

# Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial

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## Summary

**Background** Anaemia caused by iron deficiency is common in children younger than age 5 years in eastern Africa. However, there is concern that universal supplementation of children with iron and folic acid in areas of high malaria transmission might be harmful.

**Methods** We did a randomised, placebo-controlled trial, of children aged 1–35 months and living in Pemba, Zanzibar. We assigned children to daily oral supplementation with: iron (12·5 mg) and folic acid (50 µg; n=7950), iron, folic acid, and zinc (n=8120), or placebo (n=8006); children aged 1–11 months received half the dose. Our primary endpoints were all-cause mortality and admission to hospital. Analyses were by intention to treat. This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN59549825.

**Findings** The iron and folic acid-containing groups of the trial were stopped early on Aug 19, 2003, on the recommendation of the data and safety monitoring board. To this date, 24 076 children contributed a follow-up of 25 524 child-years. Those who received iron and folic acid with or without zinc were 12% (95% CI 2–23, p=0·02) more likely to die or need treatment in hospital for an adverse event and 11% (1–23%, p=0·03) more likely to be admitted to hospital; there were also 15% (–7 to 41, p=0·19) more deaths in these groups.

**Interpretation** Routine supplementation with iron and folic acid in preschool children in a population with high rates of malaria can result in an increased risk of severe illness and death. In the presence of an active programme to detect and treat malaria and other infections, iron-deficient and anaemic children can benefit from supplementation. However, supplementation of those who are not iron deficient might be harmful. As such, current guidelines for universal supplementation with iron and folic acid should be revised.

## Introduction

About three-quarters of children younger than age 5 years who live in east Africa are anaemic (haemoglobin concentration <110 g/L; 1·71 µmol/L);<sup>1</sup> much of this anaemia can be ascribed to iron deficiency. International guidelines<sup>2</sup> recommend supplementation with iron and folic acid in children younger than age 2 years in areas with a high prevalence of anaemia. This recommendation is controversial though, particularly in areas affected by malaria.<sup>3,4</sup> Provision of iron supplements can enhance child development<sup>5,6</sup> and reduce the prevalence of severe anaemia.<sup>7</sup> However, results of some studies indicate that iron deficiency protects against malaria,<sup>3,8</sup> and those of others suggest that iron supplementation results in high levels of malaria parasitaemia<sup>7,9,10</sup> and increased rates of malaria, pneumonia, and diarrhoea.<sup>3,7,10–12</sup>

Trials that have ostensibly shown that iron can be given safely in settings with endemic malaria have mostly assessed treatment of anaemic children with iron.<sup>7,9,10,13,14</sup> The only three trials<sup>15–17</sup> of iron supplements for prevention of anaemia in unscreened children in a setting affected by malaria were not designed or powered to assess the safety of the supplement with respect to

admission to hospital or death. Our aim, therefore, was to assess the effect of iron and folic acid supplementation on severe morbidity and mortality.

## Methods

### Participants

Between Jan 1, 2002, and Aug 19, 2003, we did a randomised, double-masked, placebo-controlled trial on Pemba, the smaller of the two islands of the Zanzibar archipelago. The island has a population of about 350 000, most of whom are Afro-Shiraji muslims, and has a tropical climate. Malaria is holoendemic with year-round transmission that is highest in June–September.<sup>17</sup> The intensity of malaria transmission is representative of coastal east Africa, where a yearly inoculation rate of 405 infective bites per person has been described.<sup>18</sup> *Plasmodium falciparum* accounts for nearly all serious cases of clinical malaria. Governmental malaria-control activities at the time of the trial were based on diagnosis and treatment of suspected cases. A baseline census, including a birth history for women of reproductive age, indicated an infant mortality rate of 89 per 1000 livebirths. Between January and December, 2001, we mapped and

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did a census of Pemba, giving every house a serial number. We divided the four administrative districts of the island into 375 working areas. We assigned one female community worker to every cluster so that all enrolled households could be visited in a week. We invited all children aged 1–35 months, likely to remain resident on the island, and not having severe malnutrition needing rehabilitation to participate. Enrolment into the main study was undertaken one district at a time, starting in January, 2002, and finishing in May, 2002. We started enrolment into the substudy and the first round of blood collection in March, and completed it in November, 2002. We invited all new births in the study area to be enrolled at age 1 month.

