The randomized comparative pediatric critical illness stress-induced immune suppression (CRISIS) prevention trial

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Objectives: **Nosocomial infection/sepsis occurs in up to 40% of children requiring long-term intensive care. Zinc, selenium, glutamine, metoclopramide (a prolactin secretalogue), and/or whey protein supplementation have been effective in reducing infection and sepsis in other populations. We evaluated whether daily nutriceutical supplementation with zinc, selenium, glutamine, and metoclopramide, compared to whey protein, would reduce the occurrence of nosocomial infection/sepsis in this at-risk population.**

Design: **Randomized, double-blinded, comparative effectiveness trial.**

Setting: **Eight pediatric intensive care units in the NICHD Collaborative Pediatric Critical Care Research Network.**

Patients: **Two hundred ninety-three long-term intensive care patients (age 1–17 yrs) expected to require >72 hrs of invasive care.**

Interventions: **Patients were stratified according to immunocompromised status and center and then were randomly assigned to receive daily enteral zinc, selenium, glutamine, and intravenous metoclopramide (n = 149), or daily enteral whey protein (n = 144) and intravenous saline for up to 28 days of intensive care unit stay. The primary end point was time to development of nosocomial sepsis/infection. The analysis was intention to treat.**

Measurements and Main Results: **There were no differences by assigned treatment in the overall population with respect to time until the first episode of nosocomial infection/sepsis (median whey protein 13.2 days vs. zinc, selenium, glutamine, and intravenous metoclopramide 12.1 days; $p = .29$ by log-rank test) or the rate of nosocomial infection/sepsis (4.83/100 days whey protein vs. 4.99/100 days zinc, selenium, glutamine, and intravenous metoclopramide; $p = .81$). Only 9% of the 293 subjects were immunocompromised and there was a reduction in rate of nosocomial infection/sepsis with zinc, selenium, glutamine, and intravenous metoclopramide in this immunocompromised group (6.09/100 days whey protein vs. 1.57/100 days zinc, selenium, glutamine, and intravenous metoclopramide; $p = .011$).**

Conclusion: **Compared with whey protein supplementation, zinc, selenium, glutamine, and intravenous metoclopramide conferred no advantage in the immune-competent population. Further evaluation of zinc, selenium, glutamine, and intravenous metoclopramide supplementation is warranted in the immunocompromised long-term pediatric intensive care unit patient. (Pediatr Crit Care Med 2012; 13:000–000)**

Key Words: glutamine; nosocomial infection; prolactin; selenium; sepsis; whey protein; zinc

Despite implementation of Centers for Disease Control and Prevention recommendations and bundled interventions for preventing catheter-associated bloodstream infection, ventilator-associated pneumonia, and urinary catheter-associated infections, nosocomial infection/sepsis remains a significant cause of morbidity in critically ill children requiring long-term intensive care. Critical illness stress induces lymphopenia and lymphocyte dysfunction associated with hypoprolactinemia (1) and also deficiencies in zinc and selenium (2, 3) and amino acids (4, 5). Because lymphocyte integrity is important for the immune response to fight infection, standard nutritional practice in critically ill children includes zinc, selenium, and protein. It is unknown whether additional supplementation is needed in this population at risk for stress-induced lymphocyte dysfunction and nosocomial infection/sepsis.

Metoclopramide, a prolactin secretalogue, administered at the dosage commonly used for gastrointestinal prokinesis maintains prolactin levels in the high-normal range in children. In mechanically ventilated adults, metoclopramide delayed time to onset of nosocomial pneumonia by 50% but had no effect on the rate of nosocomial pneumonia or mortality (6). In malnourished children, zinc supplementation reduced morbidity and mortality with severe pneumonia (7, 8) or diarrhea (9–11) and reduced infectious disease mortality in small-for-gestational-age infants (12). Selenium supplementation (13) or glutamine-enriched enteral nutrition (14) also reduced the risk of nosocomial sepsis in preterm neonates.

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Members of the Data Safety Monitoring Board include Jeffrey R. Fineman, MD, Jeffrey Blumer, PhD, MD, Thomas P. Green, MD, and David Glidden, PhD.

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Enteral glutamine safely maintains T_H1 lymphocyte function for bacterial killing (15, 16).

Essential amino acids are also important to overall immune function and lymphocyte function in particular (5). Whey protein provides all the essential amino acids needed to maintain immune function in immune cells. Experimental animal studies show that whey protein supplementation facilitates maturation of the immune system and is protective against rotavirus (17–21). Randomized human clinical studies of whey protein have demonstrated improved lymphocyte function and reduction in coinfection in human immunodeficiency virus-infected children, reduction in infection in severely burned children, and improved immunologic response to immunization in the elderly (22–26).

