatively correlated with both calcium and vitamin D levels. FGF23 was negatively correlated with calcium and vitamin D and positively correlated with parathyroid hormone levels, BUN and cystatin C. Parathyroid hormone levels were strongly correlated with markers of reduced kidney filtration (creatinine, BUN, Cystatin C) as well as structural injury to the kidney (urine NGAL) (Figures 4, 6). Osteopontin was negatively correlated with calcium and positively correlated with markers of reduced kidney filtration (creatinine, BUN, Cystatin C) and FGF23.

## Discussion

In this prospective observational study, we demonstrated a high prevalence of sMBD in children hospitalized with a

VOC. Hypocalcemia was the most common mineral bone abnormality followed by hyperphosphatemia, hyperparathyroidism and vitamin D deficiency, respectively. With the exception of hyperphosphatemia, all mineral bone abnormalities were associated with kidney disease. sMBD and hypocalcemia were associated with AKD and low eGFR while hyperparathyroidism and vitamin D deficiency were associated with structural injury to the kidney. Although individual features of sMBD varied by age, features associated with mortality during hospitalization (hyperparathyroidism and vitamin D deficiency) were most common in older children. In this population, two additional markers of sMBD, FGF23 and osteopontin, were associated with kidney disease and also strongly associated with mortality.

Mineral bone abnormalities are well known complications of CKD (15, 33); however, there is emerging evidence that AKI is also associated with dysregulation of bone minerals that can impact bone health (16). Data on mineral bone abnormalities in children with AKI and SCA are limited and complicated by challenges in assessing kidney disease in children with SCA due to glomerular hyperfiltration and muscle wasting which affect serum creatinine levels (22–24). In the present study, AKI was assessed using both serum creatinine and urine NGAL as a biomarker of structural kidney injury (25). There were differences in the frequency of sMBD based on AKI status. However, when AKD was evaluated incorporating Cystatin C, a more stable biomarker of glomerular filtration in the context of SCA, and markers of structural kidney injury were incorporated (i.e., macroalbuminuria, positive NGAL), AKD was identified as a risk factor for sMBD. As children with SCA have altered tubular handling of creatinine, alternative biomarkers of kidney function are useful in defining the presence of kidney disease and identifying acute structural injury (34). When defining AKI based on changes in serum creatinine alone (13, 28), children with kidney disease may be misclassified. Thus, the use of a broader AKD definition was able to identify children with AKI and pre-existing kidney disease. As CKD is defined based on a follow-up period of not less than 3 months, a criterion which was not fulfilled in our study, we were unable to differentiate between AKD and CKD.

TABLE 2 Demographic, clinical and laboratory characteristics on admission associated with mineral bone disorders.