A study worker read the consent statement to the primary caregiver and signed the form if consent was given. The study was approved by the Johns Hopkins Committee on Human Research, the WHO ethical review committee, and the Zanzibar Research Council.

### Procedures

We randomised participants to one of four groups and gave them one tablet daily (or half a tablet if <1 year old), containing: iron (12.5 mg), folic acid (50 µg), and zinc (10 mg); zinc alone; iron and folic acid; or placebo. All tablets dissolved in water or breastmilk in about 20 s, were manufactured by Nutriset (Malaunay, France) in collaboration with WHO, and were provided in blister packs of seven tablets. We gave no specific instructions as to when tablets should be taken. All children also received vitamin A: those aged 12 months or older were given 200 000 IU of vitamin A every 6 months and those aged younger than 12 months were given 100 000 IU.

Randomisation was by household. We used a permuted block allocation sequence with a block length of 16 that was generated by WHO. The supplement code, which was not known to the investigators, was maintained at WHO. To ensure masking, we labelled the strips of supplements with 16 letter codes—four for each of the groups. This letter code was hidden in the batch number on each strip of tablets. On enrolment, we assigned every child a code. Labels with the child's name on were then printed from a computer database and attached by the pharmacy to the appropriate strip of supplements.

We gave every child's guardian a card with the child's and their household's identification information and a control number printed on it for verification. We asked the family to maintain the card, show it to the community worker when they visited, and present it in hospital if the child was admitted. The children were visited weekly at home by a community worker who, at every visit, delivered a new strip of seven tablets labelled with the child's identification number, collected the previous week's strip (either empty blisters or remaining tablets), and recorded level of adherence to the supplement. The community workers obtained information about any admission to public hospital and overnight stays in private

clinics, and noted if the child had died in the previous week. Reports of admission to hospital were given to study workers who maintained records of admission provided by hospital data collection workers. If a child had died, two supervisors trained to do postmortem interviews were notified. They visited the family within 2 weeks of the child's death to try to identify the cause, using a standard method<sup>19</sup> that we had adapted and pretested for local use. We analysed reports of overnight admission to private clinics on the basis of the history given by the family; these reports were not used for the primary endpoints, but rather for secondary analyses. The community workers also identified new births for possible enrolment to the trial.

We employed two staff in each of the five hospitals to work in shifts on the ward over 18 h. For all children admitted, standard study procedures included confirming identification of the child, taking their temperature, completing a detailed morbidity questionnaire on the type and severity of illness, and obtaining a venous blood sample and thick and air-dried thin films for malaria parasite assessment. We followed-up all children admitted until discharge or death, noting their final diagnosis (or cause of death) and any treatment received.

We transported blood samples and films to the central project laboratory at the Public Health Laboratory Ivo de Carneri in Pemba. Blood films were stained with Giemsa and read by light microscope with a X100 oil immersion lens. Malarial parasite count was done until more than 200 leucocytes were counted; if no parasites were seen, assessment was extended until 500 leucocytes had been counted. Standard quality control procedures were implemented for malarial parasite counting,<sup>20</sup> with every observer reading, blinded, his or her own slides from the previous day (within-observer estimation) and those of other observers (between-observer estimation). Parasite density was calculated by using actual total leucocyte count from the Coulter counter analysis. The EDTA blood was analysed with a KX-21 automated haematology analyser for a detailed haemogram, including haemoglobin, total leucocyte count, and three-part differential. Zinc protoporphyrin was measured with a fluorometer (Aviv Biomedical, Lakewood, NJ, USA).

