BRIEF REPORT

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Serum Levels of Vitamin C and Thiamin in Children With Suspected Sepsis: A Prospective Observational Cohort Study

Brett McWhinney, BSc[1]

Jacobus Ungerer, MD[1]

Renate LeMarsey, BSc[2,3]

Natalie Phillips, MD[2,3,4]

Sainath Raman, PhD[2,3]

Kristen Gibbons, PhD[2,3]

Luregn J. Schlapbach, MD, PhD, FCICM[2,3,5,6]

on behalf of the Rapid Acute Paediatric Infection Diagnosis in Suspected Sepsis (RAPIDS) Study Investigators

OBJECTIVES: Vitamin C and thiamin have been trialed as adjunctive therapies in adults with septic shock but their role in critically ill children is unclear. We assessed serum levels of vitamin C and thiamin in children evaluated for sepsis.

DESIGN: Single-center prospective observational study. Serum levels of vitamin C and thiamin were measured on admission and association with multiple organ dysfunction syndrome (MODS) was explored using logistic regression.

SETTING: Emergency department and PICU in a tertiary children's hospital, Queensland, Australia.

PATIENTS: Children greater than 1 month and less than 17 years evaluated for sepsis.

INTERVENTIONS: Not applicable.

MEASUREMENTS AND MAIN RESULTS: Vitamin levels were determined in 221 children with a median age of 3.5 (interquartile range [IQR] 1.6, 8.3) years. Vitamin C levels were inversely correlated with severity as measured by pediatric Sequential Organ Failure Assessment (Spearman's rho = −0.16, $p = 0.018$). Median (IQR) vitamin C levels on admission were 35.7 (17.9, 54.1) µmol/L, 36.1 (21.4, 53.7) µmol/L, and 17.9 (6.6, 43.0) µmol/L in children without organ dysfunction, single organ dysfunction, and MODS, respectively ($p = 0.017$). In multivariable analyses, low levels of vitamin C at the time of sampling were associated with greater odds of MODS (adjusted odds ratio [aOR] 3.04; 95% CI, 1.51–6.12), and vitamin C deficiency was associated with greater odds of MODS at 24 hours after sampling (aOR 3.38; 95% CI, 1.53–7.47). Median (IQR) thiamin levels were 162 (138, 192) nmol/L, 185 (143, 200) nmol/L, and 136 (110, 179) nmol/L in children without organ dysfunction, single organ dysfunction, and MODS, respectively ($p = 0.061$). We failed to identify an association between thiamin deficiency and either MODS at sampling (OR 2.52; 95% CI, 0.15–40.86) or MODS at 24 hours (OR 2.96; 95% CI, 0.18–48.18).

CONCLUSIONS: Critically ill children evaluated for sepsis frequently manifest decreased levels of vitamin C, with lower levels associated with higher severity.

KEY WORDS: ascorbic acid; child; critical care; infection; organ dysfunction; sepsis; septic shock; thiamin; vitamin C

Mortality in children with septic shock admitted to PICUs in Australia and New Zealand was around 17% in population-based studies covering 2003–2015 (1–3). In adults with sepsis, treatment with intravenous vitamin C, thiamin, and hydrocortisone has been proposed as rescue therapy. The rationale for this intervention stems from the idea that vitamin C has antioxidant and immunomodulatory effects that lead to improved microvascular function, and enhanced effectiveness of glucocorticoid and catecholamine pathways (4–6). In addition, thiamin has a key role in cellular energy

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RESEARCH IN CONTEXT

- Vitamin-based therapies, in particular, ascorbic acid (vitamin C) and thiamin, have undergone extensive trials in septic adults.
- The role of vitamin C and thiamin deficiency in children with sepsis is largely unknown.
- Given that vitamin-based therapies are being used off-label in critically ill children, we aimed to assess the association of vitamin C and thiamin levels with multiple organ dysfunction syndrome (MODS) in children with infection.

metabolism, and low levels are associated with lactatemia in critically ill patients (7, 8). However, results from randomized controlled trials (RCTs) have been predominantly negative (9), but observational data in septic children suggest potential benefit of vitamin C and thiamin (10, 11). Given the differences between children and adults in host susceptibility, immunology, epidemiology, and nutritional status, it is essential to generate pediatric data on vitamin levels in critically ill children.

