REGULAR ARTICLE

blood advances

Zinc for infection prevention in children with sickle cell anemia: a randomized double-blind placebo-controlled trial

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Key Points

- Oral zinc supplementation (10 mg/day for 12 months) did not prevent severe or invasive infections in children with SCA
- Zinc deficiency, assessed by low plasma zinc, persisted in 41% of children who were randomized to zinc supplementation

Data from small clinical trials in the United States and India suggest zinc supplementation reduces infection in adolescents and adults with sickle cell anemia (SCA), but no studies of zinc supplementation for infection prevention have been conducted in children with SCA living in Africa. We conducted a randomized double-blind placebo-controlled trial to assess zinc supplementation for prevention of severe or invasive infections in Ugandan children 1.00-4.99 years with SCA. Of 252 enrolled participants, 124 were assigned zinc (10 mg) and 126 assigned placebo once daily for 12 months. The primary outcome was incidence of protocol-defined severe or invasive infections. Infection incidence did not differ between treatment arms (282 vs. 270 severe or invasive infections per 100 person-years, respectively, incidence rate ratio of 1.04 [95% confidence interval (CI), 0.81, 1.32, p=0.78]), adjusting for hydroxyurea treatment. There was also no difference between treatment arms in incidence of serious adverse events or SCA-related events. Children receiving zinc had increased serum levels after 12-months, but at study exit, 41% remained zinc deficient (<65 μg/dL). In post-hoc analysis, occurrence of stroke or death was lower in the zinc treatment arm (adjusted hazard ratio (95% CI), 0.22 (0.05, 1.00); p=0.05). Daily 10 mg zinc supplementation for 12 months did not prevent severe or invasive infections in Ugandan children with SCA, but many supplemented children remained zinc deficient. Optimal zinc dosing and the role of zinc in preventing stroke or death in SCA warrant further investigation. This trial was registered at clinicaltrials.gov as #NCT03528434.

Introduction

Most children with sickle cell anemia (SCA) reside in Africa,[1] where infections remain an important cause of morbidity and mortality.[2] Infections often precede or accompany SCA-related complications, including vaso-occlusive crises (VOC), acute chest syndrome, and stroke, and are a major cause of hospitalization.[3-5] It is estimated that SCA contributes to 5% to 16% of childhood mortality in Africa,[1] and that 35% to 40% of children with SCA die before the age of 5 years.[6] In Uganda, an estimated

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Fully anonymized data can be shared on request from an investigator with a data transfer agreement with Indiana University and the requesting investigator institution.

The full-text version of this article contains a data supplement.

20 000 children are born with SCA each year.[7] In a recent trial of hydroxyurea therapy in Uganda, children younger than 5 years with SCA had an average of 0.7 severe infections per child year and 60% of deaths in the study were attributed to infection.[8] Prevention of infections could therefore significantly decrease morbidity and mortality in African children with SCA.

Zinc is an essential element in many cellular processes, including the development of T and B cell immune responses. Studies in healthy children have demonstrated that zinc supplementation is effective in reducing the incidence of diarrhea and pneumonia in children with minimal side effects.[9-11] Zinc deficiency is common in children with SCA,[12] owing to zinc release after bone degradation in VOC[13] and hemolysis,[14] and subsequent loss of zinc in the urine.[13] Zinc deficiency adversely affects T and B cell function and cell-mediated immunity,[15,16] which might increase the risk of infection in SCA. In 2 clinical trials in the United States and 1 trial in India on adolescents or adults with SCA (total cohort size range, 32-145 individuals; dose range, 40-75 mg/day), zinc supplementation decreased incidence of infection compared with placebo or to a prior period without zinc supplementation.[17-19] In one of these studies, a small nonrandomized trial, long-term (>1 year) zinc supplementation also reduced VOC in adults with SCA.[18] A Cochrane review concluded that the small clinical trials conducted to date provide a strong rationale for a large clinical trial of zinc supplementation for prevention of infection in SCA.[20] However, to date no zinc supplementation trials for prevention of infection have been conducted in children aged <5 with SCA in Africa, which has the largest population of affected children.

We conducted a prospective randomized clinical trial to evaluate the effect of daily zinc supplementation on the incidence of severe or invasive infections in children <5 years of age with SCA in Uganda. We hypothesized that zinc supplementation would decrease the incidence of severe or invasive infections as well as SCA-related adverse events.