To create a conservative and specific definition of malaria, we assessed the distribution of malaria parasite counts in 21 160 records with information on the clinical profile and malarial counts of children. This analysis included data from 8015 records of individuals admitted to hospital and from 13 145 records from community-based cross-sectional surveys (3462 of these blood films were from children who were sick on the day of assessment and 9683 blood films were from children who were not sick in the previous 7 days and did not become ill in the next 7 days). Based on this analysis, for an illness to be defined as malaria a child needed to fulfil one of the following criteria: have a parasite count of more than 1000 per mm<sup>3</sup> and a recorded temperature of more than 38°C; have a

parasite count of more than 3000 per mm<sup>3</sup> and a history of fever; or have a parasite count of more than 8000 per mm<sup>3</sup> with or without fever. This definition has a sensitivity of 84% for detecting malaria in individuals admitted to hospital with a parasite count of more than 0, and a specificity of more than 90% (false positives of <10% in children during survey with no illness). In the baseline survey, 43% of children who were not sick had a parasite count of more than 0. We diagnosed a child with cerebral malaria syndrome if they had malaria as defined above and had been unconscious or had had a seizure; we also did an analysis, excluding episodes with only seizures.

In the absence of lumbar puncture, which was not possible in the hospitals on the island, meningitis was diagnosed when a child did not meet the definition for cerebral malaria, but had a temperature of more than 37.9°C with observed unconsciousness or neck stiffness, or a clinical diagnosis of meningitis. We recorded that a child had diarrhoea if they were younger than age 24 months and passed more than four loose or watery stools or older than age 24 months and passed more than three loose or watery stools within 24 h and were reported to have diarrhoea by the mother. Dysentery was present if the child had bloody diarrhoea. We recorded pneumonia if the child had either a cough or difficulty in breathing and a respiratory rate of greater than the WHO cutoff (50 breaths per min and 40 breaths per min in children younger and older than 12 months, respectively) and at least one severity indicator present, or if a doctor had diagnosed pneumonia with crepitation.

For cause-specific mortality of study children, we used a postmortem interview (verbal autopsy) with family members to assign cause of death. Three teams, each consisting of two doctors and one medical assistant, assigned one primary and two secondary causes of death independently. Any disagreements were resolved by discussion. These teams did not include investigators and were unaware of group allocation. For allocation of the final cause of death, all medical records were available to the medical team. For the 105 children who died in hospital, two independent doctors assigned cause of death based on hospital records alone, and these were compared with a verbal autopsy diagnosis ascertained by a third person before being given the hospital records. Of the 102 deaths that were labelled as malaria by verbal autopsy, 87 (85%) were labelled malaria by analysis of hospital records. Of 35 deaths labelled infection-related, 20 (57%) were labelled as infection-related by hospital record.

We judged children with zinc protoporphyrin concentrations of more than 80 µmol/mol haeme iron-deficient and children with a haemoglobin concentration of 70–100 g/L anaemic. We selected a cutoff of 100 g/L instead of 105 g/L or 110 g/L in this population with a baseline mean haemoglobin of 96 g/L and 98 g/L to make the definition more specific and provide more contrast between anaemic and non-anaemic children, in

that children with severe anaemia (haemoglobin <70 g/L) had already been excluded.

Our three main outcomes were: serious adverse events (a composite of hospital admissions and deaths), death during follow-up or within 30 days of stopping supplementation; and hospital admission to any of the five public hospitals in Pemba. The primary endpoints were all-cause deaths and admissions to hospital, with cause-specific deaths and admission as secondary endpoints.

