Clinical Nutrition 43 (2024) 543–551

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

# Plasma and red blood cell concentrations of zinc, copper, selenium and magnesium in the first week of paediatric critical illness

K. Veldscholte [a, 1], M. Al Fify [b, c, 1], A. Catchpole [d], D. Talwar [d], J. Wadsworth [d], I. Vanhorebeek [e], M.P. Casaer [e], G. Van den Berghe [e], K.F.M. Joosten [a], K. Gerasimidis [b, 2], S.C.A.T. Verbruggen [a, *, 2]

[a] Department of Neonatal and Paediatric Intensive Care, Division of Paediatric Intensive Care, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands
[b] Human Nutrition, School of Medicine, Dentistry and Nursing, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, Glasgow, UK
[c] Clinical Nutrition Department, Faculty of Applied Medical Science, Jazan University, Saudi Arabia
[d] Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory, Department of Clinical Biochemistry, MacEwen Building, Glasgow Royal Infirmary, Castle Street, Glasgow, UK
[e] Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

ARTICLE INFO

*Article history:*
Received 18 August 2023
Accepted 8 January 2024

*Keywords:*
Micronutrients
Trace elements
Critically ill children
Nutritional therapy
Erythrocyte
Deficits

SUMMARY

*Background & aims:* Critically ill children are at risk of micronutrient deficiencies, which might lead to poor clinical outcomes. However, the interpretation of micronutrient concentrations in plasma is complicated due to age-dependent and critical illness-dependent changes. Certain red blood cell (RBC) concentrations might reflect the overall body status more reliably than plasma levels in the presence of systemic inflammatory response. This study longitudinally examined micronutrient concentrations in both plasma and RBC in critically ill children.

*Methods:* This secondary analysis of the PEPaNIC RCT investigated the impact of early versus late initiation of parenteral macronutrient supplementation in critically ill children. All children received micronutrients when EN was insufficient (<80 % energy requirements). Blood samples were obtained on days 1, 3, 5 and 7 of Paediatric Intensive Care Unit (PICU) admission. Inductively coupled plasma mass spectrometry was used to measure zinc, selenium, and copper in plasma and selenium, copper, and magnesium in RBCs. Plasma magnesium was measured with colorimetric detection. Micronutrient concentrations were compared with age-specific reference values in healthy children and expressed using Z-scores. Changes in micronutrient concentrations over time were examined using the Friedman and post hoc Wilcoxon signed-rank tests.

*Results:* For 67 critically ill children, median (Q1; Q3) age 9.5 (5.5; 13.2) years, PIM3 score −2.3 (−3.1; −0.8), samples were available at various time points during their PICU stay. For 22 patients, longitudinal samples were available. On day 1, the median plasma Z-score for zinc was −5.2 (−5.2; −2.9), copper −1.6 (−2.9; −0.2), selenium −2.6 (−3.8; −1.0), magnesium −0.2 (−1.6; 1.3), and median RBC Z-score for copper was 0.5 (−0.1; 1.3), selenium −0.3 (−1.1; 0.7), magnesium 0.2 (−0.4; 1.3). In the longitudinal analysis, plasma zinc was significantly higher on day 5 (Z-score −3.2 (−4.6; −1.4)) than on day 1 (Z-score −5.2 (−5.2; −3.0), $p = 0.032$), and plasma magnesium was significantly higher on day 3 (Z-score 1.1 (−0.7; 4.0)) than on day 1 (Z-score −0.3 (−1.6; 0.5), $p = 0.018$). Plasma copper and selenium remained stable, and the RBC concentrations of all micronutrients remained stable during the first five days.

*Conclusions:* Most patients had low plasma zinc, copper and selenium concentrations in the first week of their PICU stay, whereas they had normal to high RBC concentrations. More research is needed to examine the relationships between micronutrients and clinical outcome.



* Corresponding author. Department of Neonatal and Paediatric Intensive Care, Division of Paediatric Intensive Care, Erasmus MC Sophia Children's Hospital, Postbus 2060, 3000 CB, Rotterdam, The Netherlands.
*E-mail address:* s.verbruggen@erasmusmc.nl (S.C.A.T. Verbruggen).
[1] Contributed equally.
[2] Contributed equally.

https://doi.org/10.1016/j.clnu.2024.01.004

**Abbreviations**

| | |
|---|---|
| BFA | body mass index (BMI)-for-age |
| CRP | C-reactive protein |
| EN | enteral nutrition |
| ICU | intensive care unit |
| MN | micronutrient |
| PEPaNIC | paediatric early versus late parenteral nutrition in critical illness |
| PELOD | paediatric logistic organ dysfunction |
| PICU | paediatric intensive care unit |
| PIM3 | paediatric index of mortality 3; |
| PN | parenteral nutrition |
| RBC | red blood cell |
| RCT | randomized controlled trial; |
| REE | resting energy expenditure |
| Q1 | first quartile |
| Q3 | third quartile |
| STRONGkids | screening tool risk on nutritional status and growth in children |
| T3 | triiodothyronine |
| T4 | thyroxine |
| rT3 | reverse triiodothyronine |
| TE | trace elements |
| TSH | thyroid stimulating hormone |
| WFA | weight-for-age |

## 1. Introduction

Micronutrients (MN) are essential in metabolism, immunity, thyroid function, and antioxidative processes [1,2]. All these processes are important during critical illness, and critically ill children often present with low concentrations of MN [1,3]. Although some studies found low MN concentrations associated with more severe illness, the direct relation between low MN concentrations during the acute phase of critical illness and clinical outcomes in critically ill children is still unclear [4,5]. Moreover, to what extent critically ill children suffer from deficiencies, i.e., low concentrations combined with an objective loss of MNs in body fluids and loss of function or onset of deficiency symptoms [6], rather than only low biochemical levels, remains unknown.

Multiple factors make the assessment of MN status in critically ill children complicated. Plasma MN concentrations might be affected by the redistribution of MN due to a systemic inflammatory response, and by changes in plasma volume, e.g., due to high loads of intravenous fluids [5,7,8]. Therefore, plasma MN levels may not always reflect MN body status well during critical illness [1]. Red blood cell (RBC) MN concentrations show a positive relation with plasma MN concentrations in the absence of the APR, but are generally less affected by acute fluctuations and the APR [9]. Therefore, measuring MN concentrations in RBCs might offer a more reliable assessment of the body's MN status during critical illness, particularly long-term stores [5,10]. Moreover, current literature has demonstrated age-dependent relationships in MN concentrations, and MN requirements and metabolism might vary depending on the developmental stage [1,11]. A recent study has developed age-dependent centile charts for both plasma and RBC micronutrients [11], allowing adjustment for age and computation of Z-score values for plotting and evaluation of temporal changes over time.