	Calcium			Phosphate		
	No hypocalcemia (n = 129)	Hypocalcemia (n = 55)	p value	No hyperphosphatemia (n = 146)	Hyperphosphatemia (n = 38)	p value
<b>Demographic characteristics</b>						
Age, years, median (IQR)	8.2 (5.3, 11.4)	10.3 (7.0, 12.8)	0.068	8.9 (5.3, 11.5)	9.7 (6.7, 13.1)	0.130
Sex, n (%) Female	57 (44.2)	19 (34.6)	0.224	60 (41.1)	16 (42.1)	0.910
<b>Complete blood count</b>						
WBC × 10 <sup>3</sup> /μl	22.4 (16.1, 33.4)	23.0 (16.8, 39.5)	0.597	22.7 (16.4, 33.3)	21.7 (16.7, 40.1)	0.716
Hemoglobin, g/dl	7.5 (6.5, 8.5)	6.4 (5.9, 7.5)	<0.0001	6.9 (6.1, 8.2)	7.6 (6.7, 8.4)	0.037
Haptoglobin, mg/L	29.1 (6.3, 144.3)	19.4 (6.3, 61.8)	0.157	30.4 (6.3, 144.3)	13.6 (6.3, 83.1)	0.199
Platelet count × 10 <sup>9</sup> /L	437 (346, 566)	348 (279, 451)	0.003	417 (301, 520)	434 (351, 575)	0.317
<b>Bone mineral measures</b>						
Ca <sup>2+</sup> , mmol/L	2.26 (2.21, 2.35)	2.11 (2.05, 2.17)	—	2.21 (2.12, 2.30)	2.23 (2.17, 2.34)	0.224
PO <sub>4</sub> , mg/dl	5.0 (4.2, 5.6)	4.7 (4.1, 6.1)	0.901	4.6 (4.1, 5.4)	6.6 (6.1, 7.2)	—
Mg <sup>2+</sup> , mmol/L	0.9 (0.9, 1.0)	1.0 (0.9, 1.1)	0.123	0.9 (0.9, 1.0)	1.00 (0.9, 1.1)	0.002
PTH, pg/ml	27.0 (18.2, 41.0)	37.0 (25.8, 52.8)	0.001	30.2 (20.4, 42.8)	33.6 (20.8, 52.8)	0.175
25-OH Vitamin D, nmol/L	62.9 (50.5, 74.7)	54.9 (38.6, 76.8)	0.033	61.4 (46.8, 77.9)	57.0 (41.9, 67.7)	0.093
FGF23, pg/ml	217 (195, 623)	314 (195, 1169)	0.065	233 (195, 651)	272 (195, 1610)	0.388
Serum Osteopontin, ng/ml	187 (134, 243)	231 (162, 320)	0.002	199 (144, 254)	208 (132, 334)	0.517
<b>Kidney function</b>						
BUN, mg/dl	3 (3, 5)	5 (3, 10)	0.002	3 (3, 6)	5 (3, 9)	0.013
Potassium, mmol/L	3.8 (3.6, 4.1)	3.7 (3.3, 4.1)	0.228	3.7 (3.5, 4.0)	4.0 (3.8, 4.3)	0.001
Cystatin C, mg/L	0.8 (0.6, 1.0)	0.9 (0.7, 1.2)	0.001	0.8 (0.6, 1.0)	1.0 (0.7, 1.3)	0.008
eGFR (CKiD, Cystatin C)	92 (72, 113)	75 (61, 93)	0.001	90 (71, 111)	70 (54, 104)	0.008
Urine NGAL, ng/ml	9.0 (5.0, 30.6)	13.4 (5.0, 38.4)	0.177	10.1 (5.0, 29.6)	8.5 (5.0, 50.0)	0.861
	Parathyroid hormone			Vitamin D		
	No hyperparathyroidism (n = 169)	Hyperparathyroidism (n = 16)	p value	No vitamin D deficiency (n = 175)	Vitamin D deficiency (n = 10)	p value
<b>Demographic characteristics</b>						
Age, years, median (IQR)	8.8 (5.4, 11.6)	10.8 (7.9, 13.9)	0.047	8.3 (5.4, 11.5)	12.5 (10.8, 15.3)	<0.001
Sex, n (%) Female	66 (39.1)	11 (68.8)	0.02	98 (41.9)	9 (56.3)	0.261
<b>Complete blood count</b>						
WBC × 10 <sup>3</sup> /μl	22.5 (16.7, 33.4)	27.1 (16.7, 50.5)	0.536	22.4 (16.7, 33.4)	28.6 (14.2, 98.4)	0.744
Hemoglobin, g/dl	7.3 (6.3, 8.3)	6.7 (6.0, 8.4)	0.620	7.1 (6.3, 8.3)	7.4 (6.4, 8.3)	0.809
Haptoglobin, mg/L	28.7 (6.3, 147.9)	24.2 (6.3, 58.8)	0.239	26.9 (6.3, 144.3)	37.9 (6.3, 72.0)	0.553

(continued)