We also did a substudy with the original objectives of assessing the effects of supplementation on haematological and zinc status, malaria prevalence, and infectious disease morbidity. Given the results from the main trial, we analysed the data from the substudy to assess the adverse effects in the groups that received iron and folic acid by baseline anaemia, iron status, and anthropometry. The substudy consisted of a random sample of clusters in each of the four districts selected after the census. Supplementation and adverse-event surveillance was the same as in the main trial. Children in the substudy visited a study clinic for baseline assessment, including physical examination, height and weight measurements, and parental interview by a doctor and trained health worker; a 3 mL venous blood sample was obtained from the child for detailed haematological analysis, erythrocyte zinc protoporphyrin, and malarial parasite count. We excluded children from the substudy if they had baseline haemoglobin concentrations of less than 70 g/L, and gave them a therapeutic iron supplement for 3 months. We gave children with clinical malaria (parasite count >5000 per mm<sup>3</sup> of blood and axillary temperature >37.5°C) a dose of sulfadoxine/pyrimethamine as per local guidelines and enrolled them to the trial. We assessed the children in the clinic again at 6 months and 12 months after enrolment. During these scheduled clinics and in the intervening period, any child diagnosed with slide-confirmed malaria was given sulfadoxine/pyrimethamine. Any child with pneumonia, sepsis, or other infection was treated with appropriate antibiotics. These diagnosis and treatment practices were more extensive than in the main trial.

### Statistical analysis

To detect a difference of 20% in overall mortality between groups with 90% power, a two-sided type I error of 5%, a design effect of 1.05, accounting for 10% loss to follow-up, and based on the assumption of no interaction of iron, folic acid, and zinc on mortality, we calculated that we needed to include about 15 000 person-years in every row (column) of the marginal comparisons—ie, iron and folic acid groups versus placebo or zinc groups versus placebo. However, at the second meeting of the data and safety monitoring board 14 months into recruitment, we noted that the mortality rate of children was much lower than originally expected. We therefore recalculated the sample size estimates. The final number needed was calculated at

about 30 000 person-years in every row (column) of the marginal comparisons.

In view of the results in the groups taking iron and folic acid supplements, however, the marginal comparisons are no longer valid, so we did power calculations for analysed outcomes with an  $\alpha$  of 0.05 (two-tailed) and actual child follow-up. For the comparison between the groups taking iron and folic acid and those taking placebo, estimated power for a mortality increase of 15% was 80%, for an increase in admission to hospital of 11% was 68%, and for an increase in adverse events of 12% was 90%. For a 16% increase in adverse events due to malaria-related causes it was 76% and for a 32% increase in other infection-related causes it was 90%. For those taking iron and folic acid compared with those taking placebo, the estimated power to detect an increase in mortality of 17% was 31%; for an increase in adverse events of 10%, it was 52%.

We based the sample size of the substudy on the effect on severe anaemia for which the study was originally designed to detect a reduction of 40% with 80% power, a type I error of 5%, a design effect of 1.25, accounting for 10% loss to follow-up, and based on the assumption of no interaction of iron, folic acid, and zinc. Given the adverse effects of iron and folic acid in the main study, we analysed the substudy data to provide additional insights about this effect. For the groups that took iron and folic acid compared with those that took placebo, the estimated power to detect a reduction in total adverse events of 24% was 51%.

We used Visual Basic and ORACLE 8i to manage data (Oracle Corporation, CA, USA). All data obtained in the field were entered by the end of the next day. The systems used had extensive range and checking facilities. We verified possible errors with field or hospital staff on a daily basis. Data collection and supplement allocation was rigorously controlled with the help of computer monitoring. For all outcomes, we used double-data entry to detect errors. Flow of information, distribution of supplements, and collection of samples between households and the central office at the Public Health Laboratory Ivo de Carneri in Pemba was ensured by supervisors on motorbikes on the same day.

For analysis of cause-specific deaths or admissions to hospital, we used exclusive categories such that an event classified in a preceding category could not be considered for the next category. As such, we first allocated malaria-related causes, then pneumonia and other infection-related causes, and finally diarrhoea and others.