This study's hypothesis is that plasma MN concentrations in critically ill children will be affected by critical illness-related changes, resulting in low plasma MN concentrations, whereas RBC MN concentrations will not be affected by these changes and thus remain within reference ranges. Therefore, this study aimed to longitudinally examine MN concentrations in plasma and RBC in critically ill children and compare this with age-dependent reference intervals to determine whether assessing MN concentrations in RBCs could provide a more reliable reflection of the body's overall MN status.

## 2. Methods

### 2.1. Study design and participants

This study is a secondary analysis of patients participating in the multicentre *paediatric early versus late parenteral nutrition in critical illness* (PEPaNIC) randomised controlled trial (RCT, ClinicalTrials.gov: NCT01536275). This secondary analysis was initiated by the Rotterdam group and executed only in Rotterdam. The study protocol and results of the RCT have been published previously [12,13]. In summary, children (age term neonate (gestational age ≥37 weeks) to 17 years) admitted to one of the participating paediatric intensive care units (PICUs) with an expected PICU stay ≥24 h, and a medium-to-high risk of malnutrition, assessed via the Screening Tool for Risk on Nutritional Status and Growth score (STRONGkids) [14], were eligible for participation. From June 18, 2012, until July 27, 2015, 1440 critically ill children were randomised to receive either early supplemental parenteral nutrition (early-PN) or to be withheld from supplemental PN during the first week of admission to the PICU (late-PN), of which 593 were included in Rotterdam. During the intervention period, data regarding the nutritional intake, treatment, and results of routine laboratory analyses were recorded daily. Written informed consent was obtained from parents or legal guardians for all participants, confirmed by the child if applicable.

### 2.2. Clinical management

Enteral nutrition (EN) was started as soon as possible in all patients and increased gradually according to the local protocol. The caloric targets were based on the body weight and calculated with the Schofield equation [15], with goals of up to 200 % of predicted resting energy expenditure (pREE) in neonates, declining to 130 % of pREE in adolescents. The EN intake was similar in both randomisation groups. Supplemental PN was provided if EN was <80 % of the daily caloric target. However, in the late-PN group, PN was only started beyond day 7 of their PICU stay. Patients from both randomisation groups received intravenous micronutrients (vitamins, minerals and trace elements (TE)) from day two until EN provided >80 % of the caloric target, either via PN or intravenous fluids [16]. Standard, weight-based trace element and vitamin mixtures were administered daily over an 8-h infusion period. These infusions comprised three commercially available products

containing TE, vitamins, and sodium chloride 0.9 %. Weight-based electrolyte mixtures were administered continuously 24 h a day. Doses were set to cover basal needs. Further details regarding the provision of micronutrients have been described previously [16].

### 2.3. Handling of blood samples

According to Dutch ethical guidelines, blood sample collections depended on the age and weight of the patient. Blood samples for this secondary analysis could only be collected in older children after obtaining the primary study samples. Blood samples were collected in heparin tubes on days 1, 3, 5, and 7 of PICU stay. The samples were centrifuged for plasma and RBC collection, after which they were stored at −80 °C. Zinc (Zn), selenium (Se), and copper (Cu) were measured in plasma, and Se, magnesium (Mg), and Cu were measured in RBCs. As zinc is highly preserved in RBCs despite the deterioration of body stores, RBC zinc is not a good biomarker of body status [11] and was therefore not measured. Measurements were performed by the Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory (STEMDRL) using inductively coupled plasma mass spectrometry, as described in Supplemental Methods 1. Plasma magnesium concentrations were routinely measured by the local clinical chemistry laboratory (colorimetric detection with xylidyl blue, Roche Diagnostics). Serum thyroid stimulating hormone (TSH) concentrations were measured using a commercially available TSH immunoradiometric assay (TSH IRMA kit; Beckman Coulter, Prague, Czech Republic). Serum total thyroxine (T4), triiodothyronine (T3), and reverse T3 (rT3) concentrations were measured using commercially available radioimmunoassays (RIA; total T4 RIA kit and total T3 RIA kit; Beckman Coulter; RIAZEN Reverse T3; ZenTech s.a., Liège, Belgium).

### 2.4. Statistical analysis

Continuous data were reported as the mean and standard deviation (SD) or as the median and first quartile (Q1) – third quartile (Q3), as appropriate. Categorical data were reported as numbers and percentages. Data analyses were performed in R Statistical Software version 4.2.1 (R core team 2022). The most important R packages for statistical analysis and data visualisation were “gamlss”, “ggpubr”, “PairedData”, “rms”, “rstatix”, “survival”, “survminer”, and “tidyverse”. The complete reference list of the used packages is shown in Supplemental Methods 2. All p-values were two-sided, and p-values of <0.05 were considered to indicate statistical significance. The statistical analyses were performed using data from the complete study population, i.e., patients with any sample available, unless stated otherwise.

The primary outcomes were the MN concentrations in plasma and RBC throughout the first week of PICU stay. Age-dependent reference intervals based on measurements in healthy children (using the same methods in the same laboratory) were available and were used to calculate Z-scores using a free access script in R (https://researchdata.gla.ac.uk/1221/) [11]. The summary statistics of the MN concentrations and Z-scores were calculated. The one-sided Wilcoxon signed-rank test was used to examine whether the distribution of Z-scores was statistically different from 0. The percentage of patients with suboptimal MN concentrations (Z-score < -2 or Z-score >2) was investigated. Additionally, as a sensitivity analysis, the summary statistics of the early-PN and late-PN groups were calculated, and differences were examined using the Mann-Whitney U test.

The secondary outcome were the longitudinal changes in MN concentrations in plasma and RBC during PICU stay. The course was only examined in a subgroup of patients with complete longitudinal measurements available, i.e., on days 1, 3 and 5. Temporal changes of MN concentrations were displayed graphically, and the Friedman and (post hoc) Wilcoxon signed-rank tests were used to examine the statistical significance of differences in MN Z-scores throughout PICU stay. As a sensitivity analysis, the summary statistics were calculated for this specific subgroup with longitudinal measurements.