The Eunice Kennedy Shriver National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network investigators hypothesized that critical illness stress-induced immune suppression-related nosocomial infection/sepsis would be more effectively prevented by prophylactic supplementation of "standard" nutritional practice with added zinc, selenium, glutamine, and metoclopramide, (ZSGM) than by prophylactic supplementation with added amino acid (whey protein). The Collaborative Pediatric Critical Care Research Network designed a randomized, double-blinded, comparative effectiveness trial with the primary hypothesis that daily ZSGM would prolong the time to development of nosocomial infection/sepsis compared to daily whey protein. In this article we report the results of the critical illness stress-induced immune suppression prevention trial.

MATERIALS AND METHODS

This randomized, double-blinded, comparative study was performed on two parallel groups of children at eight pediatric intensive care units (PICUs) in the Collaborative Pediatric Critical Care Research Network. The Institutional Review Boards of all Collaborative Pediatric Critical Care Research Network centers approved the protocol and informed consent documents. Parental permission was provided for each subject. An independent Data Safety Monitoring Board was appointed by the NICHD, and two interim safety and efficacy analyses were planned. The study was performed under an Investigational New Drug application from the Food and Drug Administration (investigational new drug 74,500), for which the DCC Principal Investigator (J.M.D.) acted as the sponsor. The study was registered with ClinicalTrials.gov (number NCT00395161).

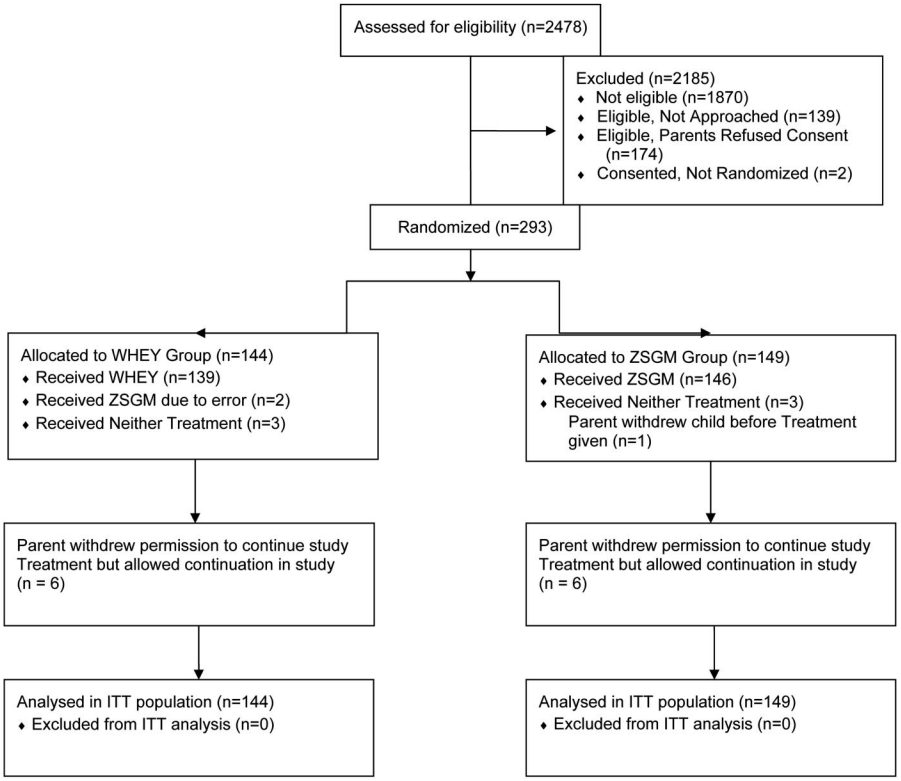

Figure 1. Screening, enrollment, randomization, and study completion.

Patients were eligible for enrollment if they: were older than 1 yr and younger than 18 yrs of age; were within the first 48 hrs of PICU admission; had an endotracheal tube, central venous catheter (new or old, tunneled or not tunneled), or urinary catheter; were anticipated to have an indwelling arterial or venous catheter for blood sampling during the first 3 days of study enrollment; and were anticipated to have venous access and an enteral feeding tube for the administration of study drugs. Patients were excluded from enrollment if they: had a known allergy to metoclopramide; were expected to have planned removal of endotracheal tube, central venous, and urinary catheters within 72 hrs after study enrollment; had suspected intestinal obstruction; had intestinal surgery or bowel disruption; had other contraindications to the enteral administration of drugs or nutrients; received chronic metoclopramide therapy before enrollment; had a known allergy to whey (cow's milk) or soy-based products; had been discharged from the PICU in the previous 28 days; had been previously enrolled in this study; or had a positive pregnancy test. Patients were also excluded if their parents indicated a lack of commitment to aggressive intensive care therapies.

The Food and Drug Administration required an interim analysis review by the Data Safety Monitoring Board before authorizing enrollment of infants younger than 1 yr. After the first interim analysis, the Data Safety Monitoring Board deferred this authorization until a second interim analysis could be reviewed. At the time of the second review, the trial was terminated for futility. Therefore, no infants younger than 1 yr were enrolled in the trial. The study commenced in April 2007 and terminated in November 2009.