Therefore, we aimed to measure vitamin C and thiamin levels in children evaluated for suspected sepsis and investigate the association between low vitamin C or thiamin levels with multiple organ dysfunction syndrome (MODS). We hypothesized that vitamin C and thiamin levels correlate with severity.

MATERIALS AND METHODS

Single-center prospective observational study nested within the "Rapid Pediatric Infection Diagnosis in Sepsis (RAPIDS)" study (approved June 06, 2019, by Children's Health Queensland Human Research Ethics Committee Brisbane, Australia; HREC/17/QRCH/85). The study was performed in accordance with the ethical standards of the committee and with the Helsinki Declaration of 1975. Reporting follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (12). Prospective consent or consent-to-continue was obtained from the parents of study participants. Children greater than 1 month old and less than 17 years old were eligible if they underwent blood culture sampling for suspected sepsis upon presentation to the emergency department (ED) or the PICU at Queensland Children's Hospital, between September 25, 2017, and February 05, 2020. Two milliliters of serum were collected concomitantly with blood cultures. Immunosuppressed patients, neonates, patients with hospital-acquired infection, and children where parents were unavailable to provide consent were excluded. Patient data were collected into the Research Electronic Data Capture database (Vanderbilt University, Nashville, TN), including demographics, history and comorbidities, infection data, and severity. Severity was assessed using the pediatric Sequential Organ Failure Assessment (pSOFA) at the time of blood sampling, and 24 hours after sampling (13, 14). Adjudication of the type and severity of infection was performed by two assessors using available clinical and microbiological information at discharge (15). Serum was centrifuged immediately at 1,000*g* for 15 minutes before aliquoting into cryotubes and transferred into a minus 80°C freezer until analysis.

Serum ascorbate concentrations were quantified with an in-house validated method using ultra-performance liquid chromatography (UPLC) coupled with a mass spectrometer. Sample preparation consisted of protein precipitation with 10% meta-phosphoric acid containing internal standard ascorbic acid-$^{13}C_6$ in a 96-well plate. Chromatographic separation was performed on an Acquity UPLC Atlantis T3 (Waters Limited, Wilmslow, United Kingdom) 3.0 µm 2.1 × 100 mm column using a water/formic acid/acetonitrile gradient over a run time of 2 minutes and the mass detected with a QDa spectrometer (Waters Corporation, Milford, MA). The measurement range starts at 0 mg/L and is linear up to 1,000 mg/L. The limit of the blank was 0.04 mg/L and the inter-run precision (coefficient of variation) was less than 7% across four quality control levels. Whole blood thiamin concentrations were quantified using Chromsystems vitamin B1 Whole Blood Kit (Munich, Germany) as per the manufacturer's instructions.

The primary outcome was defined as MODS at the time of sampling, with MODS being defined as a pSOFA score greater than 1 in at least two organ systems. Low levels of vitamin C were defined as less than 23 µmol/L and deficiency was defined as less than 11 µmol/L (16). Thiamin deficiency was defined as levels less than 70 nmol/L (8). Categorical data are reported as percentages and numbers, with continuous data

presented as medians with interquartile ranges (IQRs). Spearman's correlation coefficient was used to investigate unadjusted associations between vitamin C and thiamin levels with pSOFA. Group comparisons were undertaken using the Kruskal-Wallis test. We investigated associations of vitamin levels with MODS at the time of sampling, and at 24 hours after sampling, using bivariable and multivariable logistic regression. Backward stepwise regression was used to develop the final model; independent variables were chosen based on existing literature and those with clinical importance. In the instance where independent variables with strong clinical or statistical collinearity were available for inclusion in the multivariable model, the most clinically relevant variable was selected. All resulting models met the required assumptions. No sample size calculation was performed, and analyses were not adjusted for multiple comparisons. As such, results are considered exploratory, rather than confirmatory. All analyses were conducted using StataSE, version 17.0 (Stata Corp, College Station, TX). *p* values less than 0.05 were considered statistically significant.