The hereafter mentioned additional analyses used all data available, i.e., from the complete population. The correlations of plasma and RBC MN concentrations were examined using Spearman's rank correlation test. Other outcomes were the correlations of MN concentrations with C-reactive protein (CRP), albumin, Pediatric Logistic Organ Dysfunction (PELOD) scores [17] and weight-for-age (WFA, for children younger than 1 year) or body mass index (BMI)-for-age (BFA, for children of 1 year and older). The correlation of RBC selenium and thyroid hormones was investigated as well, as particularly selenium concentrations have been associated with thyroid function and disease [18]. The correlations were examined using Spearman's correlation test, using data from all patients on days 1 and 3. The strength of the correlation was interpreted as a correlation coefficient of 0.00–0.10, indicating a negligible, 0.10–0.39 a weak, 0.40–0.69 a moderate, 0.70–0.89 a strong, and 0.90–1.00 a very strong correlation. The association of MN concentrations and clinical outcomes was assessed using logistic regression for the odds of new infections and Cox proportional hazard analysis for time to live weaning from mechanical ventilation and time to live PICU discharge, with data censored at 90 days. To take death into account as competing risk for time-to-event outcomes, data for non-survivors were censored at 91 days. The time-to-event analyses were additionally adjusted for possible confounders: randomisation group, WFA/BFA Z-score, and PIM3 score.

The CRP concentrations were displayed graphically.

## 3. Results

For 67 patients, samples were available on one or more days, of whom 32 (48 %) were randomised to early-PN and 35 (52 %) to late-PN (Fig. 1). Twenty-two of these patients had samples available on all time points of the longitudinal analysis, i.e., on days 1, 3 and 5, with 11 patients in both randomisation groups (Fig. 1). The baseline characteristics are shown in Table 1. The median (Q1; Q3) age was 9.5 (5.5; 13.2) years, PIM3 score −2.3 (−3.1; −0.8) in the complete population of this analysis, and 11.7 (9.4; 14.1) years and PIM3 score −1.8 (−3.0; −0.5) in the subset with longitudinal samples.

### 3.1. Trace element concentrations in plasma and red blood cells of critically ill children

The summary statistics of MN concentrations in plasma and RBC in critically ill children compared to age-specific reference intervals are shown in Table 2. Zinc plasma concentrations were, on average, significantly lower than age-specific reference intervals on days 1, 3 and 5. Copper plasma concentrations were, on average, significantly lower than age-specific reference intervals on days 1 and 3, but not on days 5 and 7, whereas copper RBC concentrations were significantly higher than age-specific reference intervals on all time points.

Selenium plasma concentrations were on all days significantly lower than average, whereas selenium RBC concentrations were not significantly different than reference intervals. Magnesium plasma concentrations were significantly higher than reference intervals on day 3, but not significantly different on the other days, and magnesium RBC concentrations were on all days significantly higher than reference intervals. On days 1, 3 and 5, the majority of patients showed plasma zinc and selenium concentrations below

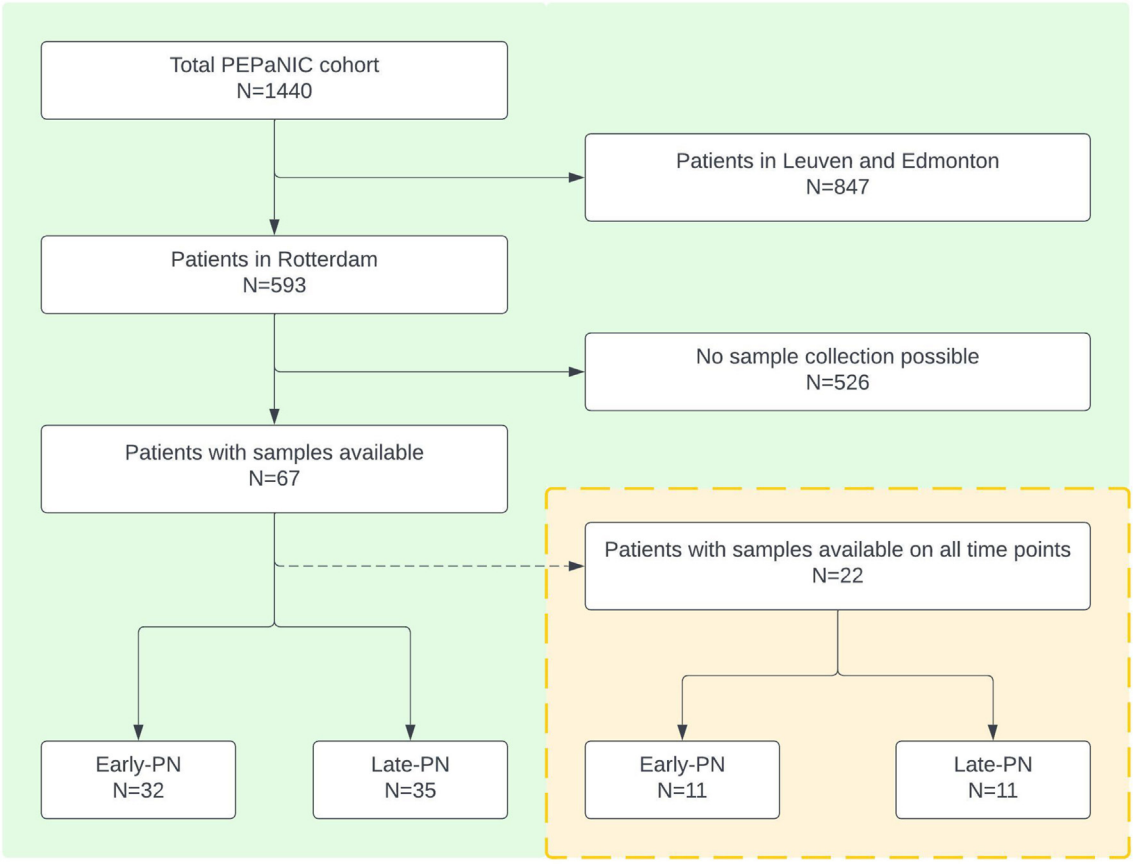


**Fig. 1.** Flowchart – inclusion of patients.
PEPaNIC: paediatric early versus late parenteral nutrition in critical illness, PN: parenteral nutrition.