After informed consent was obtained from parents, subjects were randomized by telephone according to an *a priori* design using randomized blocks of variable length, stratified according to center and immunocompromised status. Immunocompromised status was defined by the known presence of acquired immune deficiency syndrome, cancer, transplantation, primary immune deficiency, or chronic immune suppressant therapy. Children were randomized in a 1:1 ratio into the two arms of the trial in these stratified groups. All patients, medical and nursing staffs, clinical site monitors, and most DCC staff remained blinded throughout the study period. The DCC biostatistician (R.H.) prepared Data Safety Monitoring Board reports and reviewed results in the two arms but remained blinded to actual group assignment throughout the study period. Central and clinical site research pharmacists and the pharmacy site monitor were unblinded throughout the study.

Subjects were randomized to receive enteral whey protein powder and intravenous

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Table 1. Epidemiologic and clinical characteristics at admission

Factor	Whey Protein Group (N = 144)	Zinc, Selenium, Glutamine, and Metoclopramide Group (N = 149)
Age (yrs), median (range)	7.1 (1–17)	7.0 (1–17)
Female (%)	55	46
Pediatric logistic organ dysfunction, median (range)	11 (0–40)	11 (0–50)
Pediatric risk of mortality, median (range)	8 (0–34)	7 (0–31)
Organ failure index, median (range)	2 (0–5)	2 (0–6)
Immunocompromised (%)	8	9
Postoperative pediatric intensive care unit admission (%)	28	26
Primary diagnosis (%)		
Asthma	3	1
Cancer	3	1
Cardiac arrest	3	3
Cardiovascular disease	7	5
Pneumonia/bronchiolitis	22	15
Seizures	3	5
Sepsis	7	8
Shock	3	5
Trauma	17	23
Human immunodeficiency virus	0	1
Hypoxic-ischemic encephalopathy	1	1
Intoxication	1	0
Meningitis	1	2
Transplant	1	0
Other	28	29
Chronic diagnoses (%)	49	48
Malnutrition (reported as primary or secondary diagnosis) (%)	1	0
Infection status at entry (%)		
Existing infection	32	37
Existing sepsis	33	29
No infection or sepsis	35	34
Existing lymphopenia (ALC $\leq$1000/mm^3)	40% (N = 114)	37% (N = 126)
Baseline core laboratory data (%)		
Zinc deficiency	79% (N = 138)	89% (N = 141)
Prolactin deficiency	23% (N = 133)	15% (N = 134)
Selenium deficiency	57% (N = 138)	55% (N = 140)

saline (whey protein group) or ZSGM (ZSGM group). Subjects assigned to the whey protein group received 0.3 g/kg beneprotein each morning and intravenous saline every 12 hrs. Subjects assigned to the ZSGM group received zinc (20 mg), selenium (40 μg age 1–3 yrs, 100 μg age 3–5 yrs, 200 μg age 5–12 yrs, 400 μg adolescent), and glutamine (0.3 g/kg) each morning, and intravenous metoclopramide (0.2 mg/kg, maximum 10 mg) every 12 hrs. All study drugs were shipped from a central pharmacy (University of Utah) and dispensed by site research pharmacists. Subjects received study drug until discharge from the PICU or for 28 days from the time of randomization, whichever occurred earlier. Enteral drugs were administered by feeding tube and discontinued if the feeding tube was removed or if contraindications to enteral feeding developed during the study. Intravenous drugs were discontinued if the intravenous was removed.

The hypothesis of the critical illness stress-induced immune suppression Prevention trial was that daily prophylaxis with enteral zinc, selenium, and glutamine, and intravenous metoclopramide would delay the time (hours) between admission to the PICU and occurrence of nosocomial infection/clinical sepsis in PICU patients who have endotracheal tubes, central venous catheters, or urinary catheters. Nosocomial events were defined as occurring at least 48 hrs after PICU admission during the hospital stay until 5 days after discharge from the PICU; for children remaining in the PICU for >28 days after randomization, events were counted until day 33. The study protocol required that patients be randomized within 48 hrs of PICU admission and that study drug administration begin within 72 hrs of PICU admission. According to Center for Disease Control and Prevention definitions, clinical sepsis occurs when patients older than 1 yr have development of fever (≥38°C), hypotension (≤90 mm Hg), or oliguria (≤20 mL/hr) and a clinician initiates antibiotic therapy with no positive microbiological evidence and no other recognized cause. Nosocomial infection occurs when microbiologically (culture, antigen, polymerase chain reaction, or antibody) proven infection is observed in a patient with fever, hypothermia, chills, or hypotension. The treatment arm blinded Collaborative Pediatric Critical Care Research Network investigators adjudicated the presence or absence of a nosocomial clinical sepsis or infection event for every subject. Each case was reviewed independently by two investigators and presented in detail so that consensus for all outcomes was attained. All participants in this adjudication process were blinded to treatment arm through the study period.