TABLE 2 Continued

	Parathyroid hormone			Vitamin D		
	No hyperparathyroidism (n = 169)	Hyperparathyroidism (n = 16)	p value	No vitamin D deficiency (n = 175)	Vitamin D deficiency (n = 10)	p value
Platelet count × 10 <sup>9</sup> /L	418 (306, 541)	390 (244, 481)	0.287	418 (306, 533)	349 (304, 538)	0.666
<b>Bone mineral measures</b>						
Ca <sup>2+</sup> , mmol/L	2.21 (2.13, 2.32)	2.19 (2.06, 2.29)	0.206	2.2 (2.1, 2.3)	2.2 (2.1, 2.2)	0.047
PO <sub>4</sub> , mg/dl	4.8 (4.1, 5.6)	5.7 (4.9, 6.8)	0.019	5.0 (4.2, 5.6)	6.0 (3.9, 6.9)	0.404
Mg <sup>2+</sup> , mmol/L	0.9 (0.9, 1.0)	1.0 (0.9, 1.3)	0.024	0.9 (0.9, 1.0)	1.0 (0.9, 1.1)	0.212
PTH, pg/ml	27.9 (18.8, 40.2)	102.2 (76.2, 153.7)	—	29.3 (19.1, 42.7)	51.4 (34.0, 147.4)	<0.001
25-OH Vitamin D, nmol/L	61.4 (47.6, 76.7)	40.0 (28.7, 56.7)	<0.001	61.4 (47.8, 76.5)	24.6 (22.7, 26.2)	—
FGF23, pg/ml	223 (195, 558)	4,419 (248, 10350)	<0.001	225 (195, 603)	4,063 (230, 7693)	0.030
Serum Osteopontin, ng/ml	197 (138, 251)	394 (238, 568)	<0.001	202 (143, 259)	243 (198, 441)	0.045
<b>Kidney function</b>						
BUN, mg/dl	3 (3, 5)	16 (5, 56)	<0.001	3 (3, 6)	8 (4, 29)	0.057
Potassium, mmol/L	3.8 (3.5, 4.0)	4.2 (3.7, 5.3)	0.014	3.8 (3.5, 4.0)	4.3 (3.9, 5.5)	0.001
Cystatin C, mg/L	0.8 (0.6, 1.0)	1.3 (1.0, 2.4)	<0.001	0.8 (0.6, 1.0)	1.1 (0.8, 1.6)	0.130
eGFR (CKiD, Cystatin C)	90 (70, 111)	54 (31, 70)	<0.001	89 (68, 110)	65 (45, 87)	0.130
Urine NGAL, ng/ml	9.2 (5.0, 27.9)	213.4 (11.3, 1069.8)	<0.001	9.6 (5.0, 29.6)	42.3 (13.7, 983.1)	0.016