Methods

Study design and participants

Between March 2019 and December 2020, we conducted a randomized double-blind placebo-controlled trial of daily zinc supplementation vs placebo for infection prevention in Ugandan children with SCA (Zinc for Infection Prevention in Sickle cell anemia, ZIPS). The study protocol was published[21] and the study was registered on ClinicalTrials.gov (Identifier NCT03528434; registered on 17 May 2018). The ZIPS study was conducted at the Nalufenya Sickle Cell Clinic at the Jinja Regional Referral Hospital, Uganda. Study eligibility criteria included documented SCA (HbSS) supported by hemoglobin electrophoresis, age between 1.00 and 4.99 years at enrollment, weight ≥5.0 kg, and willingness to comply with all study-related treatments, evaluations, and follow-up. Children with other known chronic medical conditions (eg, HIV-infection and malignancy) or severe malnutrition (weight-for-height < −3SD according to WHO growth standards) were excluded. All children received standard maintenance care for SCA, which included daily folic acid, penicillin prophylaxis, monthly sulphadoxine-pyrimethamine for malaria prophylaxis, and an insecticide-treated mosquito net for malaria prevention. Based on recent studies showing the efficacy of hydroxyurea in all children with SCA,[8,22] hydroxyurea treatment was encouraged for all study children, particularly those meeting the Uganda Ministry of Health SCA hydroxyurea treatment criteria at the time of the study, which included >5 pain crises in the past 12 months, stroke, baseline Hb < 6g/dL, and history of or new acute chest syndrome (ACS). Hydroxyurea was administered daily at a dose of 20 ± 2.5 mg/kg, with dose adjustments for changes in weight or hematological toxicities. As per Ugandan guidelines at the time of study, the hydroxyurea dose was not titrated to maximum tolerated dose. Hydroxyurea use in children with SCA was uncommon at this site at the time of study initiation but increased over the study period as parents became more comfortable with its use. No child was on a chronic transfusion program. Indications for blood transfusion included acute anemia, acute splenic sequestration crisis, clinical stroke, and ACS that did not respond to the conventional treatment.

Randomization and masking

Block randomization was used (block sizes of 8). The randomization list was created and managed by an independent study data manager before the initiation of the study. Study investigators other than the study biostatistician, along with staff, parents, guardians, and study pharmacists were all blinded to the treatment assignment. Information on randomized treatment for each participant was provided by the data manager to the study pharmacist, who dispensed the study medication as group A or B without knowing which group was zinc or placebo. The oral dispersible zinc sulfate tablets (10 mg) or identical placebo tablets were manufactured under good manufacturing processes by Laboratoires Pharmaceutiques (Rodael, France) and were prescribed once daily for 12 months.

Study procedures

At baseline, the children underwent a detailed physical examination and relevant history of SCA-related complications, including a prior history of stroke, transfusions, and hospitalizations. The children in the study had follow-up visits at 1, 3, 6, 9, and 12 months for replenishment of zinc or placebo tablet supply, assessment of adherence (by pill counts), evaluation of adverse events, and interim measurements of height and weight.

Evaluation and clinical management of infections

Parents or guardians were asked to bring their children to the Nalufenya Sickle Cell Clinic for any illness, where they were evaluated for clinical evidence of infection by taking a clinical history and diagnostic workup, according to protocol-defined procedures. Children with a history of fever or measured axillary temperature of ≥37.5°C were assessed for malaria, urinalysis to assess signs of a urinary tract infection, and chest radiography if they had age-specific tachypnea. Children with a temperature ≥38°C and signs of severe illness underwent a blood culture. Full definitions of protocol-specified severe/invasive infections are provided in Table 1. Details of the treatment for specific infections are provided in the supplementary information document (supplemental Table 4).

Laboratory evaluations

At enrollment, complete blood count and hemoglobin electrophoresis were performed using capillary zone electrophoresis (Minicap, Sebia). Malaria was evaluated by microscopy of Giemsa-stained slides by 2 independent readers, with a third to resolve any

Table 1. Definitions of severe or invasive infections in the ZIPS study

Infection	Definition
Abscess	Opaque, fluid-filled/fluctuant collection on skin (with purulent discharge if drained)
Bacteremia	Children with a positive blood culture with a true pathogen (eg, *Staphylococcus aureus, Streptococcus pneumoniae*, *Salmonella*, other gram-negative infections)
Cellulitis	Area of reddened, warm skin in a child with a history of fever or measured axillary temperature of ≥37.5°C
Diarrhea	More than 3 loose stools in a 24-h period
Dysentery	Fever with bloody stools
Malaria	Measured fever (axillary temperature ≥ 37·5°C) or fever by history and *Plasmodium* species infection on blood smear
Meningitis/encephalitis	Fever with (1) nuchal rigidity or altered mental status and (2) CSF with >5 WBC or with positive CSF culture for meningitis-associated organisms (e.g, *S pneumoniae*, *Neisseria meningiditis*, *Haemophilus influenzae*)
Osteomyelitis	Fever with bone pain, redness of skin over bone and x-ray findings consistent with osteomyelitis
Pharyngitis/tonsillitis	Inflamed, erythematous pharynx and/or tonsils, with pharyngeal or tonsillar exudate
Pneumonia/ACS	Pneumonia: history of fever or measured axillary temperature ≥37·5°C, with age-specific tachypnea, cough, and an infiltrate and/or effusion on chest x-ray consistent with pneumonia* ACS: signs of pneumonia above plus chest pain and/or tenderness
Sepsis	Meets modified criteria for SIRS/sepsis in International pediatric sepsis consensus guidelines (2 or more of the following criteria, 1 of which must be abnormal temperature: T ≥ 38·5°C, age-specific tachycardia, age-specific tachypnea, age-specific leukopenia). Modified to remove leukocytosis because, per NOHARM study data, >80% of children with SCA at Mulago Hospital will have age-specific leukocytosis at baseline, which is an IPSC criterion for SIRS/sepsis. Because SIRS in a child with SCA is always suspected to be owing to infection, we will use the term sepsis
Sinusitis (acute)	Congestion, nasal discharge or cough for more than 10 days without improvement; or symptoms of congestion with purulent nasal discharge for >3 days
Urinary tract infection	Symptoms (fever with urinary frequency, burning or new incontinence after previous toilet training) plus urinalysis positive for LE or nitrite OR clean catch urine culture with >100 000 colonies of a single pathogen

