Baseline Serum Concentrations of Zinc, Selenium, and Prolactin in Critically Ill Children

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Objectives: To describe serum concentrations of zinc, selenium, and prolactin in critically ill children within 72 hrs of PICU admission, and to investigate relationships between these immunomodulators and lymphopenia.

Design: An analysis of baseline data collected as part of the multicenter Critical Illness Stress Induced Immune Suppression (CRISIS) Prevention Trial.

Setting: PICUs affiliated with the Collaborative Pediatric Critical Care Research Network.

Patients: All children enrolled in the CRISIS Prevention Trial that had baseline serum samples available for analysis.

Interventions: None.

Measurements and Main Results: Of 293 critically ill children enrolled in the CRISIS Prevention Trial, 284 had baseline serum samples analyzed for prolactin concentration, 280 for zinc concentration, and 278 for selenium concentration within 72 hrs of PICU admission. Lymphocyte counts were available for 235 children. Zinc levels ranged from nondetectable (< 0.1 μg/mL) to 2.87 μg/mL (mean 0.46 μg/mL and median 0.44 μg/mL) and were below the normal reference range for 235 (83.9%) children. Selenium levels ranged from 26 to 145 ng/mL (mean 75.4 ng/mL and median 74.5 ng/mL) and were below the normal range for 156 (56.1%) children. Prolactin levels ranged from nondetectable (< 1 ng/mL) to 88 ng/mL (mean 12.2 ng/mL and median 10 ng/mL). Hypoprolactinemia was present in 68 (23.9%) children. Lymphopenia was more likely in children with zinc levels below normal than those with zinc levels within or above the normal range (82 of 193 [42.5%] vs. 10 of 39 [25.6%], $p = 0.0498$). Neither selenium nor prolactin concentrations were associated with lymphopenia ($p = 1.0$ and $p = 0.72$, respectively).

Conclusions: Serum concentrations of zinc, selenium, and prolactin are often low in critically ill children early after PICU admission. Low serum zinc levels are associated with lymphopenia, whereas low selenium and prolactin levels are not. The implications of these findings and the mechanisms by which they occur merit further study. (*Pediatr Crit Care Med* 2013; 14:0–0)

Key Words: children; intensive care; lymphocytes; prolactin; selenium; zinc

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The provision of nutritional supplements with the ability to affect cells of the immune system is a promising therapy that may help to maintain immune system efficacy and prevent infection (1). Three natural immunomodulators important for lymphocyte health are zinc, selenium, and prolactin. The multicenter Critical Illness Stress Induced Immune Suppression (CRISIS) Prevention Trial evaluated the effect of zinc, selenium, glutamine, and metoclopramide (a prolactin secretagogue) vs. whey supplementation on the development of infection and sepsis in critically ill children (2). As part of the trial, baseline serum levels of zinc, selenium, and prolactin were obtained within 72 hrs of PICU admission. In this report, we describe these baseline values and their relationships to lymphocyte counts. We hypothesized that zinc, selenium, and prolactin concentrations are low in critically ill children early after PICU admission and that low concentrations are associated with lymphopenia.

METHODS

Setting and Participants

The CRISIS trial was conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (CPCCRN). The study was approved by the Institutional Review Board at each site. Parental permission was obtained for all participants.

All children admitted to a PICU affiliated with the CPCCRN between April 2007 and May 2009 were screened for enrollment in the CRISIS trial (2). Children were eligible if they were between 1 and 18 yr of age; were within the first 48 hrs of PICU admission; had an endotracheal tube, central venous catheter, or Foley catheter; were anticipated to require PICU care and have vascular access for blood sampling during the first 3 days of study enrollment; and were anticipated to have venous access and an enteral feeding tube for administration of study supplements. Major exclusions included known allergy to metoclopramide and chronic metoclopramide therapy prior to enrollment. Full exclusion criteria are reported elsewhere (2).

Clinical Data Collection

Clinical data collected within 72 hrs of PICU admission included gender, age, primary admission diagnosis, history of chronic illness, presence of sepsis or infection, immune status (compromised or not), use of dopamine, severity of illness, and lymphocyte count. Sepsis and infection were defined according to the Centers for Disease Control and Prevention (3). Immune compromised status was defined as the known presence of acquired immune deficiency syndrome, cancer, transplantation, primary immune deficiency, or chronic immune suppressant therapy. Severity of illness was quantified using the Organ Failure Index (OFI), the Pediatric Logistic Organ Dysfunction (PELOD) score, and the Pediatric Risk of Mortality III (PRISM III) score (4–6). Higher scores indicate greater severity of illness. Lymphopenia was defined as an absolute lymphocyte count < 1000 cells/mm^3 occurring anytime from PICU admission until the first study supplement was administered (within 72 hrs of PICU admission), or until the time of randomization for those few who received no study supplements.

