whereas this does not happen for the kidney, which thus can play a role in the development of accidental metformin intoxication.

DISCLOSURE
All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL
Supplementary File (PDF)
Supplementary Methods.
Supplementary References.
CARE Checklist.

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Serum Calcification Propensity in Children on Chronic Hemodialysis

Aadil Kakajiwala1, Andreas Pasch2,3,4, Rachel Rogers5, Andrew Hoofnagle6, Sherin Meloni7, Susan L. Furth7,8, Mary B. Leonard9, Lawrence Copelovitch7,8 and Michelle R. Denburg7,8

1Division of Critical Care Medicine, Children’s National Medical Center, Washington, District of Columbia, USA; 2Calciscon AG, Nidau, Switzerland; 3Institute for Physiology and Pathophysiology, Johannes Kepler University Linz, Linz, Austria; 4Department of Internal Medicine and Nephrology, Lindenhofspital Bern, Bern, Switzerland; 5Biostatistics and Data Management Core, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; 6Department of Labortaory Medicine, The University of Washington, Seattle, Washington, USA; 7Division of Nephrology, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; 8Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA; and 9Division of Nephrology, Lucile Packard Children’s Hospital, Stanford University School of Medicine, Palo Alto, California, USA

Correspondence: Aadil Kakajiwala, Division of Critical Care Medicine, Children’s National Medical Center, 111 Michigan Ave NW, Washington District of Columbia 20010, USA. E-mail: akakajiwal@childrensnational.org

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isturbances in mineral metabolism, including elevations in parathyroid hormone (PTH), phosphate, and calcium × phosphate product, occur in patients with end-stage kidney disease (ESKD) and likely play an important role in cardiovascular morbidity and mortality.1 In patients with chronic kidney disease (CKD), serum calcium and phosphate can precipitate in the vascular smooth muscle cell of the arteries, causing vascular calcification and vessel stiffening.51 Serum calcification is a tightly regulated process inhibited by small molecules (such as magnesium, bicarbonate, and pyrophosphate) and acidic plasma proteins (including albumin and fetuin-A).2–4 Dystrophic calcification of soft tissues and vascular walls can occur when these homeostatic defenses are overwhelmed.

Pasch et al. developed a functional nanoparticle based in vitro test (T50 test) that time-dependently assesses the ex vivo calcification propensity of serum. In the presence of serum proteins, supersaturated calcium and phosphate solutions form amorphous primary calciprotein particles (CPPs). These undergo a transition in shape and size into crystalline secondary CPPs (which
induce vascular smooth muscle cell calcification) in a timed and coordinated manner. The T50 test detects the changes in laser light scattering (nephelometry) to determine the one-half maximal conversion time of primary to secondary CPPs. In adults, lower T50 has been shown to predict cardiovascular and all-cause mortality in advanced CKD, hemodialysis (HD), and kidney transplant populations, with changes in serum phosphate levels demonstrating the strongest independent association with decline in T50. In adults with CKD, higher serum calcification propensity was associated with severity and progression of coronary artery calcification.

Age-specific mortality is 30- to 150-fold higher in children with ESKD than in the general pediatric population, and cardiovascular mortality is 1000-fold higher. Coronary calcification is highly prevalent among young adult HD patients and present even in children and adolescents with ESKD. The T50 test could be a useful tool to identify and monitor vascular calcification risk in children with ESKD.

The objectives of this study were to perform the first study of serum calcification propensity in children on chronic HD, quantify short-term biologic variability in T50, and determine the associations of measures of mineral metabolism with T50.

RESULTS

We previously conducted a prospective single-center study of variability in measures of mineral metabolism in children on chronic HD. The present analysis included 209 serial serum samples from 9 participants in that study. The median age of participants was 15 years (range, 3.6–17.3 years), and 7 (78%) were male. Participants had 21 to 25 serial measures each, 96% of which were drawn on Monday and Friday.

The median T50 across the 209 samples was 328 minutes with an interquartile range of 243 to 386 minutes (range, 87 to >600). The violin plots in Figure 1 show the median, variability, and probability density of T50 and calcium × phosphate product by participant. The session-to-session (within participant) coefficient of variation for T50 was 19.1%. The participants who on average had greater serum calcification propensity (lower median T50 values) had higher median calcium × phosphate products, driven by higher phosphate concentrations (Spearman rho = −0.88, P = 0.002 for calcium × phosphate and phosphate; Spearman rho = 0.02, P = 0.97 for calcium).