*Any child with a standard clinical diagnosis of pneumonia (clinical signs above) will be treated for pneumonia regardless of CXR findings as per the Mulago Hospital Sickle Cell Clinic protocol. Chest radiographs will be read by an on-call radiologist for acute clinical care, and saved for reading by a second radiologist. Specific criteria will be assessed by both radiologists, and only children who meet criteria from the WHO Radiology Working Group for pneumonia will be given a final diagnosis of pneumonia (Cherian T et al, Bulletin of WHO, 2005;83:353-359). Children who do not meet radiographic criteria will be given a final diagnosis of "respiratory infection" and not included in primary category of "severe or invasive infections" that constitute the primary study endpoint. They will be considered the secondary end point of "all clinical infections." Republished without any changes from Datta et al.[21] Originally published under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

discrepancies. To assess bacteremia, 1 to 3 mL of whole blood was inoculated into pediatric blood culture bottles (Peds Plus/F), which were incubated in an automatic BACTEC 9050 blood culture system for up to 5 days. To assess zinc levels, whole blood was collected in the morning in trace element free and additive free tubes using gloves, pipettes, and cryovials and confirmed to have low zinc levels before study initiation. Serum was stored at −20°C within 30 minutes of collection and later at −80°C. Zinc was measured using atomic absorption spectroscopy at the University of Minnesota. Samples were available for testing at both time points for 121 children in the zinc arm and 117 children in the placebo arm.

Outcomes

The primary study outcome was the incidence of severe or invasive infections. Protocol-defined secondary outcomes included the incidence of clinically defined infections, culture-confirmed bacterial infections, VOC, zinc-related adverse events, and change in height-for-age z-score.

Statistical analysis

Assuming an incidence of 0.71 severe or invasive infections per year in the placebo group, based on the NOHARM trial data,[8] a sample size of 250 children (with a 10% loss to follow-up) was estimated to have 80% power to detect a decrease of ≥40% in severe or invasive infection incidence over the 12 month treatment period. Primary analysis was performed in an intention-to-treat framework for all eligible randomized patients. The incidence of infections, serious adverse events, or sickle cell anemia-related adverse events was compared between treatment arms using negative binomial regression or Poisson mixed-effects regression models as appropriate, adjusting for time on hydroxyurea therapy. For binary outcome measures, Cox proportional hazards regression was used with hydroxyurea as a time-varying covariate. Continuous measures were compared between the treatment arms using the two-sample t-test, whereas categorical measures were compared using the χ^2 test or Fisher exact test, as appropriate. A paired *t*-test was used to compare serum zinc levels at baseline and follow-up, whereas McNemar chi-square test was used to compare the proportions with low zinc levels. A P value of <.05 was considered significant.

Ethics

Parents or legal guardians of all children provided written informed consent to participate in the study. Ethical approval was obtained from the Makerere University School of Medicine Research and Ethics Committee and Indiana University Institutional Review Board. The Uganda National Drug Authority and Uganda National Council of Science and Technology granted regulatory approval. The participating site provided administrative clearance.

Results

Study enrollment, treatment adherence and treatment-related adverse events, and use of hydroxyurea

A total of 252 children were enrolled in the study, and 250 were randomized (124 to zinc and 126 to placebo; supplemental Figure 1). Two children were excluded before randomization, as they met the exclusion criteria (1 with another chronic medical condition and 1 nonadherent to the study procedures). Among the

Table 2. Baseline demographic, clinical, and laboratory characteristics of the study cohort, according to zinc or placebo randomized intervention