Blood Specimens

Blood specimens for zinc, selenium, and prolactin concentrations were collected within 72 hrs of PICU admission prior to administration of study supplements and analyzed by a central laboratory (Mayo Clinic Laboratory, Rochester, MN). One to seven milliliters of blood were collected into a metal-free tube. Blood was allowed to clot for 30 mins at room temperature and then centrifuged at 3000 rpm for 30 mins. Serum was checked for hemolysis by visual inspection and separated into three aliquots for zinc, selenium, and prolactin assessment. Aliquots were refrigerated and shipped on a cold pack to the central laboratory by overnight mail. Serum was analyzed for zinc using inductively coupled plasma optical emission spectrometry, selenium was analyzed using inductively coupled plasma mass spectrometry, and prolactin was analyzed on a Beckman Coulter Dxl 800 using Beckman reagents (Brea, CA). Normal ranges for zinc and selenium previously established by the central laboratory are as follows: zinc, 0.60–1.20 μg/mL for children age $\leq$ 10 yr and 0.66–1.10 μg/mL for children $\geq$ 11 yr; and selenium 70–150 ng/mL for children 1–10 yr and 95–165 ng/mL for children $\geq$ 11 yr. Hypoprolactinemia was defined as a concentration of < 3 ng/mL (7, 8).

Statistical Analysis

Categorical variables are expressed as absolute counts and percentages. Continuous variables are expressed as means, medians, and ranges. Significance of associations between categorical variables was assessed by the Pearson chi-square test or Fisher's exact test. Magnitude and significance of associations between continuous variables were assessed by Spearman rank-based correlation coefficients. Reported significance levels are not adjusted for multiple comparisons as this analysis is considered exploratory.

RESULTS

A total of 293 children were enrolled into the CRISIS study, of which 284 had a baseline blood specimen available and analyzable by the central laboratory. Of these, 141 (49.6%) were female; age ranged from 1 to 18 yr (mean 8.1 yr and median 7.0 yr). Ten (3.5%) children had a primary admission diagnosis of shock. A history of chronic illness was obtained for 137 (48.2%) children. Infection was present in 98 (34.5%) children and sepsis in 89 (31.3%). Twenty-five (8.8%) children were immune compromised. Dopamine was administered on the day baseline blood specimens were obtained for 92 (32.4%) children.

Prolactin levels were obtained for all 284 children with baseline blood specimens, whereas zinc levels were assessed in 280 and selenium levels in 278. Zinc levels ranged from nondetectable

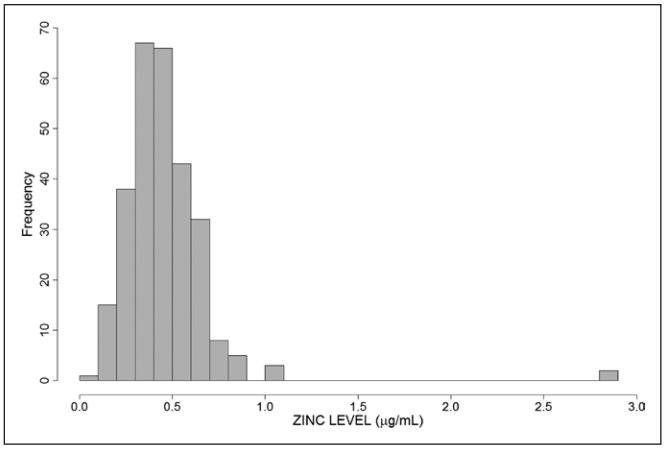

Figure 1. Baseline zinc concentration, 83.9% have zinc level below the normal range.

