Supplemental Information High-Dose Vitamin A With Vaccination After 6 Months of Age: A Randomized Trial

SUPPLEMENTAL METHODS

Setting and Population

The Bandim Health Project Health and Demographic Surveillance System covers an urban population of 102 000 individuals in the capital Bissau, and a rural population of 25 000 women and their children in 258 randomly selected villages. In the urban area, children younger than 3 years are followed through trimonthly home visits; in the rural area, children younger than 5 years are followed through 6-monthly home visits.

Three government health centers provide vaccinations during weekdays in the urban study area. When additional funding is available, vaccines also are given in outreach campaigns. In the rural area, children are vaccinated by government nurses at health centers where vaccination is most often available at a certain weekday, during outreach when funding is available, and by the Bandim Health Project team at their biannual visit. More funding was available to conduct outreach after the introduction of the new vaccines in 2008.[1] Thus, there were more opportunities to receive vaccinations in the urban than in the rural area and more opportunities after rather than before August 2008.

Identification of Eligible Children, Informed Consent and Baseline Examination

When the trial was planned, children in Guinea-Bissau received a DTP-booster at 18 months of age. This policy was discontinued in 2007 shortly before we initiated the trial. We therefore applied to the ethics committee to alter the enrollment criteria, and after approval in February 2008 we enrolled children up to 23 months of age in the rural areas. No other changes in enrollment criteria took place during the trial.

We intended to pause enrollment 1 month before a VAS campaign, but campaigns were announced with shorter notice and/or postponed several times. The interval between pausing enrollment and initiation of a campaign was 7 to 53 days. No child who received VAS within 1 month before a VAS campaign died during follow-up.

The mother was informed that it is recommended by WHO to give vitamin A with vaccines after 6 months of age, but that this policy is not implemented in Guinea-Bissau, and that we aimed to study whether it would be beneficial to implement. It was emphasized that participation was voluntary and that free consultations and essential drugs were available at the Bandim Health Project office in Bissau and at the village visits in the rural areas regardless of participation status. The same information was given in writing. If the mother accepted to participate, she would sign or fingerprint the inclusion form. The form was also signed by the enrolling a Bandim Health Project assistant and another person who witnessed the consent procedures.

From 15% of the participants dried blood spots were collected by finger prick at enrollment.[2] Vitamin A status and on-going infection were assessed by measurement of retinol binding protein and C-reactive protein.[2] We defined vitamin A deficiency as circulating retinol concentration <0.70 μmol/L by using the corresponding retinol binding protein concentration; infection/inflammation was defined as a C-reactive protein >5 mg/L[2].

Randomization and Masking

Randomization lots were prepared by the study supervisor who like all other trial personnel, was blind to the coding. Twenty lots, 10 with numbers corresponding to VAS and 10 with numbers corresponding to placebo, were packed in each envelope and separate envelopes were used for boys and girls. The mother drew a lot indicating from which of 2 numbered bottles the child should receive the supplement. Same-gender twins were randomized to the same treatment arm (the mother drawing only 1 lot). According to randomization, a vitamin A/placebo supplement was administered orally by the Bandim Health Project assistant/nurse responsible for the enrollment.

Coded vitamin A and placebo supplements were prepared by Skanderborg Pharmacy, Skanderborg, Denmark, which held the code. Two different sets of codes were used to allow breaking the code in the substudy of adverse events before completed follow-up of all children. A blinded dataset with data on the adverse events study was sent to a DSMB member in December 2010; the DSMB also received the code for the first

SUPPLEMENTAL TABLE 6 Cause of Death for the 8 Children Who Died Due to Accidents

Intervention	Gender	No. of Days After Enrollment	Cause of Death
Placebo	Girl	186	Burn
Placebo	Boy	205	Poisoning (ate mother's iron tablets)
Placebo	Boy	311	Attack by bee swarm
Vitamin A	Boy	325	Burn
Placebo	Boy	172	Drowning
Placebo	Boy	107	Drowning
Placebo	Boy	78	Car accident
Vitamin A	Girl	31	Burn

part of the trial from Skanderborg Pharmacy, Skanderborg, Denmark, and returned an unblinded dataset to the investigators. The second part of the trial was unblinded by study end, July 21, 2011.

Vaccines received at enrollment were registered by the enrolling Bandim Health Project assistant/nurse. Vaccines were UNICEF-certified vaccines delivered through the national vaccination program. The vaccines were DTP (Serum Institute of India, Pune, India and Bio Farma, Bandung, Indonesia), pentavalent (Quinvaxem from Berna Biotec, Incheon, Korea; Easyfive from Panacea, New Delhi, India; and Shan5 from Shantha, Hyderabad, India), OPV (Polio Sabin; GlaxoSmithKline, Wavre, Belgium and OPVERO, Sanofi-Pasteur, Lyon, France) MV (Measles Vaccine, Edmonston-Zagreb from Serum Institute of India, Pune, India and Rouvax, Schwarz, from Sanofi-Pasteur, Lyon, France), and YF (Stamaril, Sanofi-Pasteur, Lyon, France and Vaccin Amaril Stabilize, Institut Pasteur de Dakar, Dakar, Senegal).