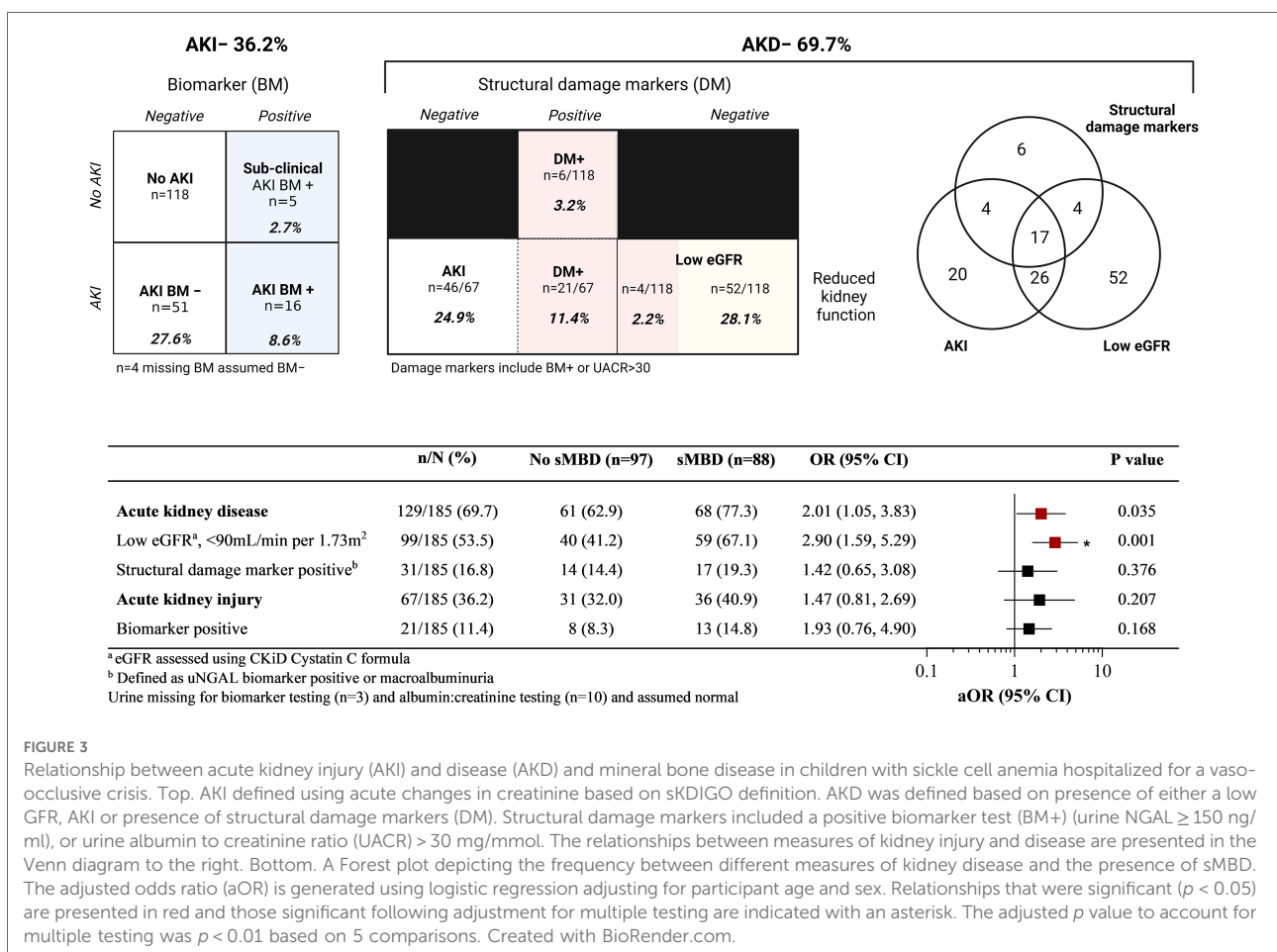
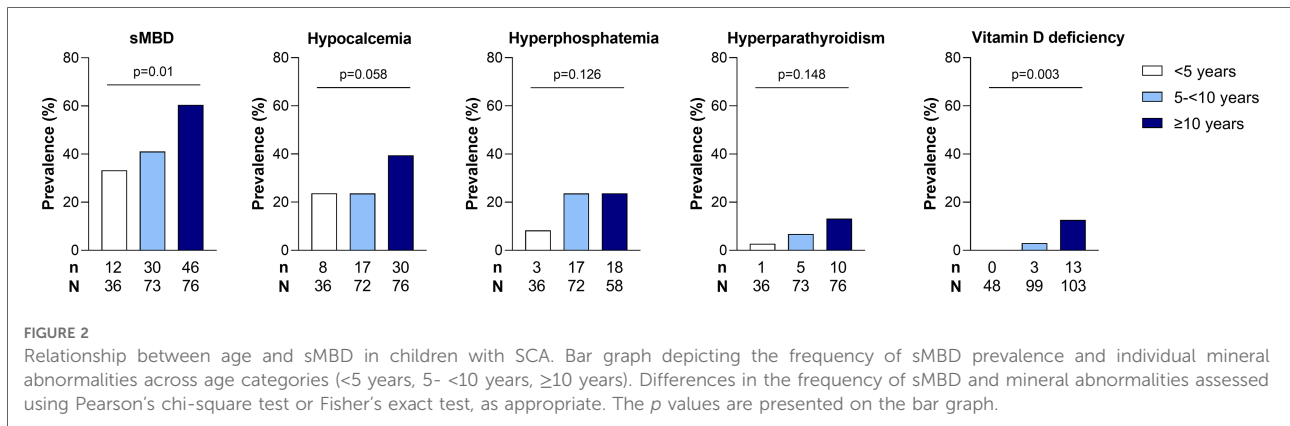
Data presented are median (interquartile range) or n (%). Differences in continuous variables were assessed using Wilcoxon rank sum test. Differences in categorical variables were assessed using Pearson's Chi-square test.

The most common mineral abnormality in our study was hypocalcemia which occurred in over a quarter of the population and was frequent across the age span. In the context of AKI, low calcium is thought to be secondary to reduced activity of 1 alpha hydroxylase enzyme and vitamin D deficiency (16). Vitamin D deficiency was comparatively uncommon in our cohort, occurring in 5.4% of children although a large proportion of children (24%) had vitamin D insufficiency. A recent multi-country study of 4,509 African children <8 years of age identified vitamin D deficiency in 0.6% of children with seasonal variations in vitamin D levels and increasing vitamin D deficiency in older children (35). Hypocalcemia may also occur as a result of increased intracellular uptake of calcium in sickled cells (36) and as a result of CKD in children with SCA (14, 31). Hypocalcemia and hyperphosphatemia are drivers for elevated parathyroid hormone levels, which were also observed in the population. Parathyroid hormone levels were negatively correlated with serum calcium and positively correlated with serum phosphate levels. The hyperphosphatemia was likely due to reduced renal clearance and was correlated with higher Cystatin C levels as a measure of reduced glomerular filtration

in these children (37). In children with sickle cell anemia, hyperphosphatemia may also be secondary to increased tubular reabsorption of phosphorus and resistance to FGF23 (38, 39).