Analyses were by intent to treat. We included data for children who migrated out of the area or withdrew from the study up to the date of censorship. Person-time analysis was done with actual follow-up as denominator. For the effect on total mortality and cause-specific mortality, we used Cox regression with exact handling for ties (STATA version 8.2). For analysis of adverse events and admissions, we used Anderson Gill time-to-event survival methods in Cox regression<sup>21</sup> with robust

estimation of standard error to account for multiple events per child or within household (SAS version 9.0, STATA version 8.2). In these analyses, a relative rate (RR) of greater than 1 indicated a higher event rate in the intervention group than in the placebo group (increased risk with intervention) and a value of less than 1 was consistent with the intervention being protective. Because there were no significant or clinically important differences between the group taking iron, folic acid, and zinc and that taking iron and folic acid, we compared the two groups combined with placebo. To assess the effect of duration of supplementation on intervention effects, we calculated Nelson-Aalen cumulative hazard estimates. In Cox regression models, we modelled an interaction term of time and treatment as a continuous variable. Effect of age was also assessed by modelling an interaction term of age and treatment. Based on Nelson-Aalen estimates, we stratified time since start of supplementation as less than 90 days and more than 90 days. We assessed trends across three age groups (0–5 months, 6–11 months, and 12 months or older) and the two time strata with the Mantel-Cox method (Strate Mantel-Cox procedure in Stata 8.2, which estimates a one step Newton approximation to the log-linear Poisson regression coefficient).

On the recommendation of the data and safety monitoring board, we stopped treatment in the iron, folic acid, and zinc group and in the iron and folic acid group on Aug 19, 2003, converting the trial into one with two arms—zinc versus placebo. The data and safety monitoring board received data from the main trial every month and established at the beginning of the trial the rule that it would do further analysis of the data only when the difference in mortality between any two groups reached a *p* value of 0.2 or less. This point was reached in July, 2003, and the additional analyses revealed significantly ( $p < 0.05$ ) higher rates of total adverse effects in the groups taking iron and folic acid than in the placebo group, and a consistency in direction and magnitude of effect for deaths and admissions to hospital. Data limited to the two groups taking iron and folic acid and for the placebo group, from enrolment until Aug 19, 2003, were stripped of identification and supplementation code, and given new identifiers by the statistician of the data and safety monitoring board. The analyses presented in this report pertain to this data set. Because the double-masked zinc trial is continuing, the statistician of the board did not provide information on that group. The effects of zinc will be presented at a later date after completion of that part of the trial.

This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN59549825.

#### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to

all the data in the study and had final responsibility for the decision to submit for publication. WHO coordinated the preparation and delivery of supplements and organisation of meetings of the data and safety monitoring board.

## Results

Figure 1 shows the trial profile. Of 24 253 households contacted between Jan 1, 2002, and Aug 19, 2003, 95% agreed to participate, providing a total enrolment of 32 155 from 36 781 contacted children (87%); 24 076 children were assigned to one of the three groups included in this analysis. We followed children until age 48 months or until the iron and folic acid-containing groups were stopped (maximum duration of follow-up 18 months). At the time of stopping the trial, mean duration of follow-up in the study was 383 days (SD 171). The total follow-up was 25 524 child-years (figure 1); follow-up for age 1–5 months, 6–11 months, and 12 months or older was 527, 2545, and 22 452 child-years, respectively. Many parents refused to enrol their children until they were 3–4 months old, which is why there are fewer numbers in the 1–5 month age group than in the other groups. The baseline characteristics of those enrolled to the three

groups in the main trial were similar (table 1), including use of bednets (overall 38% [n=9210]), transfusions done in the previous 6 months (1% [n=253]), and reported admissions to hospital in the past 6 months (6% [n=1554]). Most children were given supplements between meals. Based on available weights, the mean (SD) iron dose in mg/kg per day for infants 0–5 months old was 0·92 (0·23), for infants 6–11 months old was 0·80 (0·14), and for children aged 12 months or older was 1·15 (0·23). Adherence to the supplements during follow-up (proportion of days supplement was taken) did not differ greatly between groups (iron and folic acid 84·9% [n=2 608 443]; iron, folic acid, and zinc 84·3% [n=2 632 098]; and placebo 86·5% [n=2 711 723]).