VAS campaigns

National vitamin A and mebendazole campaigns took place in July 2007, December 2007, July 2008, January 2009, July 2009, January 2010, May 2010, December 2010, and May 2011. Vitamin A and mebendazole was given with iodine in January 2009, with MV in July 2009 and with OPV in May 2010 and May 2011. All children enrolled in the study, regardless of group assignment, were eligible to receive VAS and the other interventions given during these campaigns. Children also could have received VAS in campaigns before enrollment.

In the urban area, participation in campaigns was registered during the campaigns and through follow-up visits to all children who had not been seen during the campaigns. In the rural area, information was collected at the first visit after the campaign. Information was obtained from 87% of the children and, among these, 91% had received campaign VAS and 9% had not.

Sample Size

The planned sample size of 6000 children would be sufficient to demonstrate a 40% reduction in mortality among the 59% of the children expected to receive MV at enrollment. Because we had hypothesized a negative effect among children receiving VAS with DTP, we anticipated to demonstrate an interaction between vaccine type and VAS.

Statistical Analysis

Key enrollment information was double entered and inconsistencies resolved. *Z*-scores for weight-for-age, length-for-age, weight-for-length, and arm-circumference-for-age were based on the 2006 WHO child growth standard[3] and calculated using the WHO Anthro version 3.1 macro for Stata (Stata Corp).[4] Baseline characteristics were compared by χ^2 tests, *t* tests, or rank-sum tests. The proportional-hazards assumption was evaluated by log-log plots, Schoenfeld residuals, and by including the parameters estimated in each model as time-varying covariates to test whether their effect changed with time.

The analyses stratified by type of vaccine at enrollment had to take into account that the vaccination program changed during the conduct of the trial. Hence, we defined MV as MV given with or without YF, and DTP vaccine as either DTP vaccine or pentavalent vaccine. Combined live and inactivated vaccine was defined as MV (±YF) and DTP/pentavalent vaccines administered simultaneously. Smaller groups of children who received OPV alone or OPV+YF as their live vaccines were not

SUPPLEMENTAL TABLE 7 The Effect of VAS on All-Cause Mortality Overall and by Gender During 6 Months of Follow-up in the First 6000 Enrolled Children and When Excluding Children Who Received VAS or Placebo From the First Batch

	Rate per 1000 PYRS# (Deaths/PYRS)		MRR (95% Confidence Interval)[a]	Test of Interaction Between VAS and Gender[b]
	Vitamin A	Placebo		
First 6000 children followed				
	n = 2986	*n* = 3014		
All	19.7 (29/1469)	23.7 (35/1477)	0.83 (0.51–1.36)	
Boys	25.6 (19/743)	16.1 (12/744)	1.57 (0.76–3.24)	0.02
Girls	13.8 (10/726)	31.4 (23/733)	0.44 (0.21–0.93)	
Excluding first batch (lower potency)				
	n = 2807	*n* = 2805		
All	21.8 (30/1376)	24.1 (33/1371)	0.91 (0.55–1.49)	
Boys	27.3 (19/695)	14.3 (10/699)	1.91 (0.89–4.11)	0.01
Girls	16.1 (11/681)	34.2 (23/672)	0.48 (0.23–0.98)	

#, person-years of observation.

[a] Analyzed using Cox proportional-hazards models with age as underlying time scale.

[b] Tested using Wald test.

SUPPLEMENTAL TABLE 8 Reception of Routine Vaccines During the First 6 Months of Follow-up by Vaccine Type at Enrollment.

Vaccine at Enrollment	*n*	Routine Vaccines Registered On or Before 6 mo of Follow-up (%)	Routine Vaccines Registered by Type of Vaccine		
			DTP/Pentavalent	MV	YF
MV/MV + YF	3161	139 (4)	35 (1)	10 (0)	109 (3)
DTP/Pentavalent	2154	857 (40)	537 (25)	696 (32)	569 (26)
MV+DTP/Pentavalent	1610	229 (14)	197 (15)	6 (0)	61 (4)

considered in this stratified analysis, which focused on MV and DTP.

In addition to the analyses prespecified in the protocol, we examined whether the effect of VAS with vaccines varied by vitamin A status in the subgroup of 1102 children for whom we had assessed vitamin A status at enrollment.[2]

Ethical Considerations

WHO recommends VAS at vaccination contacts. The Bandim Health Project and the Guinean Ministry of Health considered it important to assess whether the policy would improve child survival before implementing in Guinea-Bissau. Safety reports with data on adverse events and mortality were presented to our DSMB every 3 months.

SUPPLEMENTAL RESULTS

Mortality for VAS Versus Placebo

A total of 261 pairs of enrolled children were siblings, and 1 mother had 3 children enrolled; adjusting for within-mother clustering using robust variance estimates had no effect on the estimates.