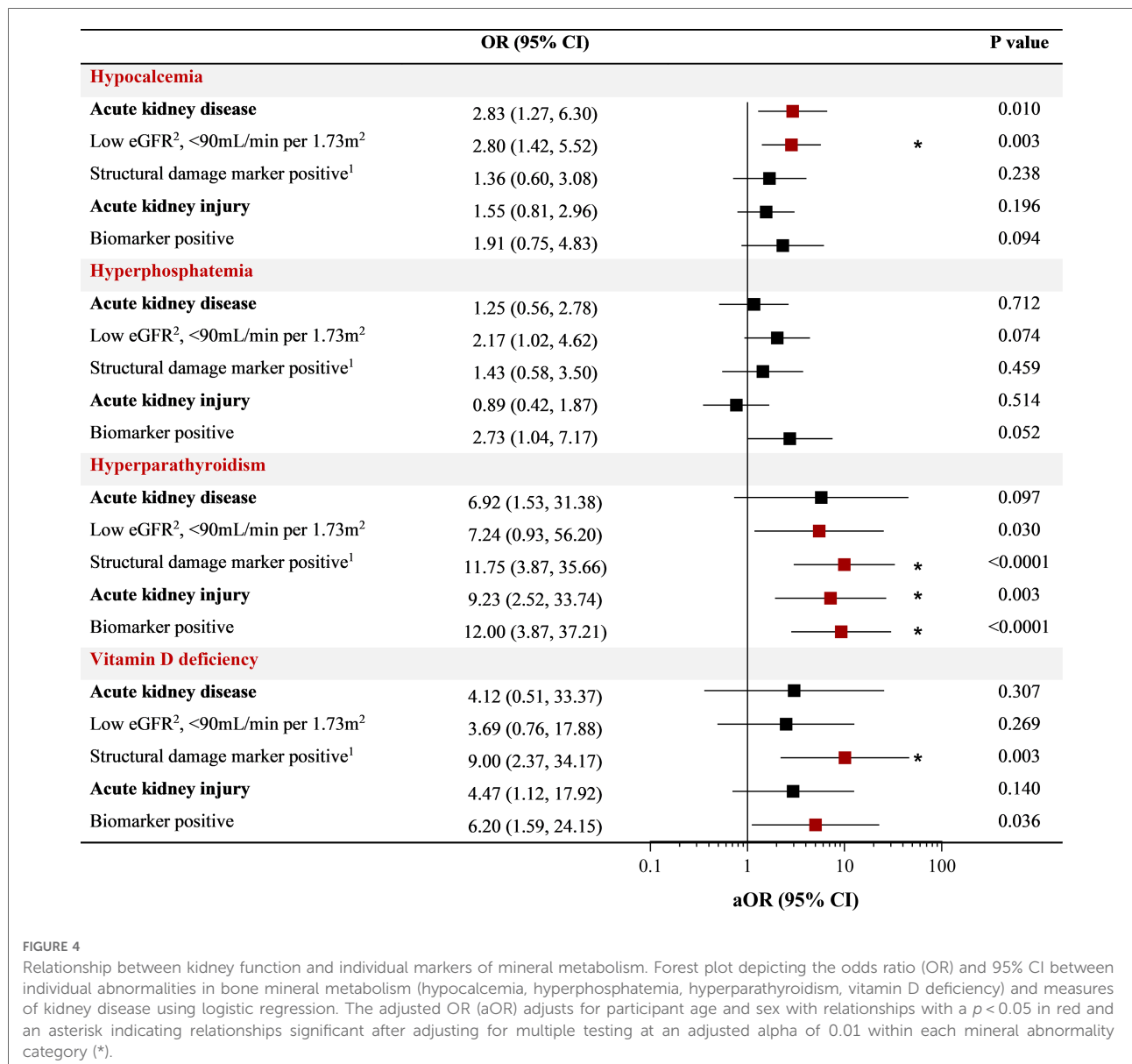
Hyperparathyroidism was associated with multiple measures of kidney function, including structural abnormalities in kidney function and AKI. This suggests that AKI may exacerbate hyperparathyroidism present in SCA (40, 41). Hyperparathyroidism occurred in 8.7% of children with SCA, was more frequent in children ≥10 years of age (13.2%) and was associated with a marked increase in both Cystatin C and urine NGAL levels (Table 2). While death was relatively infrequent in the study population, there was a substantial increase in mortality among children with hyperphosphatemia and vitamin D deficiency. Vitamin D deficiency was not seen in children <5 years of age but increased to 12.6% in children ≥10 years of age and was more frequent in the context of structural kidney injury (such as children who were biomarker positive for NGAL).