RESULTS

Two hundred twenty-one children were enrolled with a median age of 3.5 (range 0.08, 16.7) years; and 101 of 221 (45.7%) were female. Comorbidities were present in 27 of 221 (12.2%) patients. Samples were collected in the ED in 144 of 221 (65%) patients, and the other 77 of 221 upon admission to PICU. The median (IQR) time from ED/PICU admission to blood sampling was 160 (IQR 88, 264) minutes. The median length of stay in PICU was 4.5 (IQR 2.5, 9.8) days. Overall, 4 of 221 patients (1.8%) died; and 82 of 221 patients (37.1%) had organ dysfunction at the time of sampling, with a median pSOFA score of 6 (IQR 3, 10).

Vitamin C levels ranged from 0 µmol/L to 151.3 µmol/L with a median (IQR) of 29.9 (13.5, 52.0) µmol/L. Vitamin C levels were inversely correlated with pSOFA scores (**Fig. 1**, Spearman's rho = –0.16, $p = 0.018$). Median (IQR) vitamin C levels were 35.7 (17.9, 54.1) µmol/L, 36.1 (21.4, 53.7) µmol/L, and 17.9 (6.6, 43.0) µmol/L in children without organ dysfunction, single organ dysfunction, and MODS at admission, respectively ($p = 0.017$, Fig. 1*A*). Low vitamin C levels were associated with MODS at time of sampling, and MODS at 24 hours, as was vitamin C deficiency in bivariate analyses (**Supplementary Table 1**, http://links.lww.com/PCC/C420). In multivariable analyses, low vitamin C levels were associated with greater odds of MODS at the time of sampling (aOR 3.04; 95% CI, 1.51–6.12), and vitamin C deficiency was associated with greater odds of MODS at 24 hours (aOR 3.38; 95% CI, 1.53–7.47). C-reactive protein was inversely correlated with vitamin C levels (Spearman`s rho = –0.538, $p < 0.001$), but we failed to identify such a correlation for thiamin levels (Spearman`s rho = –0.136, $p = 0.062$).

Thiamin levels on admission ranged from 53 nmol/L to 438 nmol/L with a median (IQR) of 161 (129, 190) nmol/L. Median (IQR) thiamin levels were 162 (138, 192) nmol/L, 185 (143, 200) nmol/L, 136 (110, 179) nmol/L in children without organ dysfunction, single organ dysfunction, and MODS, respectively ($p = 0.06$ [Fig. 1]). We failed to identify any association between thiamin deficiency and greater odds of MODS at time of sampling (OR 2.52; 95% CI, 0.15–40.86), or greater odds of MODS at 24 hours (OR 2.96; 95% CI, 0.18–48.18; Supplementary Table 1, http://links.lww.com/PCC/C420).

WHAT THIS STUDY MEANS

- In this observational study of 221 children with suspected sepsis, vitamin C and thiamin levels were comparable to reference values in the absence of organ dysfunction.
- Vitamin C levels were inversely correlated with severity as measured by pediatric Sequential Organ Failure Assessment, and low vitamin C levels on presentation were associated with MODS in multivariable analyses.
- We failed to identify an association between thiamin levels and severity.

DISCUSSION

In this prospective observational study of children evaluated for sepsis, we observed that a substantial proportion of children had vitamin C levels below the normal range. Vitamin C levels correlated inversely with pSOFA scores and low levels were associated with greater odds of MODS. For thiamin serum levels, we failed to identify any consistent association with severity.