Secondary outcome variables of this study included the rate of nosocomial infection/sepsis per 100 PICU days, number of antibiotic-free days, incidence of prolonged lymphopenia (ALC ≤1000/mm^3 for ≥7 days), serum prolactin, zinc, and selenium levels before treatment and after 7 days of treatment, and the safety indicator 28-day mortality and adverse events. Serum zinc and selenium levels were classified as deficient if they were below the pediatric reference ranges of the core laboratory. Zinc deficiency was defined as a level <0.60 μg/mL in children aged 10 yrs or younger and <0.66 μg/mL in children aged at least 11 yrs. Selenium deficiency was defined as a level <70 ng/mL in children aged 10 yrs or younger and <95 ng/mL in children aged at least 11 yrs. Prolactin deficiency was defined as a level of <3 ng/mL across all ages. Glutamine levels were not measured because they are not considered indicative of total body reserves.

The sample size was calculated to provide 90% power to detect an inverse hazard rate of 1.5 using a two-sided nonparametric test (log-rank test) with type I error (α) of 0.05. This required accrual of subjects until 263 had nosocomial events; the estimated total sample size was 600 subjects based on initial event rate estimates. A log-rank test with a two-sided $\alpha = 0.05$, stratified by immune-compromised status, was used to compare the primary end point of freedom from nosocomial infection or sepsis (from time of admission to the PICU until up to 5 days after discharge from the PICU) between treatment arms. Outcome rates over time are presented using Kaplan-Meier freedom from event curves. In the subgroup of immunocompromised whey protein patients, median time to nosocomial infection/sepsis was derived at the 50.5% event-free time point.

In prespecified analyses complementary to the primary analysis, rates of infection were analyzed using Poisson regression analyses, which count multiple events for a single subject. Additionally, numbers and proportions of antibiotic-free days during the PICU stay were compared between study arms using the Wilcoxon rank-sum test. Incidence of prolonged lymphopenia and all-cause mortality and adverse events at 28 days after randomization were analyzed using the chi-square test or exact analogues when numbers of events were small. Four ZSGM-assigned subjects (one of

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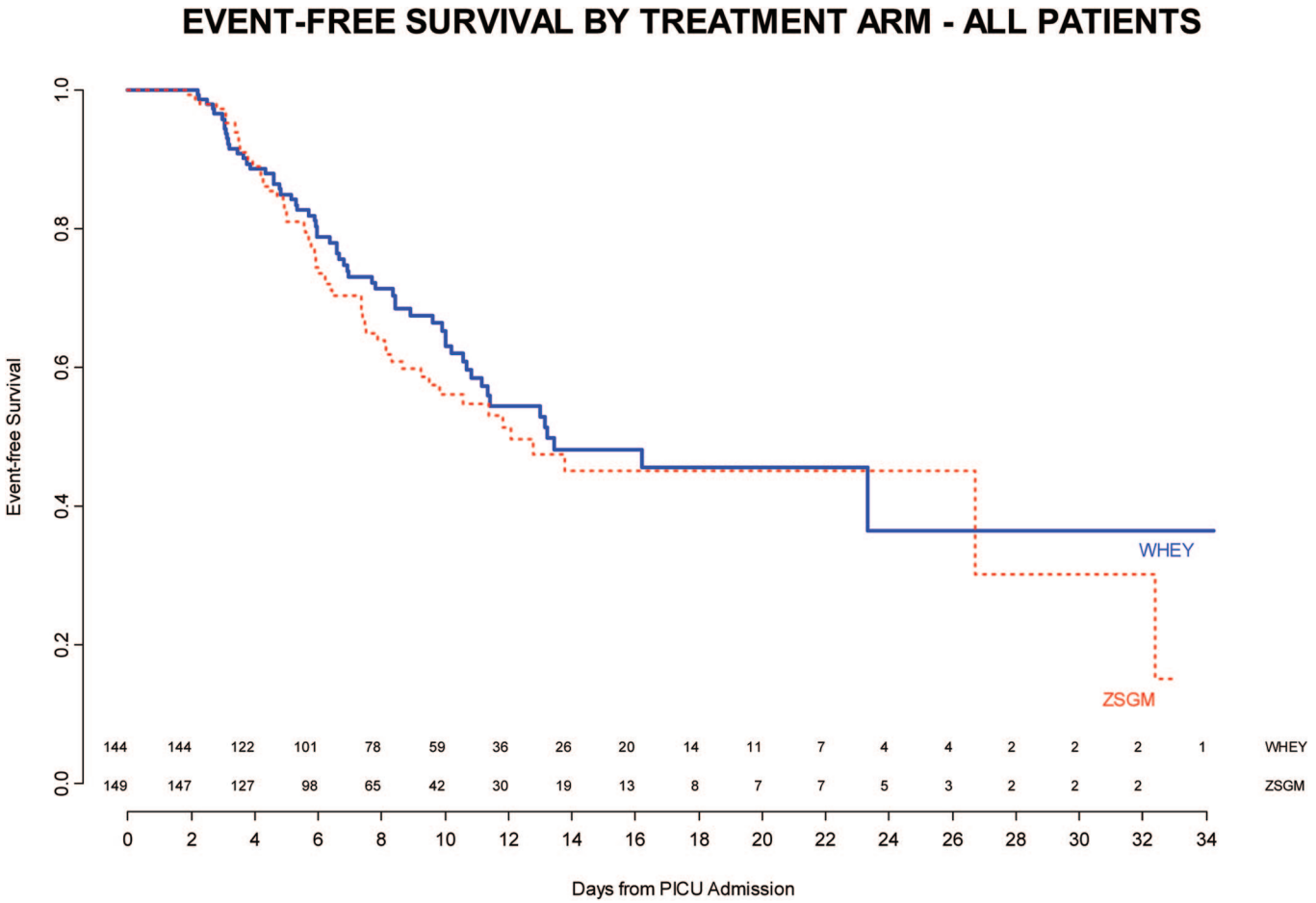