We demonstrated that FGF23 and osteopontin were elevated in sMBD and strongly associated with mortality. FGF23 is a hormone excreted by osteoblasts and osteoclasts



and regulates serum phosphate through the FGF receptor /Klotho complex in the kidneys (12). FGF23 has been found to predict mortality in patients with AKI following cardiac surgery (42), critically ill patients (43, 44), and patients with CKD (45). There is limited evidence that FGF23 may predict SCA-related mortality in adults (46), and our study provides additional evidence that increased FGF23 is associated with

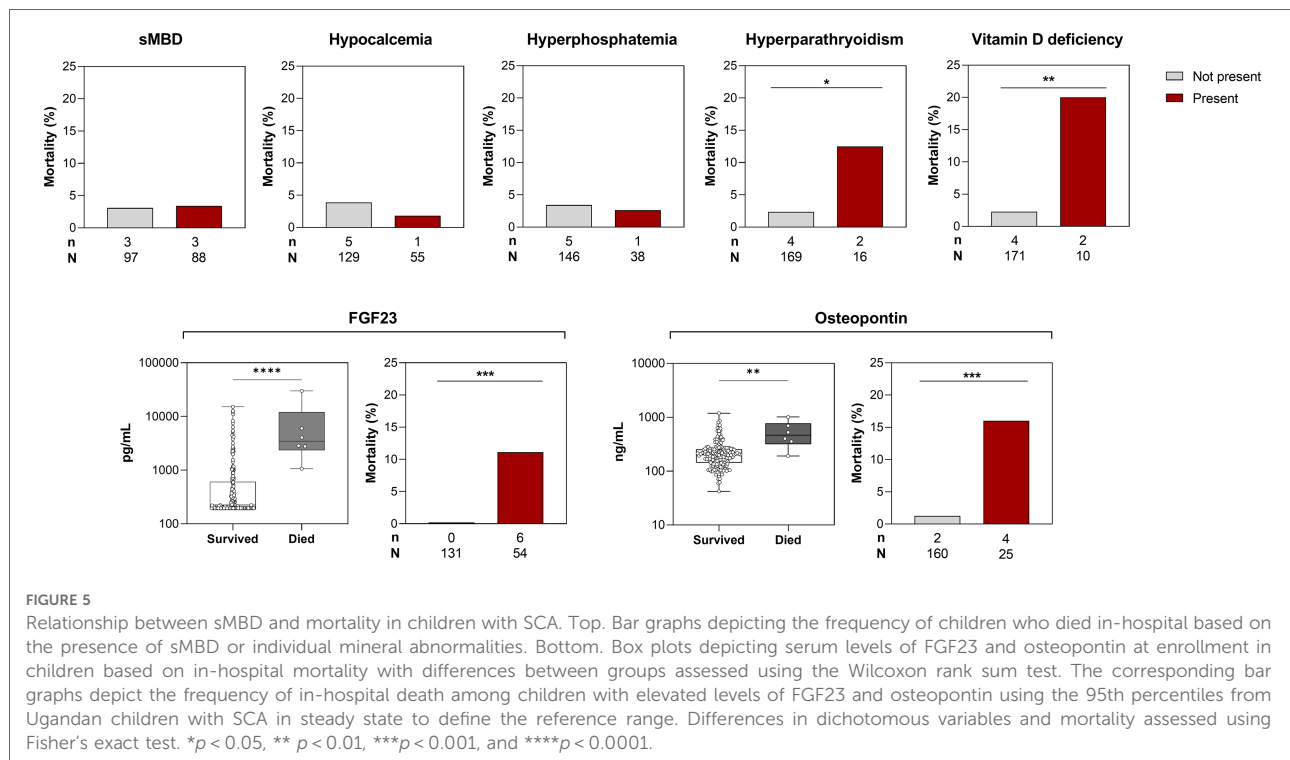
mortality in children with SCA. FGF23 reduces circulating plasma phosphate by decreasing renal reabsorption of phosphate and by blocking vitamin D activation. There was an 18-fold increase in median FGF23 levels in children with vitamin D deficiency and a negative correlation between FGF23 and 25-hydroxyvitamin D. However, we did not have measures of active 1,25 dihydroxy vitamin D to assess