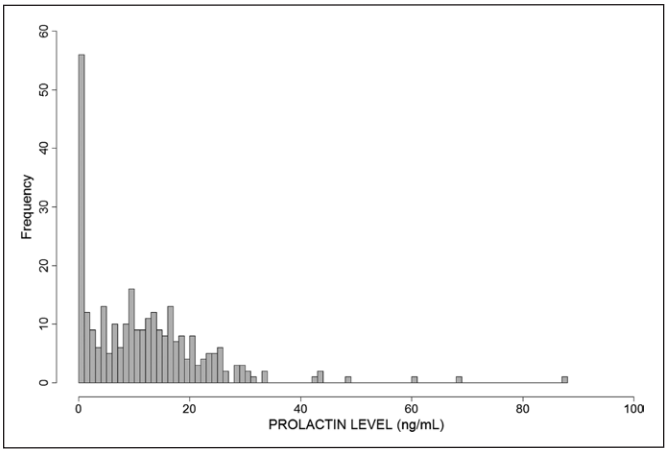

Figure 3. Baseline prolactin concentration, 23.9% have prolactin level below the normal range.

(< 0.1 µg/mL) to 2.87 µg/mL (mean 0.46 µg/mL and median 0.44 µg/mL) (**Fig. 1**). Zinc levels were below normal for 235 (83.9%) children. Selenium levels ranged from 26 to145 ng/mL (mean 75.4 ng/mL and median 74.5 ng/mL) (**Fig. 2**). Selenium levels were below normal for 156 (56.1%) children. Prolactin levels ranged from nondetectable (< 1 ng/mL) to 88 ng/mL (mean 12.2 ng/mL and median 10 ng/mL) (**Fig. 3**). Hypoprolactinemia was present in 68 (23.9%) children. Of 274 children with data for all three immunomodulators, 98 (35.8%) had low levels of one, 123 (44.9%) had low levels of two, and 35 (12.8%) had low levels of three. Only 18 (6.6%) children had levels within or above the normal range for all three immunomodulators. When only immune competent children were included in the analysis, zinc levels were below normal for 216 (84.4%), selenium levels were below normal for 138 (54.3%), and hypoprolactinemia was present in 59 (22.8%).

Lymphocyte counts were available for 235 children. Lymphocyte counts ranged from 0 to 17,200 cells/mm^3 (mean 1725 cells/mm^3 and median 1300 cells/mm^3). Lymphopenia (< 1000 cells/mm^3) was present in 93 (39.6%) children. Lymphopenia was more likely in children with zinc levels below normal than those with zinc levels within or above the normal range (82 of 193 [42.5%] vs. 10 of 39 [25.6%], p = 0.0498). Neither selenium nor prolactin concentrations were associated with lymphopenia (p = 1.0 and p = 0.72, respectively). When only immune competent children were included, lymphopenia was more significantly associated with low zinc levels (72 of 176 [40.9%] vs. 6 of 34 [17.6%], p = 0.01). Again, neither selenium nor prolactin levels were associated with lymphopenia (p = 0.69 and p = 0.39, respectively). When using a cut-off of 500 cells/mm^3 to define lymphopenia, only 23 of all 235 patients (9.8%) were lymphopenic; there was no association between lymphopenia and levels of zinc (p = 1.0), selenium (p = 0.77), or prolactin (p = 0.79).

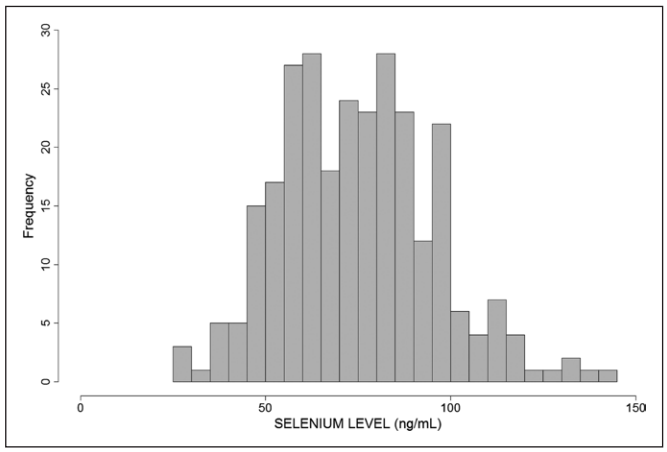

Figure 2. Baseline selenium concentration, 56.1% have selenium level below the normal range.