Figure 2. *Top*, Freedom from nosocomial sepsis according to assigned treatment for all randomized patients. Numbers above the *horizontal time axis* denote number of patients remaining at risk at each time point. $p = .29$ for log-rank test comparing curves between study arms, stratified by immune competent status. *Middle*, Freedom from nosocomial infection/sepsis according to assigned treatment for patients immunocompromised at study entry. Numbers above the *horizontal time axis* denote number of patients remaining at risk at each time point. $p = .24$ for log-rank test comparing curves between study arms. *Lower*, Freedom from nosocomial infection/sepsis according to assigned treatment for patients who were immune-competent at study entry. Numbers above the *horizontal time axis* denote number of patients remaining at risk at each time point. $p = .16$ for log-rank test comparing curves between study arms.

whom received no study treatment) had unknown 28-day survival status.

Five factors (immunocompromised status, postoperative status, gender, race/ethnicity, and center) were prespecified for subgroup analysis, and the Data Safety Monitoring Board subsequently added another factor (infection or sepsis at study entry). The intention-to-treat approach was used for all study analyses of efficacy. Analysis by treatment received was performed for the safety outcomes of mortality and occurrence of adverse events.

RESULTS

We enrolled 293 subjects (Fig. 1). Enrollment was terminated for futility after the second interim analysis indicated that the conditional power to determine a beneficial effect by ZSGM, compared to the whey protein therapy, was <10%. Figure 1 shows the screening, enrollment, randomization, and study completion results.

Table 1 shows the epidemiologic and clinical characteristics of the study population at the time of enrollment in both treatment arms. The median age of children was 7 yrs and <10% were immunocompromised on entry. Baseline characteristics were equally distributed between the study arms. Among patients assigned to whey protein, 46.5% received parenteral nutrition and 89.6% received enteral nutrition, compared to 43.0% and 90.6% of patients assigned to ZSGM. The proportion of patients who were allowed nothing by mouth during one or more PICU study days was 55% in the whey protein arm and 53% in the ZSGM arm, with the average proportion of PICU study days being allowed nothing by mouth at 14% and 13%, respectively.

Treatment with ZSGM did not delay the time until nosocomial infection/sepsis compared to treatment with whey protein (median time, whey protein 13.2 days vs. ZSGM 12.1 days; log-rank $p = .29$; Fig. 2 *top*). The median PICU stay was 10 days. Of subjects at risk for an event, approximately 50% in each treatment arm were event-free at 14 days after PICU admission. The effect of immunocompromised status on time to nosocomial infection/sepsis was not significant (median time in immunocompromised patients, whey protein 10 days vs. ZSGM 32.4 days; median time in immune competent patients, whey protein 13.2 days vs. ZSGM 11.8 days; $p = .12$) for interaction between treatment group and immunocompromised status in the time-to-event analysis (Fig. 2 *middle*, *bottom*). Other subgroup factors examined were