**Table 1**
Baseline characteristics of the PEPaNIC RCT, patients included in this secondary analysis and patients with longitudinal samples available.

| Characteristic | Patients in PEPaNIC RCT (n = 1440) | Complete population of this analysis (n = 67) | Patients with longitudinal samples (n = 22) |
|---|---|---|---|
| Late Parenteral Nutrition - no. (%) | 717 (50 %) | 35 (52 %) | 11 (50 %) |
| Median age (Q1; Q3) - years | 1.5 (0.3; 6.3) | 9.5 (5.7; 13.1) | 11.7 (9.4; 14.1) |
| Male sex - no. (%) | 830 (58 %) | 42 (63 %) | 9 (41 %) |
| Median WFA or BFA (Q1; Q3) - Z-score[a] | −0.6 (−1.7; 0.4) | −0.1 (−1.0; 0.7) | −0.4 (−0.9; −0.0) |
| STRONGkids risk concentration - no (%)[b] | | | |
| Medium [1,2,11] | 1288 (89 %) | 61 (91 %) | 20 (91 %) |
| High [3,4] | 152 (11 %) | 6 (9 %) | 2 (9 %) |
| Median severity of illness scores (Q1; Q3)[c] | | | |
| PELOD score, first 24 h in PICU | 21 (11; 31) | 12 (11; 22) | 21 (11; 22) |
| PIM3 score | −3.5 (−4.4; −2.4) | −2.3 (−3.3; −0.9) | −1.8 (−3.0; −0.5) |
| Emergency admission - no. (%) | 783 (54 %) | 58 (87 %) | 20 (91 %) |
| Diagnostic group - no. (%) | | | |
| Surgical | | | |
| Cardiac | 547 (38 %) | 4 (6 %) | 1 (5 %) |
| Other | 426 (30 %) | 23 (34 %) | 10 (45 %) |
| Medical | | | |
| Neurologic | 103 (7 %) | 5 (7 %) | 2 (9 %) |
| Other | 364 (25 %) | 35 (52 %) | 9 (41 %) |
| Condition at admission - no. (%) | | | |
| Mechanical ventilation | 1261 (88 %) | 62 (93 %) | 20 (91 %) |
| Mechanical hemodynamic assistance[d] | 44 (3 %) | 3 (5 %) | 1 (5 %) |

[a] WFA Z-score denotes weight-for-age Z-score (for children <1 year), BFA Z-score body-mass index-for-age Z-score (for children ≥1 year) and BMI body mass index (the weight in kilograms divided by the square of the height in meters).
[b] Scores on the Screening Tool for Risk on Nutritional Status and Growth (STRONGkids) range from 0 to 5, with a score of 0 indicating a low risk of malnutrition, a score of 1–3 indicates medium risk, and a score of 4–5 indicates high risk.
[c] Pediatric Logistic Organ Dysfunction (PELOD) scores range from 0 to 71, with higher scores indicating more severe illness. A higher Pediatric Index of Mortality 3 (PIM3) score indicates a higher risk of mortality.
[d] Mechanical hemodynamic assistance is provided by extracorporeal membrane oxygenation (ECMO).

**Table 2**
Summary statistics of the micronutrients per day in critically ill children.

| Day | Micronutrient | Plasma μmol/L or mmol/L* | Plasma Z-score | P for Z ≠0 | Plasma low (Z < −2) | Plasma high (Z > 2) | RBC nmol/g Hb or μmol/L* | RBC Z-Score | P for Z≠0 | RBC low (Z < −2) | RBC high (Z > 2) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 (n = 65) | Zinc | 6.1 (4.6; 9.2) | −5.2 (−5.2; −2.9) | <0.001 | 75 % | 6 % | | | | | |
| | Copper | 13.2 (9.6; 16.0) | −1.6 (−2.9; −0.2) | <0.001 | 42 % | 3 % | 46.7 (43.1; 57.0) | 0.5 (−0.1; 1.3) | <0.001 | 3 % | 13 % |
| | Selenium | 0.6 (0.4; 0.7) | −2.6 (−3.8; −1.0) | <0.001 | 63 % | 0 % | 5.3 (4.8; 6.1) | −0.3 (−1.1; 0.7) | 0.14 | 5 % | 3 % |
| | Magnesium | 0.8 (0.7; 0.9) | −0.2 (−1.6; 1.3) | 0.95 | 19 % | 22 % | 8.1 (7.4; 9.0) | 0.2 (−0.4; 1.3) | 0.04 | 3 % | 13 % |
| 3 (n = 37) | Zinc | 8.5 (5.8; 11.0) | −3.6 (−5.2; −0.7) | <0.001 | 59 % | 3 % | | | | | |
| | Copper | 15.3 (11.7; 18.4) | −0.8 (−1.9; 0.6) | 0.04 | 24 % | 8 % | 53.4 (45.8; 56.3) | 1.1 (0.4; 1.7) | <0.001 | 0 % | 16 % |
| | Selenium | 0.5 (0.4; 0.7) | −2.8 (−3.8; −1.4) | <0.001 | 65 % | 3 % | 5.4 (5.0; 6.0) | −0.2 (−0.8; 0.5) | 0.57 | 8 % | 3 % |
| | Magnesium | 0.8 (0.8; 0.9) | 0.6 (−0.1; 2.8) | 0.004 | 3 % | 30 % | 8.4 (7.7; 8.9) | 0.7 (−0.1; 1.4) | 0.003 | 0 % | 14 % |
| 5 (n = 23) | Zinc | 8.4 (7.7; 10.3) | −3.3 (−4.6; −1.5) | <0.001 | 70 % | 9 % | | | | | |
| | Copper | 15.3 (13.0; 18.0) | −0.3 (−1.5; 0.6) | 0.33 | 0 % | 13 % | 51.8 (45.5; 58.4) | 1.1 (0.4; 1.7) | <0.001 | 0 % | 14 % |
| | Selenium | 0.5 (0.4; 0.7) | −2.6 (−3.9; −1.2) | <0.001 | 61 % | 0 % | 5.6 (5.3; 6.0) | 0.1 (−0.3; 0.6) | 0.59 | 5 % | 0 % |
| | Magnesium | 0.8 (0.8; 0.9) | 0.8 (−0.5; 2.1) | 0.10 | 5 % | 25 % | 8.6 (8.0; 9.1) | 1.2 (0.5; 1.7) | 0.002 | 0 % | 9 % |
| 7 (n = 11) | Zinc | 11.5 (9.2; 14.6) | −0.4 (−2.7; 1.3) | 0.52 | 36 % | 27 % | | | | | |
| | Copper | 20.0 (13.0; 27.1) | 1.1 (−1.4; 3.2) | 0.41 | 18 % | 27 % | 46.7 (43.8; 49.5) | 0.4 (0.2; 1.1) | 0.04 | 9 % | 9 % |
| | Selenium | 0.7 (0.5; 1.0) | −0.7 (−2.9; −1.6) | 0.04 | 45 % | 0 % | 5.7 (5.5; 5.9) | 0.1 (−0.1; 0.5) | 0.52 | 0 % | 0 % |
| | Magnesium | 0.8 (0.8; 0.9) | 0.3 (−0.5; 2.5) | 0.38 | 10 % | 40 % | 8.7 (8.2; 9.1) | 1.4 (0.8; 1.6) | 0.007 | 0 % | 9 % |

Z-scores were calculated based on reference intervals in healthy children [11]. The one-sided Wilcoxon signed-rank test was used to examine whether the distribution of Z-scores was statistically different from 0. *Plasma zinc, copper, and selenium are measured in μmol/L, and magnesium in mmol/L, red blood cell zinc, copper, and selenium are measured in nmol/g Hb, and magnesium in μmol/L.

reference intervals (Z-score < -2 on day 1: 75 % and 63 %, day 3: 59 % and 65 %, day 5: 70 % and 61 %, respectively). A substantial amount of patients also showed low plasma copper concentrations (Z-score < -2) on day 1 (42 %), but to a lesser extent on the other days. Only a few patients showed levels below reference intervals in any of the RBC concentrations.