The overall incidences of adverse events, deaths, and admissions to hospital in the control group in the main trial were 0·11, 0·015, and 0·10 per child-year at risk. Of the 425 deaths, 105 (25%) occurred in hospital. Overall there was a higher risk of a serious adverse event (death or severe morbidity leading to admission) in the groups that received iron and folic acid with or without zinc than in the placebo group (difference 12%, 95% CI 2–23; table 2); there was a similar trend for mortality and admission (table 2).

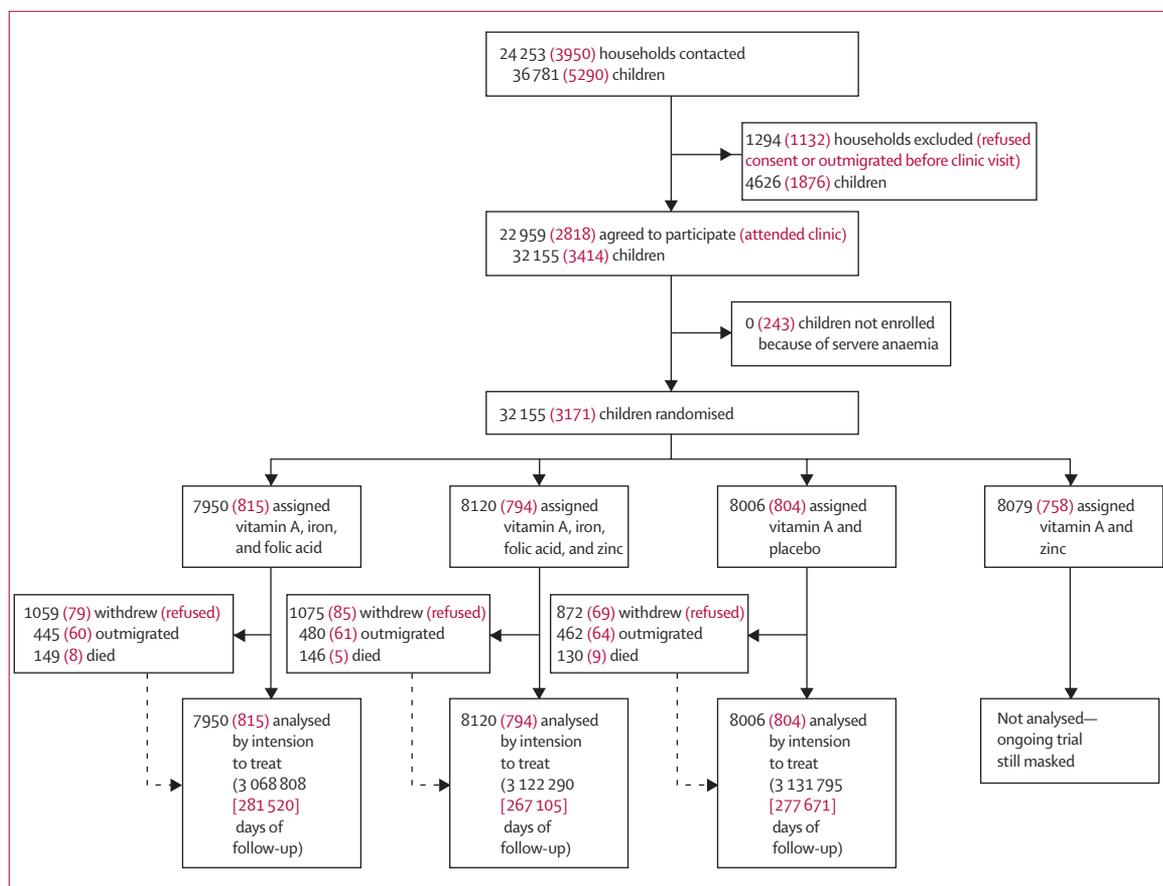


Figure 1: Trial profile

Text in red refers to substudy.