Table 1 shows the results of linear mixed effects regression analysis of factors associated with calcification propensity. Univariate analysis showed that T50 was significantly longer (i.e., better) on Fridays versus Mondays (P < 0.01). Higher serum magnesium, calcium, and fetuin-A concentrations were associated with longer T50 (all P < 0.0001), whereas higher serum phosphate (P < 0.0001) and PTH (P = 0.05) were associated with shorter T50. In multivariable analysis, higher serum magnesium, calcium, and fetuin-A concentrations remained independently associated with longer T50 (all P ≤0.01), and higher serum phosphate remained independently associated with shorter T50 (P < 0.0001). After adjusting for these factors, the day of sample collection and PTH were no longer significantly associated with serum calcification propensity. Importantly, 88% of the variability in T50 was explained by this multivariable model. Given that serum albumin was only measured weekly, it was not included in the primary multivariable analysis. In univariate analysis, higher albumin was associated with longer T50: beta 72.26 (95% confidence interval, 30.04, 114.47; P = 0.001). Additional adjustment of the multivariable model shown in Table 1 for serum albumin...
Table 1. Linear mixed effects models of factors associated with serum calcification propensity (T50)

| Variable          | n (%) or median (IQR) | Beta T50 (min) | 95% CI       | P value | Beta T50 (min) | 95% CI       | P value |
|-------------------|-----------------------|----------------|--------------|---------|----------------|--------------|---------|
| Gender, male      | 7 (78)                | -0.47          | (-261.00, 220.06) | 0.85    | -              |             | -       |
| Age, yr           | 15.0 (4.6, 17.1)      | -8.23          | (-24.32, 7.87)  | 0.27    | -              |             | -       |

Day of collection

| Monday            | 96 (47) (reference) | -              |             | -       | -              |             | -       |
| Wednesday         | 8 (4)               | 45.07          | (-3.53, 93.67) | 0.07    | 16.54          | (-20.33, 53.42) | 0.35 |
| Thursday           | 1 (<1)               | -17.16         | (-152.33, 118.02) | 0.79    | -22.52         | (-123.51, 78.46) | 0.64 |
| Friday             | 102 (49)             | 30.00          | (11.32, 48.66)  | <0.01   | 10.06          | (-7.79, 27.92)  | 0.25  |
| Calcium, mg/dl    | 10.0 (9.4, 10.7)     | 36.77          | (26.10, 47.44)  | <0.0001 | 15.31          | (3.67, 26.95)   | 0.01  |
| Phosphorus, mg/dl | 5.5 (4.4, 8.0)       | -23.89         | (-30.19, -17.57) | <0.0001 | -23.83         | (-26.15, -15.51) | <0.0001 |
| Magnesium, mmol/l | 0.95 (0.90, 1.06)    | 351.48         | (253.86, 449.09) | <0.0001 | 209.66         | (122.15, 297.16) | <0.0001 |
| PTH, pg/ml        | 386.2 (185.7, 712.3) | -0.04          | (-0.08, 0.00)   | 0.05    | -0.01          | (-0.04, 0.02)   | 0.52  |
| Fetuin-A, g/l     | 0.43 (0.39, 0.48)    | 486.34         | (343.08, 629.62) | <0.0001 | 334.69         | (208.23, 481.16) | <0.0001 |

CI, confidence interval; IQR, interquartile range; PTH, parathyroid hormone.

DISCUSSION

This is the first study demonstrating the use of T50 to assess serum calcification propensity in pediatric ESKD. We evaluated twice-weekly measures of T50 in 9 children on maintenance HD over a 12-week period. We found that the performance of the T50 test in children on HD is comparable to prior data in adults. Higher serum phosphate and lower serum magnesium and fetuin-A were independently associated with greater calcification propensity. The positive association with serum calcium, however, was discrepant from prior studies in adults with CKD and ESKD.