The summary statistics of MN concentrations in the subgroup with longitudinal data are shown in Supplemental Table 1 and were similar to the entire cohort. No difference between the early-PN and late-PN groups was observed regarding MN concentrations on days 3 and 5 (Supplemental Table 2).

### 3.2. The course of micronutrients during PICU stay

Temporal changes in MN concentrations are shown in Fig. 2. Plasma zinc was significantly higher on day 5 than on day 1 (p = 0.032, Fig. 2). The concentrations of copper and selenium in plasma and RBC remained stable from admission to day 5 of PICU stay (Fig. 2). The magnesium concentration in plasma was significantly higher on day 3 than on day 1 (p = 0.018), but remained stable in RBC (Fig. 2).

### 3.3. The correlations between micronutrients and clinical characteristics

The correlations between plasma and RBC Z-scores were weak to moderate (Supplemental Table 3). Plasma zinc and selenium were inversely correlated with plasma CRP and PELOD score and positively correlated with albumin on day 1 (plasma zinc on day 1 with CRP: ρ −0.38, p = 0.004, PELOD: ρ −0.25, p = 0.047, albumin: ρ 0.48, p=<0.001, plasma selenium on day 1 with CRP: ρ −0.41, p = 0.002, PELOD: ρ −0.32, p = 0.010, albumin: ρ 0.70, p < 0.001) but not with WFA/BFA, plasma zinc on day 3 was inversely correlated with CRP and positively with albumin (plasma zinc on day 3 with CRP: ρ −0.58, p=<0.001, albumin: ρ 0.55, p = 0.001) but not with PELOD and WFA/BFA, and plasma selenium on day 3 was inversely correlated with CRP and PELOD score and positively correlated with albumin (plasma selenium on day 3 with CRP: ρ −0.58, p < 0.001, PELOD: ρ −0.47, p = 0.003, albumin: ρ 0.63, p < 0.001, Supplemental Table 4) but not with WFA/BFA. In contrast, RBC selenium was not significantly correlated with any clinical characteristics (Supplemental Table 4). Copper and magnesium concentrations in plasma and RBC were not significantly correlated with any clinical characteristics, except for a positive correlation between plasma copper on day 3 and WFA/BFA (ρ 0.37, p = 0.036) (Supplemental Table 4).

Regarding the correlation between RBC selenium and thyroid hormones, RBC selenium on day 1 was positively correlated with rT3 concentrations (ρ 0.28, p = 0.038) but not with other thyroid hormones (Supplemental Table 5). RBC selenium was not significantly correlated with any thyroid hormone on day 3 (Supplemental Table 5).

Apart from significantly higher odds of live weaning from mechanical ventilation with higher selenium plasma Z-score on day 3 (HR 1.27, 95 % CI 1.06; 1.52, p = 0.011), none of the MN concentrations on days 1 or 3 were univariately associated with the likelihood of live weaning from mechanical ventilation, live PICU discharge, or the odds of new infections (Supplemental Table 6). In the adjusted analysis, a higher plasma selenium Z-score on day 3 still was associated with higher odds of live weaning from mechanical ventilation (adjusted HR 1.62, 95 % CI 1.19; 2.20, p = 0.002), and also higher plasma copper z-score was associated with higher odds of live weaning from mechanical ventilation (adjusted HR 1.45, 95 % CI 1.07; 1.95, p = 0.015). The rest of the MN concentrations were not significantly associated with odds of live weaning from mechanical ventilation or PICU discharge in the analyses adjusted for possible confounders.

The CRP concentrations per day are shown in Supplemental Fig. 1.

## 4. Discussion

This study investigated the concentrations of micronutrients in critically ill children, both in plasma and RBC, in their first week in the PICU. The study's findings indicate zinc and selenium plasma concentrations were lower than reference intervals in most critically ill children, with a significant proportion also showing low concentrations in copper. However, RBC concentrations of these micronutrients remained within reference interval or were even high throughout the first week of PICU stay. The study also found inverse correlations between plasma zinc and selenium with CRP