Characteristic	Zinc (N = 124)	Placebo (N = 124)
Age at enrollment, mo	32.3 ± 13.4	32.6 ± 13.8
Male, n (%)	60 (48.4)	57 (46.0)
Sleeps under an insecticide-treated bednet every night, n (%)	110 (88.7)	104 (83.9)
Weight-for-height z-score	−0.34 ± 0.91	−0.44 ± 0.93
Height-for-age z-score	−1.29 ± 0.94	−1.32 ± 1.10
Prevalence of wasting (weight-for-height z-score < −2)	5 (4.0)	6 (4.8)
Prevalence of stunting (height-for-age z-score < −2)	30 (24.2)	33 (26.6)
Had prior episode of dactylitis, n (%)	105 (84.7)	106 (85.5)
Had prior stroke, n (%)	3 (2.4)	2 (1.6)
Had prior blood transfusion, n (%)	69 (55.7)	68 (54.8)
On hydroxyurea therapy before enrollment, n (%)	2 (1.6)	1 (0.8)
On daily penicillin prophylaxis before enrollment, n (%)	95 (76.6)	93 (75.0)
On malaria prophylaxis before enrollment, n (%)	97 (78.3)	96 (77.4)
Hemoglobin concentration, g/dL	8.2 ± 1.3	8.3 ± 1.3
Fetal hemoglobin %	13.4 ± 7.3	14.0 ± 8.6
Absolute reticulocyte count, ARC, × 10^9/L	137 ± 47	142 ± 48
White blood cell count, WBC, 10^9/L	18.8 ± 6.5	19.5 ± 10.0
Creatinine, mg/dL	0.30 ± 0.06	0.30 ± 0.06
HIV positive, n (%)	0 (0)	0 (0)
Had measles vaccination before enrollment, n (%)	110 (88.7)	117 (94.4)
Had pneumococcal vaccination before enrollment, n (%)	124 (100)	124 (100)

Plus-minus values are means ± SD.

randomized children, 2 children in the placebo arm later met the exclusion criteria after repeat hemoglobin electrophoresis, and confirmatory genetic analyses revealed that these children did not have HbSS (1 HbAS and 1 HbAA). Demographic, clinical, and laboratory parameters at enrollment did not differ significantly between children randomized to the zinc and placebo treatment arms (Table 2). Intention-to-treat analysis included those eligible to be randomized to treatment.

Adherence to study medication was high, as assessed by manual pill counts performed at scheduled visits, with children taking on average 97.2% and 97.0% of zinc or placebo tablets, respectively.

The frequency of treatment-related adverse events (vomiting and nausea) recorded during scheduled visits was similar between the zinc and placebo groups. The mean percentage (standard deviation [SD]) for vomiting was 0.33% (0.45) vs 0.33% (0.73) ($P = .99$) and for nausea was 0% (0) vs 0.16% (0.36) ($P = .37$), respectively.

Only 3 children were on hydroxyurea at the beginning of the study, but an additional 117 children were initiated on hydroxyurea during the study. There was no significant difference in the proportion of children initiated on hydroxyurea therapy or in the total person-days over the study period in the zinc arm compared with the placebo arm, 43.6% vs 50.8% ($P = .25$) and mean (SD) of 231 (87) vs 218 (84) ($P = .43$), respectively.

Incidence of severe or invasive infections

Sepsis, malaria, gastroenteritis, and pharyngitis/tonsillitis were the most common forms of severe or invasive infections (Figure 1). In the zinc and placebo arms, 105 (84.7%) and 100 (80.7%, $P = .40$) children had severe or invasive infections, respectively, and 88 (66.9%) and 73 (58.9%, $P = .19$) had more than 1 severe or invasive infection, respectively. There was no difference in the incidence of severe or invasive infections in children in the zinc vs placebo treatment arms, with 282.2 vs 270.5 severe or invasive infections per 100 person-years, respectively, corresponding to an adjusted incidence rate ratio (aIRR), adjusted for time on hydroxyurea, of 1.04, (95% confidence interval [CI], 0.81-1.32; $P = .78$) (Figure 1). Culture-confirmed bacterial infections (bacteremia, urinary tract infections) did not differ in incidence between the zinc and placebo arms (bacteremia, aIRR, 0.64; 95% CI, 0.09-4.39; $P = .65$; urinary tract infections, aIRR, 0.91; 95% CI, 0.51-1.64; $P = .81$; Figure 1).

Incidence of clinically defined infections

There was no difference in the incidence of clinically defined infections, aIRR, 1.08, (95% CI, 0.88-1.33; $P = .45$) or in the incidence of specific clinically defined infections between the treatment arms (Figure 2).

Incidence of serious adverse events and SCA-related complications

The incidence of serious adverse events per 100 person-years was 6.7 in the zinc group and 13.7 in the placebo group (aIRR, 0.45; 95% CI, 0.06-3.43; $P = .44$; Figure 3). There were 9 deaths in the study, 2 in the zinc group (1.7%) and 7 in the placebo group (6.0%) (adjusted hazard ratio (aHR), 0.28; 95% CI, 0.06-1.35; $P = .11$). Five deaths occurred at the Jinja Referral Hospital and 4 at home or in another health care facility. Standardized evaluation of clinical records or parental reports established the likely cause of death as pneumonia with accompanying respiratory failure in 5 children, sepsis with accompanying heart failure in 1 child, severe malaria in 1 child, and an unspecified cause in 2 children. Among the 9 children who died, 4 were identified as zinc deficient and 5 zinc sufficient by serum zinc measures at the time of enrollment.

There was no significant difference in the incidence of the 2 most frequent SCA-related events, VOC/dactylitis and anemia requiring blood transfusion, between the treatment arms (Figure 3). Two participants had a stroke (defined as a new gross neurological deficit on neurological examination), and both were in the placebo group.