The distribution of T50 test values in our pediatric population (median, 328) was higher than that observed in adults with ESKD. In 2785 adults on hemodialysis in the EVOLVE trial, the median T50 was 212, and 328 minutes represented the 90th percentile. Importantly, however, the distribution of T50 measures in our cohort matched that of studies in adult CKD populations: mean 329 ± 95 minutes in 184 older adults with stage 3 or 4 CKD and 321 minutes (interquartile range, 270–366) in 1274 participants in the CRIC study cohort. In adults with CKD, the risk of death was >2-fold higher among those in the lowest tertile of T50 (mean 227 ± 44) compared with those in the highest tertile (434 ± 58), independent of renal and cardiovascular risk factors and other biochemical factors, including phosphate.

Consistent with prior data in adults on chronic HD, we found that fetuin-A and magnesium, although not measured routinely in the clinical setting, were positively associated with T50, whereas phosphate was inversely associated with T50. Fetuin-A is a negative acute-phase reactant and potent inhibitor of ectopic calcification. Low fetuin-A concentrations have been demonstrated in adults on HD and associated with both cardiovascular and all-cause mortality. Fetuin-A concentrations have been shown to be significantly decreased in children on dialysis and may play an important role in the vascular calcification in pediatric ESKD. Recent data indicate that magnesium protects against high phosphate-induced vascular calcification via delayed secondary CPP maturation and randomized controlled trials of magnesium supplementation in CKD stages 3 and 4 and increasing dialysate magnesium in HD have shown improvements in T50.

The strong inverse relationship of T50 with serum phosphate is consistent with the central role of hyperphosphatemia in vascular calcification and with prior studies of calcification propensity in adults with CKD and ESKD. In these prior studies, serum calcium was either also inversely associated with T50 (P = 0.05) or not associated with T50 in multivariable analysis. In contrast, our study demonstrated that higher calcium levels were associated with increased T50 in multivariable analysis. This discrepant finding warrants further confirmation but may be related to differences in calcium balance requirements in growing children compared to adults, or to the fact that total calcium measurements reflect unbound and bound calcium, the latter predominantly to albumin. Indeed, although only available for a subset of samples, with adjustment for serum albumin, serum calcium was no longer associated with T50. Recent CKD mineral and bone disorder guidelines for CKD stage 3 to 5D emphasize avoidance of hypercalcemia in adults and maintaining serum calcium in the age-appropriate normal range for children.

Current management of CKD mineral and bone disorder is aimed at maintaining serum calcium, phosphate, and PTH within target ranges in children with
ESKD. Our prior study in this cohort highlighted the considerable session-to-session variability in measures of mineral metabolism that dictate CKD mineral and bone disorder management and impact on clinical decision making. With a coefficient of variation of 19.1%, T50 had less short-term intraindividual variability as compared to PTH (coefficient of variation, 38%). Because the calcification cascade is a functional interplay of multiple factors in addition to calcium and phosphate, many of which are not routinely measured, simply targeting the goals of these individual measures and assessing the calcium × phosphate product is inadequate to address vascular calcification risk. The T50 test is a composite functional measure that depends on the interaction of multiple nontraditional cardiovascular risk factors and has potential use not only for risk assessment but for monitoring treatment response. It has already been used to evaluate interventions to reduce serum calcification propensity in clinical trials.

Our study has several limitations. Given that this was a single-center study of 9 participants, the generalizability of the results is limited. Given the small sample size and duration of the study, we were unable to evaluate the association between calcification propensity and clinical outcomes. However, the association of the T50 test with coronary artery calcification and its ability to predict cardiovascular and all-cause mortality has been clearly demonstrated in multiple cohorts of adults with CKD and ESKD.

In summary, the performance of T50 test in children on HD is comparable to prior data in adults. Higher serum phosphate and lower serum magnesium and fetuin-A were associated with greater calcification propensity. The results of our study provide critical pilot data for larger studies evaluating T50 as a modifiable risk factor for vascular calcification in children with CKD and its potential clinical utility.

**DISCLOSURE**

AP is an employee and holds stock in Calciscon AG, Nidau, Switzerland, which commercializes the T50 test. All the other authors declared no competing interests.

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**SUPPLEMENTARY MATERIAL**

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

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