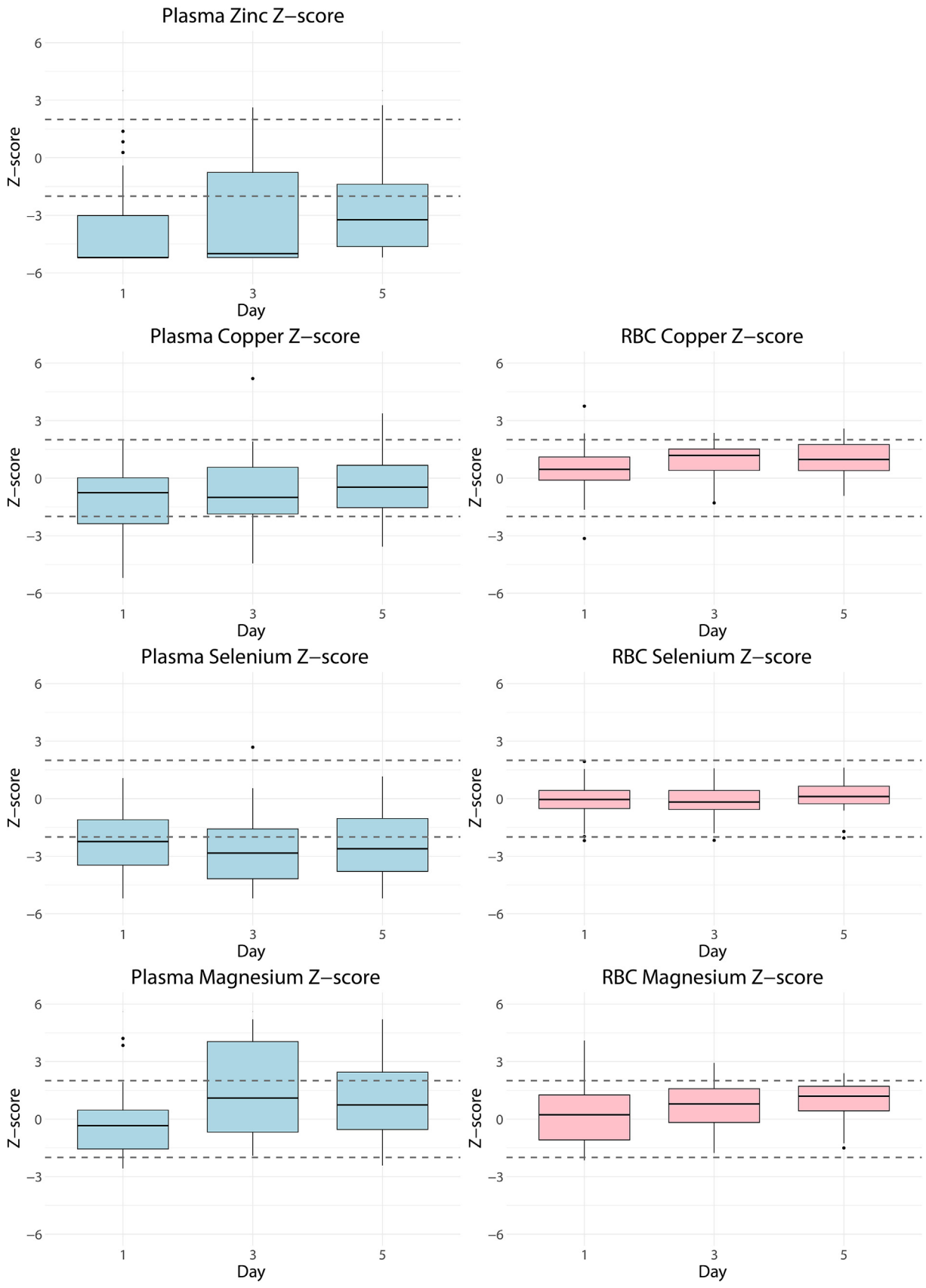


**Fig. 2.** Boxplots of the course of the micronutrient concentrations in plasma and red blood cells in patients with longitudinal samples (n = 22)
The area between the dashed lines represents the reference interval in healthy children [11]. The course was only examined in a subgroup of patients with complete longitudinal measurements available on days 1, 3 and 5 (n = 22).
RBC: red blood cell.

and PELOD score and positive correlations with albumin concentrations. These correlations were not observed with RBC concentrations. These findings support our hypothesis that plasma MN concentrations in critically ill children are affected by critical illness-related changes, whereas RBC MN concentrations remain unaffected.

### 4.1. Comparison of micronutrient concentrations with other studies

The study's findings are consistent with other studies that have reported lower plasma or serum MN concentrations in critically ill children compared to healthy controls [3,19–26]. For example, multiple studies found lower plasma zinc and selenium concentrations in critically ill children, with one study reporting 91 % of critically ill children with a systemic inflammatory response had plasma selenium concentrations below the lower limit of reference interval on admission [26]. The current study found plasma concentrations below reference intervals in 63 % of critically ill children on day 1. The differences can be attributed to factors such as age [11], systemic inflammatory response, and regional selenium deficiencies. The study's findings of an initial drop in plasma copper align with previous studies in healthy adults undergoing elective surgery and in critically ill children [3,9]. The hypothesis is that this initial drop is the result of ceruloplasmin being redistributed from the blood to the interstitium [9]. Moreover, Valla et al. found lower plasma copper concentrations in critically ill children with more severe oxidative stress and hypothesized that these lower copper concentrations are a reflection of copper being mobilised to serve as a coenzyme of superoxide dismutase [3].

Data regarding MN in RBC are scarce and conflicting. One study in critically ill children with a systemic inflammatory response reported a 31 % prevalence of low RBC selenium on PICU day 1 [24], while the current study reported a prevalence of up to 8 %. However, direct comparisons are challenging due to differences in methods and adjustments. Studies in critically ill adults have also shown inconsistent results for RBC selenium concentrations [27–29].

The findings of normal to high RBC magnesium concentrations are contrary to an Indian study that found an incidence of 17 % hypomagnesaemia in RBC in critically ill children [30]. However, the Indian study used reference intervals from adults and did not provide concentrations, which makes a direct comparison impossible. Studies in critically ill adults have generally found normal to high RBC magnesium [31,32], which aligns with the current study's findings.

This study is the first to investigate RBC copper concentrations in critically ill children. Only one study in critically ill adults examined RBC copper and found values mostly within reference intervals or below reference intervals [28], whereas the current study found normal to high concentrations.

The discrepancies regarding RBC concentrations might be partly explained by differences in nutritional status or intake of MN before the study, as RBC concentrations reflect long-term body status during health. Although RBC MN concentrations were not correlated with nutritional status upon admission in the current study, this does not imply there is no correlation between reduced intake and RBC MN concentrations, as reduced intake of MN is not necessarily accompanied by weight loss.

### 4.2. Critical illness and micronutrient concentrations

Several processes during critical illness affect plasma MN concentrations, such as the release of cytokines and capillary leak that accompany the systemic inflammatory response. These processes result in the redistribution of MN from the circulation to tissues, organs, and interstitial fluid, resulting in low plasma MN concentrations [1,5,28]. The study's data support these hypotheses, with inverse correlations of plasma zinc and selenium with CRP as a marker of the APR and positive correlations of plasma zinc and selenium with plasma albumin. However, RBC concentrations of MN were not affected by these processes, as hypothesised.

The current study found no strong or consistent indications for an association between MN concentrations and clinical outcomes or thyroid function. Nevertheless, as MN are essential in multiple important processes, it is unlikely that MN status does not impact clinical outcomes at all.

### 4.3. Low plasma micronutrients – maladaptation or adaptation?

Whereas some studies have described low plasma MN concentrations as being related to impaired outcomes, strong evidence for the causal relation between MN concentrations and clinical outcomes is still lacking. Apart from one study that found no beneficial effects on infections and sepsis of daily supplementation with zinc, selenium, glutamine and metoclopramide [33], no intervention studies investigating the impact of MN supplementation on clinical outcomes have been performed in critically ill children. In critically ill adults, consistent and robust evidence for the beneficial effects of supplementation of MN on hard endpoints, e.g., mortality, is still lacking as well [34,35].

The question remains whether this lack of strong evidence is due to methodological issues, either in the supplementation, in the design of the studies, or due to wrong assumptions. Although speculative, an alternative explanation might be that the low plasma MN concentrations result from an adaptive, beneficial response during critical illness. This might explain the normal or even high RBC concentrations. Hence, the association between low plasma MN concentrations and impaired outcome might reflect the low plasma concentrations being a marker for the severity of illness and a manifestation of the intensity of such adaptive response rather than a causal relationship and a reflection of maladaptation. One might wonder whether the supplementation of MN has added value in this case.