Mortality by Vaccine at Enrollment

Controlling for age, gender, VAS-placebo, and stratified for rural-urban enrollment, which was important for mortality level, children who received DTP/pentavalent vaccines or DTP/pentavalent vaccines with MV had higher mortality than the children receiving MV alone, the MRRs being 1.67 (0.86–3.26) and 2.04 (1.06–3.90), respectively.

The gender-differential effects of VAS were similar with the old Expanded Program on Immunizations vaccines (MRR = 1.65 [0.65–4.20] in boys and MRR = 0.51 [0.17–1.48] in girls) and the new Expanded Program on Immunizations vaccines (MRR = 2.06 [0.77–5.48] in boys and MRR = 0.37 [0.16–0.88] in girls).

Effect of VAS by Vitamin A Status

Vitamin A status and C-reactive protein levels were assessed among 1102 children at enrollment. Two-thirds of children (724/1102) were vitamin A deficient, equally as many boys (372/555) and girls (352/547).[2] During infection, circulating retinol and retinol-binding protein decrease as part of the acute-phase response.[5] Excluding samples from children with infection/inflammation (31%, 346/1102) lowered the prevalence of vitamin A deficiency slightly to 60% (455/756). Among children for whom vitamin A status was assessed, VAS was associated with an MRR of 2.36 (0.61–9.15); 1.41 (0.24–8.44) among the deficient children and 4.74 (0.53–42.5) among nondeficient children.

Cause of Death

Based on the interviews conducted after the registration of a death, the most common symptoms in the illness leading up to death were fever (36%) and diarrhea/vomiting (32%) (Supplemental Table 9). The gender-differential effects were similar for fever (MRR = 2.76 [0.89–8.66] for boys and 0.67 [0.24–1.88] for girls) and for diarrhea/vomiting

SUPPLEMENTAL TABLE 9 Main Symptom Preceding Death, Nonaccident Deaths Only

Group assignment	All		All				Dry Season				Rainy Season			
	All		Boys		Girls		Boys		Girls		Boys		Girls	
	VAS	Placebo	VAS	Placebo	VAS	Placebo	VAS	Placebo	VAS	Placebo	VAS	Placebo	VAS	Placebo
No. of deaths (% of deaths within the subgroup)														
Fever	30 (45)	19 (27)	18 (46)	8 (25)	12 (44)	11 (28)	13 (57)	6 (50)	5 (50)	6 (26)	5 (31)	2 (10)	7 (41)	5 (31)
Cough/difficulties breathing	3 (5)	6 (8)	3 (8)	2 (6)	0 (0)	4 (10)	1 (4)	0 (0)	0 (0)	4 (17)	2 (13)	2 (10)	0 (0)	0 (0)
Diarrhea/vomiting	20 (11)	24 (17)	10 (26)	10 (31)	10 (37)	14 (36)	6 (26)	1 (8)	4 (40)	10 (43)	4 (25)	9 (45)	6 (35)	4 (25)
Presumed malaria	7 (11)	12 (17)	4 (10)	8 (25)	3 (11)	4 (10)	2 (9)	4 (33)	1 (10)	2 (9)	2 (13)	4 (20)	2 (12)	2 (13)
Other[a]	3 (5)	6 (8)	3 (8)	4 (13)	0 (0)	2 (5)	1 (4)	1 (8)	0 (0)	1 (4)	2 (13)	3 (15)	0 (0)	1 (6)
Unknown	3 (5)	4 (6)	1 (3)	0 (0)	2 (7)	4 (10)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)	0 (0)	2 (12)	4 (25)
Different distribution between VAS and placebo groups[b]	0.30		0.32		0.36		0.30		0.49		0.46		0.67	
Different distribution between boys and girls[b]			All: 0.15/VAS: 0.34/Placebo: 0.21				All: 0.11/VAS: 0.85/Placebo: 0.05				All: 0.03/VAS: 0.41/Placebo: 0.06			

Symptom categories are mutually exclusive. Children with diarrhea and fever have been classified as diarrhea, cough and fever as cough, and children with reported malaria and fever in the description of the symptoms as presumed malaria.

[a] Four deaths with convulsions as main symptom (2 VAS, 2 placebo), 3 with prolonged disease with failure to thrive and underweight (all placebo), and 2 with congenital malformations (1 VAS, 1 placebo).

[b] Tested by χ^2 test.

(MRR = 1.49 [0.42–5.29] for boys and MRR = 0.46 [0.16–1.32] for girls).

The symptoms preceding death did not vary significant by season of enrollment ($P = .31$ with 6 months of follow-up, $P = .06$ with 12 months of follow-up) or by gender ($P = .73$ with 6 months of follow-up, $P = .15$ with 12 months of follow-up). The symptoms preceding death differed between the genders when stratified by season of enrollment (Supplemental Table 9), with more girls than boys having diarrhea/vomiting as a main symptom leading up to death in the dry season and more girls than boys having fever as the main symptom leading up to death in the rainy season. However, the small numbers and crude classification of the symptoms do not permit any conclusion on whether differential morbidity explains the gender-differential effects.

Two-thirds of the nonaccident deaths (54/80 within 6 months) occurred during the rainy season. In the rainy season, diarrhea/vomiting was the most common symptom (37%).

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