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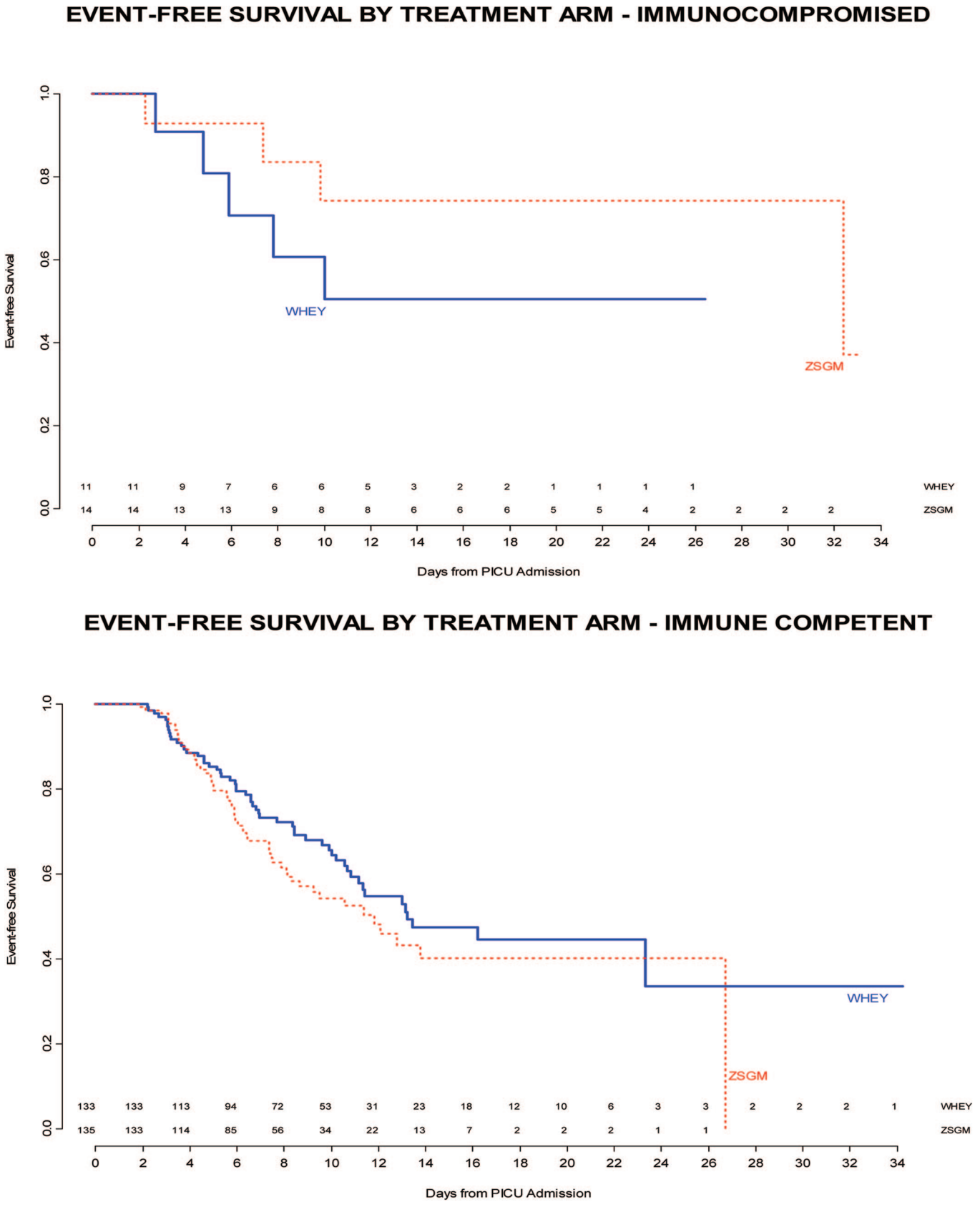

Figure 2. Continued.

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Table 2. Rates of nosocomial infection/sepsis, days of invasive lines, urinary catheterization, and mechanical ventilation by treatment group

Variable	Whey Protein Group (N = 144)	Zinc, Selenium, Glutamine, and Metoclopramide Group (N = 149)	*p*
Total events (infection or sepsis)	116	110	
Total pediatric intensive care unit days	1993	1793	
Total study days[a]	2402	2205	
Mean events/patient/100 study days	4.83	4.99	.81
95% confidence interval	4.01–5.77	4.12–5.99	
Therapeutic risk factors			
Days in pediatric intensive care unit (mean/median)	13.8/11	12/9	.16
Ventilator days (mean/median)	9.4/6	7.9/5	.13
Central venous catheter days (mean/median)	10.2/7	9.1/7	.49
Endotracheal tube days (mean/median)	8.8/6	7.3/5	.14
Urinary catheter days (mean/median)	8.0/6	6.9/5	.54
Total ventilator days	1352	1171	
Total respiratory infections	43	53	
Mean respiratory infections/patient	3.18	4.53	
Per 100 ventilator days (95% confidence interval)	2.33–4.24	3.43–5.87	.08
Total urinary catheter days	1155	1023	
Total urinary tract infections	12	8	
Mean urinary tract infections/patient	1.04	0.78	
Per 100 urinary catheter days (95% confidence interval)	0.57–1.76	0.37–1.48	.54
Total central venous catheter days	1465	1353	
Total bacteremia infections	11	11	
Mean bacteremia infections/patient	0.75	0.81	
Per 100 central venous catheter days (95% confidence interval)	0.40–1.30	0.43–1.41	.85

[a]Study days indicates days in pediatric intensive care unit plus additional days after pediatric intensive care unit discharge that patient was followed-up for events (5 days unless patient was discharged from hospital earlier).

not significantly associated with the primary end point.

There was no difference in the rate of nosocomial infection or clinical sepsis per 100 PICU days between the ZSGM and whey protein groups (Table 2; $p = .81$). Examining study days in the PICU, median number of antibiotic-free days (2 vs. 1; $p = .09$) and proportion of days (17% vs. 10%; $p = .19$) did not differ between subjects assigned to whey protein vs. ZSGM. There was no significant difference in the incidence of prolonged lymphopenia (ALC $\leq$1000/mm^3 for $\geq$7 days) between subjects assigned to whey protein (12/144 [8.3%] vs. ZSGM 5/149 [3.4%]; $p = .07$). In the study population, 41% receiving whey protein and 42% receiving ZSGM had development of nosocomial infection or sepsis. Approximately one-third of patients had development of nosocomial infection and one-fifth had development of sepsis. Days of invasive lines, urinary catheterization, and mechanical ventilation and rates of site-specific infections based on denominators of ventilator days, urinary catheter days, and central venous catheter days were not significantly different between the treatment arms (Table 2). Distribution of events, sites of infection, and infecting organisms were also generally similar between the treatment groups (Table 3).