whether elevated FGF23 was inversely correlated with activated vitamin D. Osteopontin is a glycoprotein expressed mostly by the bones and in varying amounts from other cells such as macrophages, smooth muscle, endothelial and epithelial cells and is involved in inhibiting bone mineralization (10, 11). Studies among patients with sepsis (47) and critical illness (48, 49) reported elevated osteopontin among patients with a fatal outcome although there is limited data on the role of osteopontin in patients with SCA. Osteopontin is upregulated in vasculature in the setting of ischemia, and may also be released from renal tubular cells during acute kidney injury which can influence acute lung injury in mice (50–52). With findings from our study that highlight the role of both FGF23 and osteopontin in predicting mortality in SCA, there is need

for further research to understand the interplay between bone biomarkers and kidney function in predicting mortality in children during a VOC.

Our study had several strengths including the comprehensive evaluation of sMBD parameters (calcium, phosphate, PTH, vitamin D, FGF23) in a relatively large cohort of children with SCA alongside a comprehensive assessment of kidney function (23, 24, 53). While the study was underpowered to assess biomarkers of mortality as there were only six deaths in the study population, the data were consistent with bone biomarkers being elevated in children who died while experiencing an acute pain crisis requiring hospitalization. A limitation of the study was that bone biopsies and bone density assessments were not conducted



due to resource constraints (15). Additional studies are needed to evaluate serum biochemical abnormalities in conjunction with biopsy findings and to delineate the dynamics of biochemical markers of sMBD in children over clinical recovery, as well as regarding long term bone changes in relation to child growth.

In summary, mineral bone abnormalities were common in children with SCA hospitalized with vaso-occlusion and were associated with the presence of kidney disease and increased with age. As children with SCA experience frequent VOC that can accelerate the development of chronic bone disease over childhood (53), routine evaluation of bone mineral disorders is warranted. Osteopontin is a biomarker of bone disease or of acute ischemic injury and is associated with sMBD and mortality and may represent a novel biomarker to assess bone health or predict mortality in SCA. Finally, this study presents critical insight into sMBD in children with SCA in Africa, where the majority of people living with SCA reside but for which there are limited data. Recommendations regarding the appropriate management of mineral bone disorders remain problematic because they differ based on location, resource availability, the context in which it occurs and is further complicated in pediatric populations with unique needs across development. Based on the early age of sMBD onset in this cohort, we recommend policies promoting earlier assessment of kidney and bone health in this vulnerable population and the development of context-specific guidelines to support evidence-based care.