Critical illness might lead to increased MN requirements due to hypermetabolism and increased losses, e.g., by diarrhoea or blood loss [5,36]. Moreover, providing sufficient MN might be difficult, e.g., due to feeding intolerance, fluid restrictions [5,36], or insufficient MN prescription [16]. Commercially available products of MN, as also used in the current study, do not meet all ESPGHAN recommendations [16]. These issues together might lead to deficiencies, when also the adaptation process might have its limits. Electrolytes, such as magnesium, are needed in higher quantities than the TE, so problems might occur faster for electrolytes than for the TE. Probably the duration and severity of illness, the duration and extent of reduced MN intake and the specific MN might all affect whether and when a deficiency will occur.

### 4.4. Strengths and limitations

The most important strengths of this study are the availability of RBC concentrations and age-specific reference intervals based on samples measured in the same laboratory [11]. Moreover, this study provides an overview of the natural course in critically ill children who are not actively supplemented but only receive MN in doses covering basal needs.

Due to the limitations, this study should be regarded as a hypothesis-generating study. One of the limitations is the lack of neonates and infants due to limitations on the amount of blood that

could be taken according to ethical guidelines. It cannot be ruled out that results might be different in younger children than our studied population. Secondly, as is common in PICU studies, the number of patients decreased over time. Therefore, the results of this study apply mainly to the acute phase of critical illness. Thirdly, the single-centre design might hamper the generalisability of the results to centres with different nutritional and glycaemic control protocols. Fourthly, data on MN intake prior to critical illness was lacking. Fifth, although the study's findings indicate that RBC concentrations might reflect the overall body status more reliably than plasma concentrations during critical illness, the long half-life of RBC implies that RBC concentrations could serve as a marker for long-term stores rather than for assessing acute deficiencies or recent supplementation [7]. Moreover, RBC concentrations might not be used in patients who have received blood products [7]. In addition, as the study was not designed to investigate this specific subject, the study was limited by its sample size. The study might have been underpowered to e.g., detect differences between the early-PN and late-PN group, longitudinal changes, and associations with clinical outcomes. Lastly, as we did not have data regarding MN losses and did not measure functional tests of MN, we cannot distinguish between low biochemical concentrations and deficiencies. Therefore, whether the observed changes are a physiological adaptation or maladaptation remains questionable.

### 4.5. Future directions

Studies in larger patient cohorts investigating the impact of MN status on clinical outcomes are warranted, preferably also including neonates and infants and children with long PICU stay (>2 weeks). In addition to RBC measurements, measuring the functionality of MN by assessing its functions, such as copper-zinc superoxide dismutase (SOD) for copper or glutathione peroxidase (GSHpx) activity for selenium, might be considered.

## 5. Conclusion

This study found that critically ill children often showed plasma zinc, selenium and copper concentrations below reference intervals, whereas the concentrations of these micronutrients in red blood cells were normal to high compared with age-specific reference intervals in the first week of PICU stay. We speculate that this results from an adaptation response during critical illness. Measuring MN biomarkers in the presence of a systemic inflammatory response is likely unreliable and may lead to bad practice. Therefore, we advocate using the proposed ESPGHAN recommendations [7]. As this was a hypothesis-generating study, more studies in larger samples are warranted.

## Funding statement

This study was supported by ERC Advanced Grants AdvG-2012-321670 from the Ideas Program of the European Union 7th framework program and AdvG-2017-785809 to GVdB; by the Methusalem programme of the Flemish Government (through the University of Leuven to GVdB and IV, METH14/06); by the Agency for Innovation through Science and Technology, Flanders, Belgium (through the University of Leuven to GVdB, IWT/110685/TBM); by the Sophia Children's Hospital Foundation (SSWO) to SCATV; by the Stichting Agis Zorginnovatie to SCATV; by the Erasmus Trustfonds to SCATV; and by a European Society for Clinical Nutrition and Metabolism (ESPEN) research grant to SCATV. The funders of the study had no role in the design of the study, the collection, analysis, and interpretation of data, or in the writing of the manuscript.

## Conflict of interest

KG has received research grants, hospitality and speakers fees from Nestle Health Science, Danone-Nutricia, Baxter, Abbott, Janssen, Abbvie, Servier, Mylan and DrFalk. SCATV holds an unrestricted research agreement funded by Danone Nutricia Research, The Netherlands.

## Author contributions

K. Veldscholte: conceptualization, methodology, formal analysis, data curation, writing – orginal draft, visualisation, project administration; M. Al Fify: methodology, validation, investigation, writing-review and editing; A. Catchpole: methodology, writing-review and editing, resources; D. Talwar: methodology, writing-review and editing, resources; J. Wadsworth: methodology, writing-review and editing, resources; I. Vanhorebeek: writing-review and editing, resources; M.P. Casaer: writing-review and editing; G. Van den Berghe; writing-review and editing, resources; K.F.M. Joosten: conceptualization, writing-review and editing, resources; K. Gerasimidis: conceptualization, writing-review and editing, resources, supervision; S.C.A.T. Verbruggen: conceptualization, methodology, writing-review and editing, resources, supervision.

## Acknowledgements

We are very grateful to the children and their parents or guardians participating in the PEPaNIC study. We thank the research team members and clinical staff involved in the execution of the PEPaNIC study. Moreover, we would like to thank Joke Dunk for her help in setting up the Research Collaboration Agreement and sending the samples.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.01.004.

## References

[1] Dao DT, Anez-Bustillos L, Cho BS, Li Z, Puder M, Gura KM. Assessment of micronutrient status in critically ill children: challenges and opportunities. Nutrients 2017;9(11).
[2] Shenkin A. Micronutrients in health and disease. Postgrad Med 2006;82(971):559–67.
[3] Valla FV, Bost M, Roche S, Pitance M, Cuerq C, Ridout J, et al. Multiple micronutrient plasma level changes are related to oxidative stress intensity in critically ill children. Pediatr Crit Care Med 2018;19(9):e455–63.
[4] Ong C, Han WM, Wong JJ, Lee JH. Nutrition biomarkers and clinical outcomes in critically ill children: a critical appraisal of the literature. Clin Nutr 2014;33(2):191–7.
[5] Marino LV, Valla FV, Beattie RM, Verbruggen S. Micronutrient status during paediatric critical illness: a scoping review. Clin Nutr 2020;39(12):3571–93.
[6] Shenkin A, Berger MM. Micronutrients: a low blood concentration is not equivalent to deficiency. Clin Nutr 2022;41(11):2562–4.
[7] Gerasimidis K, Bronsky J, Catchpole A, Embleton N, Fewtrell M, Hojsak I, et al. Assessment and interpretation of vitamin and trace element status in sick children: a position paper from the European society for paediatric gastroenterology hepatology, and nutrition committee on nutrition. J Pediatr Gastroenterol Nutr 2020;70(6):873–81.
[8] Duncan A, Talwar D, McMillan DC, Stefanowicz F, O'Reilly DS. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. Am J Clin Nutr 2012;95(1):64–71.
[9] Oakes EJ, Lyon TD, Duncan A, Gray A, Talwar D, O'Reilly DS. Acute inflammatory response does not affect erythrocyte concentrations of copper, zinc and selenium. Clin Nutr 2008;27(1):115–20.
[10] Berger MM, Talwar D, Shenkin A. Pitfalls in the interpretation of blood tests used to assess and monitor micronutrient nutrition status. Nutr Clin Pract 2023;38(1):56–69.