Zinc levels below the normal range were less likely among children with chronic illness than those without (107 of 135 [79.3%] vs. 128 of 145 [88.3%], p = 0.04), and also less likely among children with infection or sepsis than those without (146 of 183 [79.8%] vs. 89 of 97 [91.8%], p = 0.01). Zinc levels below the normal range were not associated with a primary diagnosis of shock at admission (9 of 10 [90.0%] vs. 226 of 270 [83.7%], p = 1.0). Zinc levels below the normal range were not associated with immune status (19 of 24 [79.2%] immune compromised vs. 216 of 256 [84.4%] immune competent, p = 0.56). Selenium levels below the normal range were more likely among children with chronic illness than those without (84 of 135 [62.2%] vs. 72 of 143 [50.3%], p = 0.046), and also more likely among children with infection or sepsis than those without (115 of 182 [63.2%] vs. 41 of 96 [42.7%], p = 0.001). Selenium levels below the normal range were not associated with a primary diagnosis of shock at admission (8 of 9 [88.9%] vs. 148 of 269 [55.0%], p = 0.08). Selenium levels below the normal range were not associated with immune status (18 of 24 [75.0%] immune compromised vs. 138 of 254 [54.3%] immune competent, p = 0.051). Hypoprolactinemia was not related to chronic illness (p = 0.62), the presence of infection or sepsis (p = 0.95), or a primary diagnosis of shock at admission (p = 0.71). Hypoprolactinemia was not associated with immune status (9 of 25 (36.0%) immune compromised vs. 59 of 259 (22.8%) immune competent, p = 0.14). Hypoprolactinemia was more likely among children receiving dopamine than those

TABLE 1. Spearman Correlations of Laboratory Values with Severity of Illness Indices

	Zinc ($n = 280$)	Selenium ($n = 278$)	Prolactin ($n = 284$)	Lymphocytes ($n = 235$)
OFI	−0.16[a] ($p = 0.009$)	−0.33 ($p < 0.001$)	−0.18 ($p = 0.003$)	0.06 ($p = 0.33$)
PELOD	−0.23 ($p < 0.001$)	−0.22 ($p < 0.001$)	−0.16 ($p = 0.006$)	−0.09 ($p = 0.15$)
PRISM	−0.23 ($p < 0.001$)	−0.14 ($p = 0.02$)	−0.17 ($p = 0.005$)	−0.16 ($p = 0.01$)

OFI = Organ Failure Index; PELOD = Pediatric Logistic Organ Dysfunction; PRISM III = Pediatric Risk of Mortality.
[a]Values represent rank-based Spearman's rho values.

not receiving dopamine (60 of 92 [65.2%] vs. 8 of 192 [4.2%], $p < 0.0001$). Zinc, selenium, and prolactin concentrations were inversely related to severity of illness indices (**Table 1**).

DISCUSSION

Our findings demonstrate that serum concentrations of zinc, selenium, and prolactin are often low in critically ill children early after PICU admission. Many children had low levels of more than one of these immunomodulators. Lower concentrations of zinc, selenium, and prolactin were associated with greater severity of illness. Low zinc concentrations were associated with lymphopenia, whereas low selenium and prolactin concentrations were not.

Zinc is a trace element necessary for normal immune functioning, oxidative stress responses, wound healing, and growth (9). Zinc homeostasis is tightly maintained through gastrointestinal absorption and excretion, urinary excretion, and tissue redistribution (10). Less than 0.1% of the body's total zinc content is present in blood, thus a low serum level may not represent true deficiency. The low serum zinc levels observed in our study and the inverse relationship between zinc level and severity of illness are consistent with other research. In a study of 20 critically ill children, Cvijanovich et al (11) found low plasma zinc levels in all; zinc levels correlated inversely with markers of inflammation (e.g., C-reactive protein) and degree of organ failure. The low zinc levels observed in our study were also associated with lymphopenia. True zinc deficiency has consistently been shown to result in thymic atrophy, lymphopenia, and impaired cell- and antibody-mediated immunity (9). Whether the low zinc levels observed early in critical illness contribute to the development of lymphopenia will require further study.

Selenium is a trace element important to the antioxidant defense system, and leukocyte and NK cell function (12). Similar to our findings in critically ill children, selenium levels have also been shown to be decreased among critically ill adults with systemic inflammatory response syndrome and to be inversely related to severity of illness (13). Potential explanations for low serum selenium concentrations among critically ill patients include tissue redistribution and ongoing losses, similar to that described for low serum zinc.