	Main study			Substudy		
	Iron and folic acid	Iron, folic acid, and zinc	Placebo	Iron and folic acid	Iron, folic acid, and zinc	Placebo
Age (years), mean (SD)	18.1 (11.1)	18.1 (11.1)	18.4 (11.1)	23.0 (11.1)	23.3 (11.2)	22.0 (11.3)
Boys	4037 (51%)	4085 (50%)	4073 (51%)	412 (51%)	429 (54%)	380 (47%)
Employment						
Father (fishing or farming)	4245 (53%)	4366 (54%)	4281 (54%)	391 (48%)	386 (49%)	414 (52%)
Mother (housewife)	5913 (74%)	5954 (73%)	5969 (75%)	641 (79%)	644 (81%)	643 (80%)
Education						
Father (illiterate)*	3278 (41%)	3288 (41%)	3287 (41%)	290 (36%)	276 (35%)	284 (35%)
Mother (illiterate)*	4041 (51%)	4136 (51%)	4086 (51%)	350 (43%)	374 (47%)	388 (48%)
History of sleeping under bednet†						
Always	2144 (27%)	2161 (27%)	2150 (27%)	298 (37%)	298 (38%)	310 (39%)
Sometimes	927 (12%)	933 (12%)	895 (11%)	101 (12%)	65 (8%)	76 (10%)
Haemoglobin (g/L), mean (SD)	..	..	..	9.6 (1.3)	9.7 (1.3)	9.8 (1.3)
Zinc protoporphyrin (μmol/mol of haeme), mean (SD)	..	..	..	146.9 (93.3)	144.2 (97.6)	143.5 (94.5)
Anthropometric status‡						
Normal	..	..	..	501 (66%)	478 (65%)	485 (66%)
Wasted	..	..	..	36 (5%)	45 (6%)	43 (6%)
Stunted	..	..	..	208 (27%)	192 (26%)	185 (25%)
Wasted and stunted	..	..	..	15 (2%)	16 (2%)	15 (2%)

Data are number (%) unless otherwise indicated. \*Unable to read or write. †Index child. ‡Weights and heights available for 2219 children (iron groups 1491, placebo 728); defined as 2 SD for National Center for Health Statistics reference population.

Table 1: Baseline characteristics

The rates of adverse events (RR 0.96, 95% CI 0.87–1.06), deaths (1.04, 0.83–1.31), and admissions (0.94, 0.85–1.05) did not differ greatly between the two groups that were taking iron and folic acid (table 3). By comparison with the placebo group, the case fatality ratio for individuals admitted to hospital tended to be higher in the group taking iron and folic acid (1.31, 0.79–2.18) than in group taking iron and folic acid with zinc (0.93, 0.55–1.57), but these were not significantly different from each other: 1.24 (0.76–2.05). For admission to private clinics (n=55), the RR comparing placebo with both groups taking iron and folic acid combined was 1.42 (0.81–2.49; p=0.22).

Age did not significantly affect the risk of an adverse event (p=0.41), death (p=0.56), or hospital admission (p=0.28; table 2). The interaction terms of age and supplementation effect for adverse events (RR 1.0, 95% CI 0.99–1.00), mortality (0.99, 0.97–1.01), and hospital admission (1.0, 0.99–1.01) were also not significant. In analysis of cumulative dosing/duration of

supplementation, Nelson-Aalen curves indicate a similar cumulative hazard between groups for the first 90 days of supplementation (figure 2), with subsequent separation of curves. Interaction of duration and effect of supplementation was not significant for adverse events, deaths, or hospital admission (all three RR 1.0, 95% CI 0.99–1.0). Modification of supplementation effect by duration of supplementation (90 days or less vs more than 90 days; Mantel-Cox method) was not significant for adverse events (p=0.48), deaths (p=0.45), or admission to hospital (p=0.63). We noted similar results when we analysed data by actual number of tablets consumed (data not shown).