In the immunocompromised population, the rate of nosocomial infection/sepsis was reduced in the ZSGM group compared with the whey protein group (unadjusted $p = .006$ for interaction between treatment group and immunocompromised status; Table 4). The causes for immune compromise in whey protein compared to the ZSGM groups were bone marrow transplant two vs. three, other organ transplant five vs. one, cancer one vs. three, human immunodeficiency virus zero vs. one, severe neutropenia one vs. one, chronic high-dose steroids/immune suppressants one vs. three, congenital immunodeficiency one vs. one, and therapeutic hypothermia zero vs. one.

Among subjects with available baseline values, zinc deficiency was present at baseline in 235 of 280 (84%), selenium deficiency in 156 of 278 (56%), and prolactin deficiency in 68 of 284 (24%) (Table 1). Among whey protein and ZSGM subjects, standard of care included the common use of zinc (44% vs. 39%), selenium (40% vs. 38%), glutamine (8% vs. 7%) primarily as part of routine TPN, and metoclopramide (3% vs. 5%) for facilitation of nasoduodenal tube placement or gastroesophageal reflux, respectively. Seven-day levels of all three measures were significantly higher in ZSGM patients than whey protein patients, and change from baseline was also higher ($p < .001$ for all six comparisons). At 7 days, zinc deficiency was present in 19 of 83 (23%) ZSGM subjects vs. 36 of 80 (45%) whey protein subjects, selenium deficiency in 10 of 84 (12%) ZSGM subjects vs. 23 of 80 (29%) whey protein subjects, and prolactin deficiency in 3 of 84 (4%) ZSGM subjects vs. 14 of 81 (17%) whey protein subjects. Controlling for presence of baseline deficiencies, ZSGM subjects with 7-day measures showed significantly lower 7-day deficiency rates compared to whey protein subjects for zinc ($p = .001$ by Cochran-Mantel-Haenszel test), selenium ($p = .009$), and prolactin ($p = .014$).

Overall 28-day mortality was 8.1% among the 284 children who received whey protein or ZSGM and had known 28-day status. There was no significant difference in 28-day mortality by treatment received between whey protein and ZSGM (8/139 [5.8%] vs. 15/145 [10.3%]; $p = .16$). Among the 287 children receiving treatment, there were 2624 adverse events, including 113 serious adverse events with no significant differences by treatment received for whey protein and ZSGM. Among 139 subjects receiving only whey protein treatment, adverse events were reported in 126 (90.6%) and serious adverse events were reported in 37 (26.6%), whereas among 148 subjects receiving ZSGM regimen adverse events were reported in 135 (91.2%) and serious adverse events were reported in 39 (26.4%). There were also no differences in specific adverse events, including diarrhea (whey protein 12.2% vs. ZSGM 12.2%), dysrhythmias (arrhythmia, extrasystole, nodal rhythm; whey protein 4.3% vs. ZSGM 4.1%), and abnormal movement (akathisia, choreoathetosis, dyskinesia, dystonia; whey protein 2.9% vs. ZSGM 2.0%).

DISCUSSION

Similar to previous literature, we observed that nosocomial infection/sepsis occurred in >40% of long-term PICU patients, with <50% of these children being free from nosocomial infection or

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Table 3. Nosocomial infection/sepsis and sites of nosocomial infection by treatment group

Variable	Whey Protein Group (N = 144)		Zinc, Selenium, Glutamine, and Metoclopramide Group (N = 149)	
Patients with events				
One or more events (%)	41		42	
Nosocomial infection (%)	31		35	
Nosocomial sepsis (%)	22		17	
Total infections	73		83	
Site of infection				
Lower respiratory	41		52	
Upper respiratory	2		1	
Urinary tract	12		8	
Skin or soft tissue	6		6	
Bacteremia	11		11	
Other	1		5	
		N = 100		N = 107
Total infecting organisms				
Fungi		16		21
Candida albicans		6		4
Candida tropicalis		4		3
Yeast		0		7
Candida glabrata		1		3
Candida lusitanae		2		2
Other		3		2
Gram-negative bacilli		43		40
Pseudomonas aeruginosa		14		14
Haemophilus influenza		5		8
Stenotrophomonas maltophilia		4		3
Enterobacter cloacae		4		2
Klebsiella pneumoniae		3		3
Other		13		10
Gram-positive bacilli		1		1
Clostridium dificile		1		1
Gram-negative cocci		2		2
Moraxella catarrhalis		2		2
Gram-positive cocci		35		37
Staphylococcus aureus		13		18
Staphylococcus coagulase negative		1		8
Enterococcus faecalis		2		2
Staphylococcus epidermidis		3		1
Other		16		8
Virus		2		3
Undetermined		1		3