Because stroke and death are the 2 most severe outcomes in SCA, we evaluated the composite end point of stroke or death. Stroke or death over the study period occurred in 11 children, 2 in the zinc arm (none on hydroxyurea) and 9 in the placebo arm (3 on hydroxyurea) (aHR, 0.22; 95% CI, 0.05-1.00; $P = .05$). The association of zinc with reduced death or stroke persisted when 5 children with stroke before study initiation were excluded from the analysis (aHR, 0.12; 95% CI, 0.02-0.98; $P = .046$). Among all children started on hydroxyurea, there were no significant differences according to zinc or placebo treatment in hemoglobin level (mean [SD], 8.7 [1.6] vs 8.4 [1.4]; $P = .34$) or MCV (mean [SD],

Event	Zinc	Placebo	Crude incidence rate ratio (95% CI)	*P* value	Adjusted incidence rate ratio (95% CI)	*P* value
	No. of events (incidence rate per 100 person–yr)					
Any severe or invasive infection*	339 (282.2)	317 (270.5)	1.03 (0.83–1.29)	.77	1.04 (0.81–1.32)	.78
All severe or invasive infections requiring hospitalization*	159 (132.4)	120 (102.4)	1.28 (0.97–1.70)	.08	1.28 (0.94–1.73)	.12
Specific infections						
Abscess	1 (0.8)	3 (2.6)	0.32 (0.02–4.88)	.41	0.47 (0.00–309)	.82
Bacteremia*	7 (5.8)	10 (8.5)	0.68 (0.26–1.79)	.44	0.64 (0.09–4.39)	.65
Cellulitis	12 (10.0)	11 (9.4)	1.06 (0.47–2.41)	.88	0.96 (0.31–2.94)	.94
Gastro–enteritis	52 (43.3)	46 (39.2)	1.10 (0.67–1.80)	.72	1.11 (0.64–1.90)	.71
Dysentery†	0 (0)	1 (0.9)	–	–	–	–
Malaria	74 (61.6)	59 (50.3)	1.20 (0.79–1.82)	.38	1.16 (0.76–1.78)	.48
Osteomyelitis†	0 (0)	0 (0)	–	–	–	–
Pharyngitis/Tonsillitis*	45 (37.5)	57 (48.6)	0.77 (0.48–1.23)	.27	0.75 (0.46–1.24)	.26
Pneumonia/Acute chest syndrome*	8 (6.7)	7 (6.0)	1.12 (0.40–3.08)	.83	1.15 (0.11–11.6)	.90
Sepsis*	92 (76.6)	69 (58.9)	1.30 (0.94–1.79)	.11	1.29 (0.91–1.81)	.15
Sinusitis (acute)*	28 (23.3)	33 (28.2)	0.83 (0.48–1.42)	.49	0.82 (0.45–1.51)	.52
Urinary Tract Infection*	20 (16.6)	21 (17.9)	0.93 (0.50–1.71)	.81	0.91 (0.51–1.64)	.81

Figure 1. Incidence of severe or invasive infections. Negative binomial regression analysis with and without adjustment for time treated with hydroxyurea. The bars and point estimates for the CIs correspond to crude incidence rate ratios. *The adjusted result is from a Poisson mixed-effects regression model, as the negative binomial counterpart did not converge or had a non-positive variance estimated for the random intercept. †No convergence (no event in at least 1 treatment arm).

90.3 [10.2] vs 87.5 [9.2]; $P = .11$) at 12-month follow-up. Both of these laboratory measures typically increase with hydroxyurea therapy; therefore, these findings suggest that there were no significant differences in hydroxyurea adherence in children receiving zinc vs placebo. Among the baseline characteristics, only the presence of stunting differed significantly between those who died (5/9, 55.6%) and those who survived (58/239, 24.3%; $P = .046$; supplemental Table 5).

Event	Zinc	Placebo	Crude incidence rate ratio (95% CI)	*P* value	Adjusted incidence rate ratio (95% CI)	*P* value
	No. of events (incidence rate per 100 person–yr)					
All clinically defined infections	648 (539.5)	576 (491.4)	1.07 (0.88–1.31)	.49	1.08 (0.88–1.33)	.45
All clinically defined infections required hospitalization	165 (137.4)	127 (108.4)	1.25 (0.92–1.69)	.15	1.25 (0.91–1.71)	.16
Specific infections						
Lower respiratory tract infection*	129 (107.4)	113 (96.4)	1.10 (0.81–1.50)	.55	1.09 (0.79–1.51)	.60
Clinical cellulitis	9 (7.5)	2 (1.7)	4.39 (0.91–21.25)	.07	3.72 (0.16–86.3)	.41
Clinical malaria*	20 (16.6)	21 (17.9)	0.94 (0.47–1.91)	.87	0.99 (0.43–2.29)	.97
Cholecystitis*	7 (5.8)	5 (4.3)	1.37 (0.39–4.81)	.62	1.16 (0.07–18.7)	.92
Conjunctivitis*	14 (11.7)	13 (11.1)	1.05 (0.49–2.24)	.90	1.07 (0.42–2.76)	.88
Fever of unknown cause	35 (29.1)	23 (19.6)	1.48 (0.88–2.51)	.14	1.48 (0.80–2.73)	.21
Measles†	3 (2.5)	0 (0)	–	–	–	–
Otitis media	10 (8.3)	8 (6.8)	1.22 (0.47–3.14)	.68	1.09 (0.16–7.24)	.93
Tinea capitis	15 (12.5)	6 (5.1)	2.45 (0.80–7.45)	.12	1.72 (0.20–14.5)	.62
Tinea corporis*	3 (2.5)	6 (5.1)	0.49 (0.12–1.95)	.31	0.50 (0.02–11.5)	.66
Upper respiratory tract infection	328 (273.1)	284 (242.3)	1.11 (0.88–1.40)	.38	1.12 (0.87–1.43)	.38
Clinical urinary tract infection*	4 (3.3)	10 (8.5)	0.39 (0.12–1.24)	.11	0.42 (0.05–3.84)	.44
Varicella*	10 (8.3)	6 (5.1)	1.63 (0.59–4.47)	.35	1.60 (0.29–8.92)	.59
Other clinical infection	61 (50.8)	79 (67.4)	0.75 (0.51–1.09)	.13	0.73 (0.49–1.08)	.12