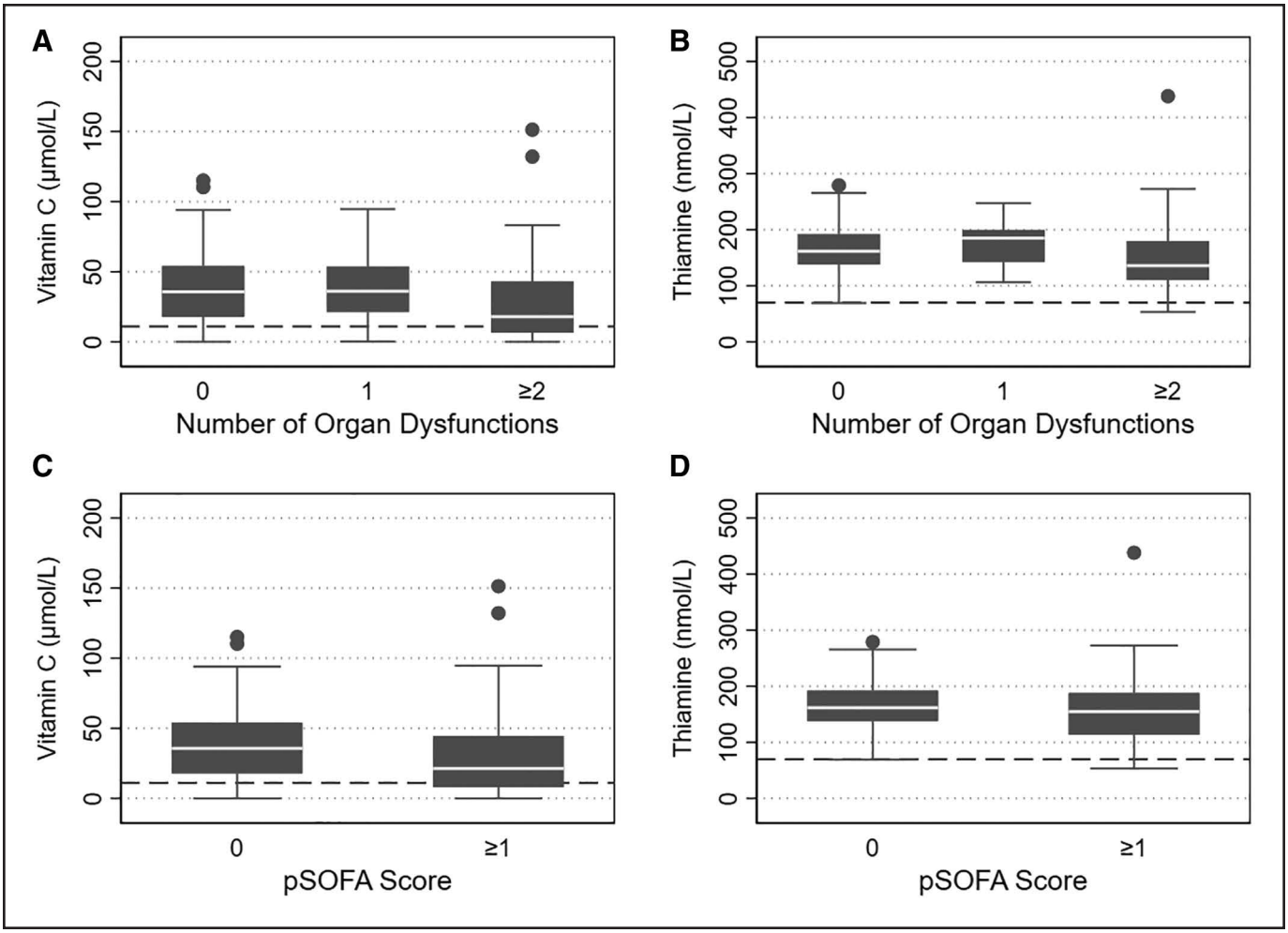

Figure 1. Serum levels of vitamin C (**A**, **C**) and thiamin (**B**, **D**) are shown for $n = 221$ children evaluated for sepsis in relation to the degree of organ dysfunction at the time of blood sampling (**A**, **B**: no organ dysfunction, $n = 139$; single organ dysfunction, $n = 19$, multiple organ dysfunction, $n = 63$), and in relation to the pediatric Sequential Organ Failure Assessment score (pSOFA, **C**, D). *Box-whisker plots* indicate the median and interquartile range. The *dashed horizontal lines* indicate the threshold to define vitamin C deficiency (< 11 µmol/L), and thiamin deficiency (< 70 nmol/L), respectively. pSOFA = pediatric Sequential Organ Failure Assessment.