[11] Al Fify M, Nichols B, Arailoudi Alexiadou L, Stefanowicz F, Armstrong J, Russell RK, et al. Development of age-dependent micronutrient centile charts and their utility in children with chronic gastrointestinal conditions at risk of deficiencies: a proof-of-concept study. Clin Nutr 2022;41(4):931–6.
[12] Fivez T, Kerklaan D, Mesotten D, Verbruggen S, Wouters PJ, Vanhorebeek I, et al. Early versus late parenteral nutrition in critically ill children. N Engl J Med 2016;374(12):1111–22.
[13] Fivez T, Kerklaan D, Verbruggen S, Vanhorebeek I, Verstraete S, Tibboel D, et al. Impact of withholding early parenteral nutrition completing enteral nutrition in pediatric critically ill patients (PEPaNIC trial): study protocol for a randomized controlled trial. Trials 2015;16:202.
[14] Hulst JM, Zwart H, Hop WC, Joosten KF. Dutch national survey to test the STRONGkids nutritional risk screening tool in hospitalized children. Clin Nutr 2010;29(1):106–11.
[15] Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39(Suppl 1):5–41.
[16] Eveleens RD, Witjes BCM, Casaer MP, Vanhorebeek I, Guerra GG, Veldscholte K, et al. Supplementation of vitamins, trace elements and electrolytes in the PEPaNIC Randomised Controlled Trial: composition and preparation of the prescription. Clin Nutr ESPEN 2021;42:244–51.
[17] Leteurtre S, Duhamel A, Salleron J, Grandbastien B, Lacroix J, Leclerc F, et al. PELOD-2: an update of the PEdiatric logistic organ dysfunction score. Crit Care Med 2013;41(7):1761–73.
[18] Ventura M, Melo M, Carrilho F. Selenium and thyroid disease: from pathophysiology to treatment. Internet J Endocrinol 2017;2017:1297658.
[19] Heidemann SM, Holubkov R, Meert KL, Dean JM, Berger J, Bell M, et al. Baseline serum concentrations of zinc, selenium, and prolactin in critically ill children. Pediatr Crit Care Med 2013;14(4):e202–6.
[20] Saleh NY, Abo El Fotoh WMM. Low serum zinc level: the relationship with severe pneumonia and survival in critically ill children. Int J Clin Pract 2018;72(6):e13211.
[21] Negm FF, Soliman DR, Ahmed ES, Elmasry RA. Assessment of serum zinc, selenium, and prolactin concentrations in critically ill children. Pediatr Health Med Therapeut 2016;7:17–23.
[22] Cvijanovich NZ, King JC, Flori HR, Gildengorin G, Wong HR. Zinc homeostasis in pediatric critical illness. Pediatr Crit Care Med 2009;10(1):29–34.
[23] Wang G, Feng X, Yu X, Xu X, Wang D, Yang H, et al. Prognostic value of blood zinc, iron, and copper levels in critically ill children with pediatric risk of mortality score III. Biol Trace Elem Res 2013;152(3):300–4.
[24] de Almeida CB, Leite HP, Lopes Junior E, Konstantyner T, Franco M. Erythrocyte and plasma selenium in children with acute inflammatory response. J Trace Elem Med Biol 2022;74:127068.
[25] Broman M, Lindfors M, Norberg A, Hebert C, Rooyackers O, Wernerman J, et al. Low serum selenium is associated with the severity of organ failure in critically ill children. Clin Nutr 2018;37(4):1399–405.
[26] Iglesias SB, Leite HP, Paes AT, Oliveira SV, Sarni RO. Low plasma selenium concentrations in critically ill children: the interaction effect between inflammation and selenium deficiency. Crit Care 2014;18(3):R101.
[27] Cirino Ruocco MA, Pacheco Cechinatti ED, Barbosa Jr F, Navarro AM. Zinc and selenium status in critically ill patients according to severity stratification. Nutrition 2018;45:85–9.
[28] Stefanowicz F, Gashut RA, Talwar D, Duncan A, Beulshausen JF, McMillan DC, et al. Assessment of plasma and red cell trace element concentrations, disease severity, and outcome in patients with critical illness. J Crit Care 2014;29(2):214–8.
[29] Stefanowicz FA, Talwar D, O'Reilly DS, Dickinson N, Atkinson J, Hursthouse AS, et al. Erythrocyte selenium concentration as a marker of selenium status. Clin Nutr 2013;32(5):837–42.
[30] Singhi SC, Singh J, Prasad R. Hypo- and hypermagnesemia in an Indian pediatric intensive care unit. J Trop Pediatr 2003;49(2):99–103.
[31] Huijgen HJ, Soesan M, Sanders R, Mairuhu WM, Kesecioglu J, Sanders GT. Magnesium levels in critically ill patients. What should we measure? Am J Clin Pathol 2000;114(5):688–95.
[32] Saur P, Niedmann PD, Brunner E, Kettler D. Do intracellular, extracellular or urinary magnesium concentrations predict renal retention of magnesium in critically ill patients? Eur J Anaesthesiol 2005;22(2):148–53.
[33] Carcillo JA, Dean JM, Holubkov R, Berger J, Meert KL, Anand KJ, et al. The randomized comparative pediatric critical illness stress-induced immune suppression (CRISIS) prevention trial. Pediatr Crit Care Med 2012;13(2):165–73.
[34] Koekkoek KWA, Berger MM. An update on essential micronutrients in critical illness. Curr Opin Crit Care 2023;29(4):315–29.
[35] Dresen E, Pimiento JM, Patel JJ, Heyland DK, Rice TW, Stoppe C. Overview of oxidative stress and the role of micronutrients in critical illness. J Parenter Enter Nutr 2023;47(Suppl 1):S38–49.
[36] Shenkin A. The key role of micronutrients. Clin Nutr 2006;25(1):1–13.