Figure 2. Incidence of clinically defined infections. Negative binomial regression analysis with and without adjustment for time treated with hydroxyurea. The bars and point estimates for the CIs correspond to crude incidence rate ratios. *The adjusted result is from a Poisson mixed-effects regression model, as the negative binomial counterpart did not converge or had a non-positive variance estimated for the random intercept. †No convergence (no event in the placebo group).

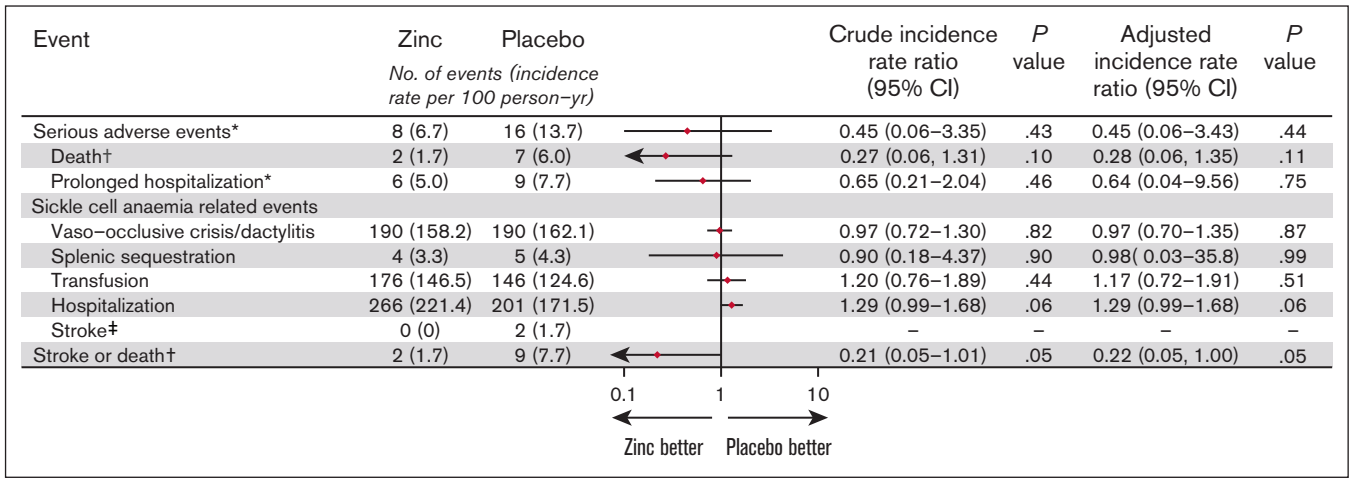

Figure 3. Incidence of adverse events. Hazard and incidence rate ratios obtained by Cox regression and negative binomial regression, respectively, with and without adjustment for time treated with hydroxyurea. The bars and point estimates for the CIs correspond to the crude incidence rate ratios. *The adjusted result is from a Poisson mixed-effects regression model as the negative binomial counterpart did not converge or had a non-positive variance estimated for the random intercept. †Hazard ratio is computed for this binary outcome with hydroxyurea as a time-varying covariate. Incidence rate ratios are computed for other outcomes. ‡ No convergence (no event in the zinc group).

Zinc levels and zinc deficiency at enrollment and 12 month follow-up

Zinc deficiency (serum zinc < 65 μg/dL) was common in children with SCA at enrollment (64.5% in children in the zinc arm and 60.7% in children in the placebo arm, Table 3). Children randomized to zinc treatment, but not those randomized to placebo, had an increase in zinc levels and a decrease in the frequency of zinc deficiency from enrollment to the 12 month follow-up visit (Table 3). However, 41.3% of zinc supplemented children remained zinc deficient even after 12 months of supplementation (Table 3). No differences were observed in the incidence of severe or invasive infections, clinically defined infections, or SCA-related adverse events with zinc vs placebo treatment in children who were zinc deficient or those who were zinc sufficient at enrollment (supplemental Table 1). Similarly, the incidence of severe or invasive infections or clinically defined infections did not differ between zinc and placebo treatment in children who were always zinc sufficient, went from sufficient to deficient, went from deficient to sufficient, or were always deficient (supplemental Tables 2 and 3). Only children who remained zinc deficient at 12 months had an IRR of <1 for severe or invasive infections, but this difference was not statistically significant (supplemental Table 2).