## Data availability statement

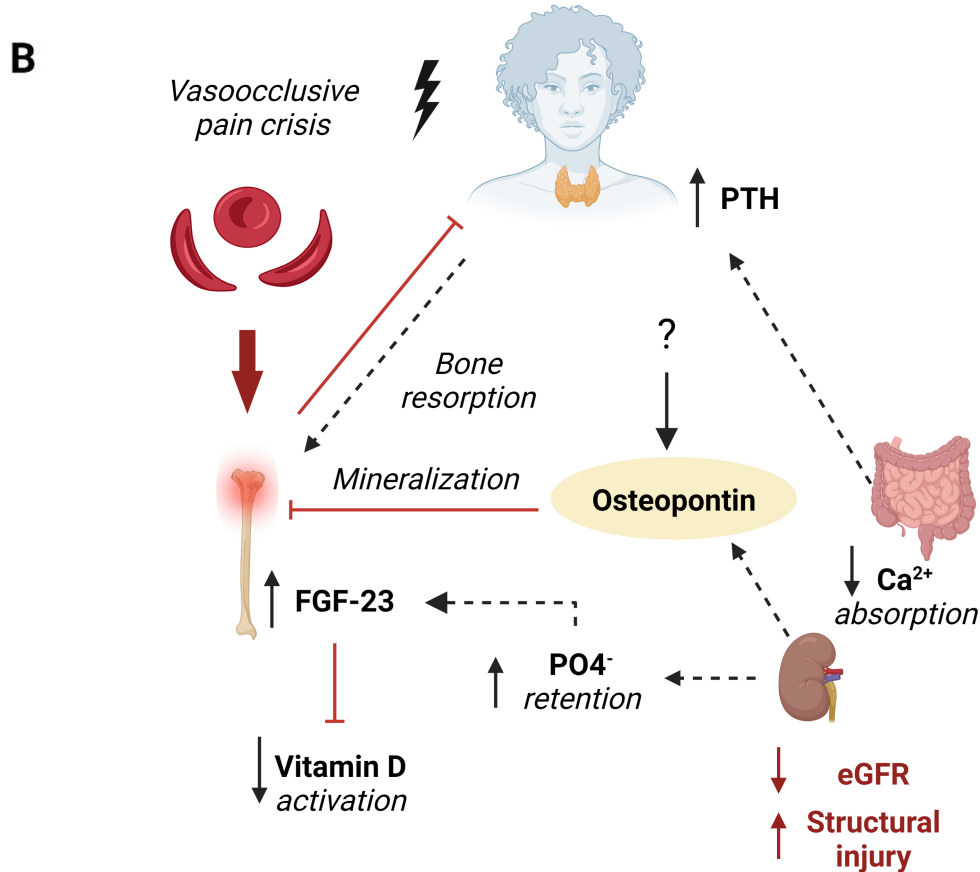
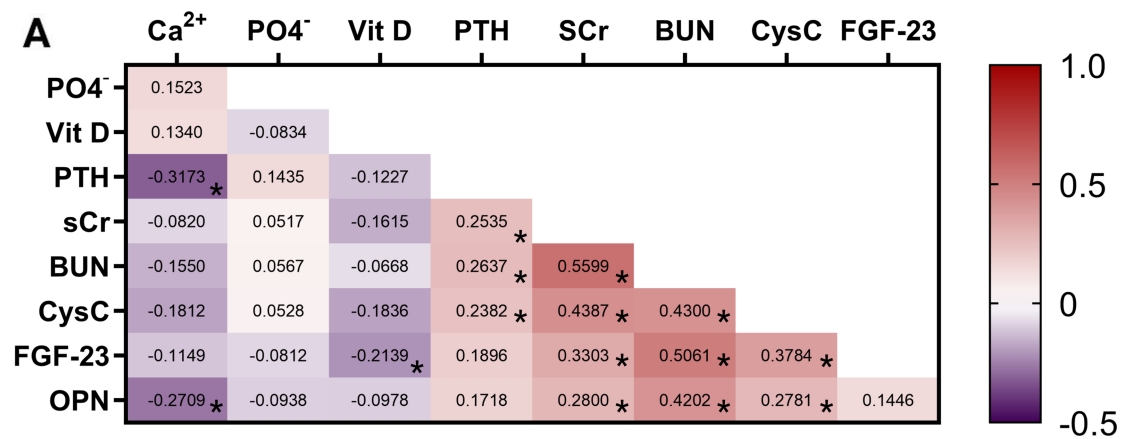
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

This study involved human participants and was reviewed and approved by the Institutional Review Board from Makerere University School of Biomedical Sciences Research and Ethics Committee (SBSREC) (first approval date 13th May 2018, IRB number; SBS-S46) and the Uganda National Council for Science and Technology (approval date 07th September 2018, approval number; HS 2443). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin and assent was obtained for children 8 years of age and older.

## Author contributions

AB, SK, JL, RK: participated in the conceptualization and design of the study. ALC and AB: designed the laboratory assessments and AB: wrote the first draft of the manuscript. Data analysis was conducted by ALC and AB. All authors participated in data interpretation, editing and writing of the



**FIGURE 6**

The bone-kidney axis during a vaso-occlusive event. (A) Heatmap depicting the Spearman correlation coefficients between serum measures of mineral metabolism and kidney function. Positive relationships are depicted in red while negative relationships are shown in purple. Relationships significant following adjustment for multiple testing are indicated with an asterisk based on an adjusted alpha of 0.005. (B) Conceptual diagram depicting the relationship between measures of bone mineral metabolism and kidney function during a vaso-occlusive crisis demonstrating the relationship depicted in the heatmap. Low serum calcium levels and increased phosphate levels lead to hyperparathyroidism with resultant bone resorption. The high phosphate levels due to reduced kidney clearance led to high FGF23 which is known to inhibit vitamin D activation. Osteopontin released by various cells and upregulated in AKI, inhibits bone mineralization. Abbreviations: calcium (Ca<sup>2+</sup>), phosphate (PO<sub>4</sub><sup>-</sup>), Vitamin D (Vit D), parathyroid hormone (PTH), serum creatinine (SCr), blood urea nitrogen (BUN), Cystatin C (CysC), fibroblast growth factor 23 (FGF23), Osteopontin (OPN). Created with BioRender.com.

manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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