Vitamins have gained interest as potential adjunctive therapies in critically ill patients (4). A network meta-analysis in adults with sepsis indicated that high-dose vitamin C treatment was associated with decreased mortality, albeit with low certainty (17). In an animal model, vitamin C used in much higher doses than in previous RCTs was effective in mitigating sepsis-related organ dysfunction (18). In children, systematic reviews indicate a high safety profile (7, 10, 19, 20). Our study was designed to characterize vitamin C and thiamin levels in a prospective cohort of children evaluated for sepsis, with different microbiological etiologies, age groups, comorbidities, and variable degrees of illness severity. Vitamin measurements were determined at admission to the ED or PICU to minimize the effect of critical illness-associated malnutrition on vitamin levels. Children with organ dysfunction in our cohort had even lower vitamin C levels compared to previous studies of general PICU patients which revealed overall decreases in micronutrients (11, 21, 22), and compared to adults with sepsis (16). Endotoxinemia during sepsis leads to widespread activation of immune and endothelial cells, platelets, and the coagulation system, all of which cause a dramatic increase in multiple oxidative mechanisms and contribute to vitamin C depletion (18).

Our study has several limitations. First, vitamin measurements were only obtained upon admission, and we were thus unable to longitudinally assess serum vitamin levels. Second, we did not obtain measures of skin-fold thickness, body mass indices, blood transfusions, or a detailed nutritional history of patients. The

cohort represents a metropolitan pediatric population in a high-income country, and we did not collect information on eligible children who were not enrolled. Hence, the vitamin levels may not be generalizable to children in less-resourced settings who may be more prone to malnutrition. Third, although we observed associations between vitamin C levels and pSOFA, a causal link between organ dysfunction and consumption of vitamin C cannot be elucidated with our data. Notably, an association of low vitamin levels with greater odds of MODS does not necessarily justify vitamin supplementation, as levels may be directly affected by factors relating to critical illness and therapies, and may reflect adaptive rather than pathological mechanisms.

In conclusion, in this cohort of children with suspected sepsis, serum vitamin C levels were frequently low. Whether vitamin C-based therapies in children with sepsis improve outcomes needs to be assessed in future RCTs.

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1 *Pathology Queensland, Queensland Health, Brisbane, QLD, Australia.*

2 *Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia.*

3 *Paediatric Intensive Care Unit, Queensland Children`s Hospital, Brisbane, QLD, Australia.*

4 *Emergency Department Queensland Children's Hospital, Brisbane, QLD, Australia.*

5 *Department of Intensive Care and Neonatology, Zurich, Switzerland.*

6 *Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland.*

Members of the Rapid Pediatric Infection Diagnosis in Sepsis (RAPIDS) Study Group are listed by study site: Queensland Children`s Hospital–Luregn J. Schlapbach, MD, PhD; Sainath Raman, MBBS, PhD; Nathalie Sharp, BSc; Natalie Phillips, MBBS, MPhil; Adam Irwin, MBChB, PhD; Ross Balch, PhD; Amanda Harley, PhD; Kerry Johnson, BSc. Gold Coast University Hospital–Shane George, MBBS, MPH; Keith Grimwood, MBChB, MD; Peter J. Snelling, MBBS, MPHTM. Townsville University Hospital–Arjun Chavan, MBBS, MD; Eleanor Kitcatt, MBBS; Luke Lawton, MBBS. Thursday Island Hospital–Allison Hempenstall, MD, MPH; Pelista Pilot. The University of Queensland–Kristen S. Gibbons, PhD; Renate Le Marsney, MPH; Antje Blumenthal, Dr. rer. Nat; Devika Ganesamoorthy, PhD; Carolyn Pardo, BSc; Jessica Kling, MD, PhD; Stephen McPherson, PhD; Anna D. MacDonald, PhD; Seweryn Bialasiewicz, PhD; Trang Pham, Meng. The University of Melbourne–Lachlan Coin, PhD.

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Dr. Schlapbach designed the study and supervised all aspects of study conduct, analyses, and wrote the first draft of the article. Mr. McWhinney and Dr. Ungerer contributed to the study design, performed vitamin measurement, and contributed to article drafting. Mr. LeMarsey and Dr. Gibbons performed the main analyses and contributed to article drafting. Drs. Phillips and Raman contributed to study design, recruitment, and article drafting. All authors have reviewed and approved the final article.

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For information regarding this article, E-mail: luregn.schlapbach@kispi.uzh.ch

The data are owned by the University of Queensland. The authors can be contacted for data inquiries.

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