Height-for-age z-scores did not differ significantly between children in the zinc and placebo arms at enrollment (mean [SD], −1.29 [0.94] vs −1.32 [1.10]; $P = .86$) and 12 month follow-up (mean [SD], −1.23 [0.92] vs −1.25 [1.00]; $P = .84$), as did the prevalence of stunting (24.2% vs 26.6% at enrollment; and 21.5% vs 18.0% at 12 month follow-up; all $P > .05$).

Discussion

In this randomized, double-blind, placebo-controlled clinical trial evaluating daily oral zinc supplementation for infection prevention in Ugandan children < 5 years of age with SCA, zinc administered at a dose of 10 mg per day for 12 months did not reduce the incidence of severe or invasive infections or clinically defined infections. A substantial proportion of children remained zinc-deficient despite a year of zinc supplementation. This unexpected finding suggests that higher doses of zinc may be required to resolve zinc deficiency and potentially decrease the risk of infection. In the post hoc analysis, the occurrence of stroke or death was lower in children treated with zinc than in those treated with placebo. Further studies on the efficacy of zinc supplementation to prevent these severe outcomes are warranted, although such studies would require a very large sample size.

The ZIPS study is the first large randomized clinical trial of zinc supplementation for infection prevention in young children with SCA in Africa. In contrast to previous studies in adults with SCA, in which zinc supplementation (dose range, 50-75 mg/day; 6-9 times the US recommended daily allowance for adults) reduced the incidence of infections,[17,18] children with SCA in the ZIPS study did not reduce the incidence of infections with zinc supplementation. It is possible that the zinc dose was insufficient to prevent infections in children with SCA. We chose the 10 mg study dose of zinc because it is 2 to 3 times the recommended daily allowance for this age group, and multiple previous studies showed benefits of zinc supplementation in healthy children at a dose of 10 mg (30 of 66 studies reviewed in a systematic review of zinc supplementation randomized clinical trials used a 10 mg dose),[23] as did 1 trial evaluating the effects of zinc on growth in US children with sickle cell disease.[24] Higher doses, such as the 20 mg treatment dose used for diarrhea, have been associated with an increased frequency of vomiting in young children being treated with zinc for 14 days.[25] Because the study involved a 1 year administration, we chose the dose considered to have the greatest safety while still providing efficacy.

In the ZIPS cohort, high rates of zinc deficiency were observed at baseline (64% and 60% in the zinc and placebo arms, respectively). Although zinc levels increased in children in the zinc supplementation arm, many zinc supplemented children (41.3%) remained hypozincemic after 12 months of treatment. Continuing urinary losses of zinc in SCA,[13] together with poor absorption, or

Table 3. Mean serum zinc levels (μg/dL), proportion with low zinc levels (<65 μg/dL), and hematological indices at baseline and month-12 follow-up

Zinc treatment arm and measure	Baseline	Month 12	*P* value*
Zinc arm (N = 121)			
Serum zinc, μg/dL, mean ±SD	61.4 ± 11.5	72.2 ± 20.6	**.001**
Zinc deficient (<65 μg/dL), n (%)	78 (64.5)	50 (41.3)	**.001**
Hematological indices			
MCV, fL, mean ±SD	78.7 ± 10.7	85.7 ± 10.2	**.001**
Hemoglobin, g/dL, mean±SD	8.2 ± 1.3	8.1 ± 1.6	.30
Placebo arm (N = 117)			
Serum zinc, μg/dL, mean ± SD	62.9 ± 12.1	64.4 ± 12.6	.32
Zinc deficient (<65 μg/dL), n (%)	71 (60.7)	64 (54.7)	.32
Hematological indices			
MCV, fL, mean ±SD	77.6 ± 8.9	83.8 ± 9.8	**.001**
Hemoglobin, g/dL, mean±SD	8.3 ± 1.3	8.0 ± 1.3	**.02**

*Paired *t*-test used to compare continuous measures, McNemar's chi-square test used to compare proportion with low zinc levels.