sepsis at 14 days and median time to nosocomial infection or sepsis being just less than 14 days (27). Similar to previous reports, we also found a high incidence of critical illness stress-related zinc and selenium deficiency, as well as prolactin deficiency in 24% and lymphopenia in nearly 40% of the patients (1, 28). The observation that nearly 95% of subjects had deficiencies at enrollment supports the study design that used the multimodal ZSGM supplement strategy and analyzed the effects on both the *pre hoc* stratified immune competent and immunocompromised populations. We observed more frequent resolution of zinc, selenium, and prolactin deficiencies at 7 days with ZSGM, but this pharmacokinetic effect was not matched with the hypothesized pharmacodynamic effect. The ZSGM supplement did not prevent persistent lymphopenia or nosocomial infection/sepsis compared with essential amino acid supplementation from whey protein in the overall study population.

We stratified the randomization of patients to nutriceutical treatment arms according to immunocompromised status because we thought it was biologically plausible that the T_H2 phenotype-dominant immunocompromised group of patients would benefit differentially from ZSGM supplementation. In this regard, we did observe a reduction in the nosocomial infection/sepsis rate with use of ZSGM in this at-risk population. Because <10% of our general PICU population was immunocompromised, the small sample size leads us to view these findings rather cautiously. Repeated study is needed, perhaps in a specialized PICU network with a larger immunocompromised population, or in a PICU network with a larger number of centers to properly assess the significance of this signal.

With regard to limitations, several study design and performance variables require the reader's consideration. First, this trial was performed in the "standard practice" setting. Protein, zinc, and selenium are an accepted part of "standard" pediatric enteral and parenteral nutrition in the intensive care setting (29, 30) and no effort was made to control this nutritional practice. The study results cannot be applied to patients who are without any nutrition in the PICU. Second, our trial compared the effectiveness of two nutriceutical strategies to one another, rather than to placebo. The research planning committee wanted to follow previous study designs of glutamine supplementation in newborns that used single amino acids as "placebos" to address potential criticism that an apparent effect in a glutamine arm could be an effect of protein nutrition rather than of glutamine *per se*. This rationale is problematic because all amino acids have specific immune cell effects and therefore are not true placebos (5). Whey protein was the only Food and Drug Administration-approved amino acid supplement available to us. Because whey protein is marketed as immune nutrition, we designed a comparative effectiveness trial rather than a true placebo-controlled trial. A true placebo arm without any zinc, selenium, or protein was considered outside of human subjects standards. Two ongoing adult trials comparing the use of a dopamine-2 antagonist to placebo in mechanical ventilation and zinc, selenium, and glutamine supplements to placebo in severe sepsis (NCT0013978, NCT00300391) will give information on the effect of these supplements in the absence of concomitant protein supplementation. Approximately ten patients in each treatment arm either did not receive the assigned treatment, or they had their treatments stopped prematurely on parental request. *Post hoc* analysis excluding these patients did not change the overall findings of the study. Fourth, a low number of antibiotic-free days in the subjects enrolled in either arm of this study was discovered. This calls into question whether high antibiotic use diminished any effects of the nutriceuticals. However, *post hoc* analysis found no association between extent

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Table 4. Rates of nosocomial infection/sepsis per 100 days by treatment group and immunocompromised status

Variable	Whey Protein Group (N = 11)	Zinc, Selenium, Glutamine, and Metoclopramide Group (N = 14)	*p*
Immunocompromised patients			
Total events (infection or sepsis)	12	4	
Total pediatric intensive care unit days	165	217	
Total study days	197	255	
Mean events/patient/100 study days (95% confidence interval)	6.09 (3.33–10.32)	1.57 (0.53–3.73)	.011

Variable	Whey Protein Group (N = 133)	Zinc, Selenium, Glutamine, and Metoclopramide group (N = 135)	*p*
Immune-competent patients			
Total events (infection or sepsis)	104	106	
Total pediatric intensive care unit days	1828	1576	
Total study days	2205	1950	
Mean events/patient/100 study days (95% confidence interval)	4.72 (3.87–5.69)	5.44 (4.47–6.55)	.30

of antibiotic use and evidence of treatment effect.

CONCLUSION

Nosocomial infection and sepsis remains a prevalent public health problem in critically ill children with long-term stay in the PICU. Implementation of Centers for Disease Control and Prevention-recommended practices is the first step in prevention. Our study was performed on the premise that evaluation of the comparative effectiveness of prophylactic nutritional support strategies could inform further improvement. The novel multimodal strategy designed and used to reduce critical illness stress-induced zinc, selenium, glutamine, and prolactin deficiencies was successful in part in reversing these deficiencies. It conferred no advantage in nosocomial infection and sepsis prevention in immune-competent children compared to whey-based amino acid supplementation. Further study of the ability of ZSGM supplementation to prevent nosocomial infection and sepsis in the immunocompromised PICU population is warranted.

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