Prolactin is a counterregulatory stress hormone that suppresses glucocorticoid-induced apoptosis of lymphocytes (14). Prolactin has a circadian peak during nighttime hours and is enhanced by sleep (15). Various drugs and endogenous substances are also known to affect prolactin levels (16). For example, dopamine is the predominant physiologic inhibitor of prolactin release. Prolonged hypoprolactinemia (i.e., > 7 days) has been shown to be a risk factor for lymphopenia and lymphoid depletion in children with nosocomial sepsis and multiple organ failure (7). However, in our study, baseline hypoprolactinemia was not associated with low lymphocyte counts within 72 hrs of PICU admission. These findings might suggest that the association between hypoprolactinemia and lymphopenia during critical illness develops over time.

Strengths of this study include the large sample size and use of a central laboratory for analysis of serum zinc, selenium, and prolactin concentrations. Limitations include the lack of tissue levels and inability to assess total body stores. Timing of prolactin levels was not standardized and thus the prevalence of hypoprolactinemia could be underestimated. Correlations between zinc, selenium, and prolactin concentrations and severity of illness indicators are low likely due to the many variables affecting severity of illness. As multiple significance assessments were performed in this exploratory study, there is the possibility of type I error for some associations found to be statistically significant. We conclude that serum concentrations of zinc, selenium, and prolactin are often low in critically ill children early after PICU admission. The implications of these findings and the mechanisms by which they occur merit further study.

REFERENCES

1. Mizock BA: Immunonutrition and critical illness: An update. *Nutrition* 2010; 26:701–707
2. Carcillo JA, Dean JM, Holubkov R, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Collaborative Pediatric Critical Care Research Network (CPCCRN): The randomized comparative pediatric critical illness stress-induced immune suppression (CRISIS) prevention trial. *Pediatr Crit Care Med* 2012; 13:165–173
3. Garner JS, Jarvis WR, Emori TG, et al: CDC definitons of nosocomial infections. *In:* APIC Infection Control and Applied Epidemiology: Principles and Practice. Olmsted RN (Ed). St. Louis, MO, Mosby, 1996, A1–A20
4. Doughty L, Carcillo JA, Kaplan S, et al: The compensatory anti-inflammatory cytokine interleukin 10 response in pediatric sepsis-induced multiple organ failure. *Chest* 1998; 113:1625–1631
5. Leteurtre S, Martinot A, Duhamel A, et al: Validation of the paediatric logistic organ dysfunction (PELOD) score: Prospective, observational, multicentre study. *Lancet* 2003; 362:192–197
6. Pollack MM, Patel KM, Ruttimann UE: PRISM III: An updated Pediatric Risk of Mortality score. *Crit Care Med* 1996; 24:743–752

7. Felmet KA, Hall MW, Clark RS, et al: Prolonged lymphopenia, lymphoid depletion, and hypoprolactinemia in children with nosocomial sepsis and multiple organ failure. *J Immunol* 2005; 174:3765–3772
8. Wiedemann G, Jonetz-Mentzel L: Establishment of reference ranges for prolactin in neonates, infants, children and adolescents. *Eur J Clin Chem Clin Biochem* 1993; 31:447–451
9. Prasad AS: Zinc in human health: Effect of zinc on immune cells. *Mol Med* 2008; 14:353–357
10. King JC, Shames DM, Woodhouse LR: Zinc homeostasis in humans. *J Nutr* 2000; 130:1360S–1366S
11. Cvijanovich NZ, King JC, Flori HR, et al: Zinc homeostasis in pediatric critical illness. *Pediatr Crit Care Med* 2009; 10:29–34
12. Vincent JL, Forceville X: Critically elucidating the role of selenium. *Curr Opin Anaesthesiol* 2008; 21:148–154
13. Manzanares W, Biestro A, Galusso F, et al: Serum selenium and glutathione peroxidase-3 activity: Biomarkers of systemic inflammation in the critically ill? *Intensive Care Med* 2009; 35:882–889
14. Buckley AR, Buckley DJ: Prolactin regulation of apoptosis-associated gene expression in T cells. *Ann N Y Acad Sci* 2000; 917: 522–533
15. Lange T, Dimitrov S, Born J: Effects of sleep and circadian rhythm on the human immune system. *Ann N Y Acad Sci* 2010; 1193: 48–59
16. Molitch ME: Drugs and prolactin. *Pituitary* 2008; 11:209–218