increased loss from increased intestinal permeability from VOC-associated intestinal injury[26] could explain the high percentage of children remaining zinc-deficient even after supplementation. The average increase in serum zinc levels in the present study in the zinc supplementation arm was 10.8 μg/dL over 1 year. In the study by Prasad et al in adults, zinc levels increased by 13.8 μg/dL and 28 μg/dL in year 1, and year 3, respectively.[18] Given the high percentage of children who remained zinc-deficient despite treatment, it is possible that the immunological benefits of zinc supplementation (eg, greater natural killer cell activity, a higher $CD4^+$to $CD8^+$ T cell ratio, and increased T cell differentiation, B cell development and antibody production reported in earlier studies were not achieved in this study).[15,27] Increased baseline inflammation in children in Africa with SCA[5] would also be expected to lower plasma zinc levels through upregulation of the zinc transporter ZIP14 (*SLC39A14*), leading to increased hepatocellular uptake of zinc.[28] Finally, although use of plasma levels is the standard way to evaluate zinc deficiency, its low sensitivity is well recognized and normal plasma concentrations do not rule out a functional response to zinc supplementation.[29] The size of the exchangeable zinc pool size, which represents ~10% of total body zinc and reflects metabolically active zinc, has recently been shown to respond to zinc supplementation and may be less impacted by inflammation, but measurement requires isotope methodology and it has not been linked to functional outcomes.[30] Pilot studies with increasing zinc doses may be required to determine the dose that can raise zinc levels to sufficiency but still be well tolerated.

The finding of a lower incidence of stroke or death in children in the zinc supplementation arm is unexpected. This outcome was not prespecified because we did not expect the number of deaths in this study to be over a 1 year period, and the study was not designed or powered to evaluate this outcome; therefore, these findings must be viewed as preliminary. However, the striking differences (2 strokes or deaths in the zinc arm and 9 in the placebo arm) suggest that these outcomes deserve further investigation, especially because most study deaths were attributed to an infectious cause. It is possible that zinc prevents life-threatening infections rather than moderate to severe infections. Protection occurred in children with zinc deficiency or sufficiency at study enrollment, raising the possibility of direct protective effects of zinc on the immune response,[17] rather than solely correcting zinc deficiency. Zinc can also reduce the levels of endothelial activation[17] and increase vasorelaxation,[31] which could lower stroke risk. The findings on the occurrence of stroke or death are preliminary, but given their importance as outcomes in SCA, merit further investigation in future studies. Recent evidence supports the role of hydroxyurea in stroke prevention.[32,33] Our data suggest that adherence to hydroxyurea was not a major contributor to the differences in the risk of stroke or death in children in the zinc vs placebo treatment arms in this study.

In this study, we did not identify the beneficial effects of zinc on other SCA-related complications, including VOC, splenic sequestration, or transfusion. In a prior pilot study showing the benefits of high-dose zinc supplementation against VOC,[18] benefits were seen only in the second and third years of supplementation. It is not clear why it should take 2 or more years for zinc supplementation to affect the risk of VOC. The lack of benefit from zinc supplementation against VOC in the ZIPS study could be because of a too-low dose, too short a course, or lack of benefit in children despite the benefit in adults in the pilot study.

This study had several strengths, including a randomized, double-blind, placebo-controlled trial design, large sample size, and a high rate of retention during follow-up. Infections were systematically and rigorously evaluated and defined using standardized criteria, allowing for a comprehensive description of infections during the study period. We were able to initiate hydroxyurea treatment in 47% of the children, which improved the clinical care for SCA. This study highlights the capacity to conduct rigorous research to evaluate interventions to improve the clinical management of SCA, especially in settings where the burden and etiology of infections vary from high-income settings. Important study limitations were the lack of evaluation of multiple doses of zinc to determine optimal zinc dosing to resolve zinc deficiency and prevent infection, and the absence of evaluation of urinary zinc losses, which could potentially indicate the postulated increase in zinc requirement and, therefore, optimal zinc dosage. Although serum zinc is the most widely used indicator of zinc levels, it may not represent the true body zinc status because levels can be affected by several factors, including inflammation, fasting (and eating), and diurnal rhythm. In addition, we measured zinc levels only at baseline and at the 12 month follow-up, and zinc levels were not measured at the time of the actual infection. Malaria and penicillin prophylaxis and hydroxyurea treatment likely decreased the risk of infection and SCA-related complications, but because all children received prophylaxis medications and the rates of hydroxyurea prescription were similar in children in the zinc and placebo arms, they likely did not affect the risk of infection according to the treatment arm.

In conclusion, the administration of daily oral zinc given at a standard dose of 10 mg did not reduce the incidence of severe or invasive infections in children <5 years of age with SCA in the context of a high prevalence of zinc deficiency and high infection burden. Future studies are needed to explore approaches to reduce zinc deficiency in high-risk populations and evaluate whether higher doses can improve outcomes. The potential of zinc

supplementation to prevent stroke or death in children warrants further investigation.

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Authorship

Contribution: R.O. and C.C.J. designed the study; R.N., R.O., A.T., and C.C.J. supervised the study; C.B. and M.J.G. supervised data management and quality control and performed initial data analysis; W.T. and J.H.J. performed final data analysis; R.N. wrote the first draft of the manuscript; M.-S.R., D.D., and A.L.C. performed laboratory analyses, supervised laboratory processes, analyzed the results, and monitored the study; S.E.C., N.F.K., and R.E.W. provided guidance throughout the study on zinc supplementation and treatment of SCA-related complications; C.B., M.J.G., R.N., R.O., and C.C.J. verified the underlying data and provided final data interpretation; and all authors had full access to the study data, participated in the editing of the manuscript, and accepted responsibility of submission of the manuscript for publication.

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