The effects of supplementation on cause-specific serious adverse events did not differ significantly between the treatment groups that did and did not receive zinc (table 3). The combined treatment groups had a higher risk (RR 1.16, 95% CI 1.02–1.32) for serious adverse events due to clinical malaria than the placebo group; we noted a similar trend for deaths (1.08, 0.84–1.40) and hospital admissions (1.18, 1.02–1.36). For cerebral

	Adverse events			Mortality			Hospital admission		
	Number	RR (95% CI)	p	Number	RR (95% CI)	p	Number	RR (95% CI)	p
Overall	2135	1.12 (1.02–1.23)	0.02	295	1.15 (0.93–1.41)	0.19	1840	1.11 (1.01–1.23)	0.03
<b>Age group</b>									
1–5 months	85	1.10 (0.75–1.60)	0.63	17	1.71 (0.63–4.64)	0.29	68	1.01 (0.67–1.52)	0.98
6–11 months	590	1.04 (0.90–1.20)	0.58	88	1.03 (0.71–1.48)	0.89	502	1.04 (0.89–1.22)	0.59
12–23 months	975	1.19 (1.06–1.33)	0.004	111	1.29 (0.90–1.84)	0.16	864	1.17 (1.04–1.33)	0.01
≥24 months	485	0.99 (0.85–1.16)	0.94	79	0.98 (0.67–1.42)	0.90	406	1.00 (0.84–1.18)	0.98
<b>Duration of supplementation</b>									
≤3 months	751	1.07 (0.94–1.21)	0.30	101	1.03 (0.73–1.45)	0.87	650	1.08 (0.94–1.23)	0.29
>3 months	1384	1.13 (1.03–1.25)	0.01	194	1.21 (0.94–1.57)	0.15	1190	1.12 (1.01–1.24)	0.03

Table 2: Effect of supplementation with iron and folic acid with or without zinc on serious adverse events, deaths, and admissions to hospital overall, by age group and by duration of supplement compared with placebo.

	Iron and folic acid (child-years' follow up 8402)			Iron, folic acid, and zinc (child-years' follow up 8548)			Placebo (child-years' follow up 8574)
	Number	RR (95%CI)	p	Number	RR (95% CI)	p	Number
<b>Overall</b>							
Adverse events	1036	1.10 (0.99–1.22)	0.09	1099	1.14 (1.03–1.27)	0.01	965
Deaths	149	1.16 (0.92–1.47)	0.21	146	1.12 (0.88–1.42)	0.35	130
Hospital admissions	887	1.08 (0.97–1.21)	0.16	953	1.14 (1.03–1.28)	0.02	835
<b>Cause-specific adverse events</b>							
Malaria-related causes	467	1.16 (1.00–1.34)	0.05	476	1.16 (1.01–1.34)	0.04	411
Infection-related causes	232	1.25 (1.01–1.53)	0.04	265	1.40 (1.14–1.71)	0.001	190
Smear positive	71	1.37 (0.96–1.95)	0.09	97	1.84 (1.31–2.57)	0.00	53
Smear negative	161	1.20 (0.94–1.53)	0.14	168	1.23 (0.97–1.56)	0.09	137
Diarrhoea	87	0.92 (0.68–1.25)	0.62	92	0.96 (0.72–1.29)	0.79	96
Smear positive	21	0.93 (0.51–1.70)	0.82	25	1.09 (0.61–1.94)	0.77	23
Smear negative	66	0.92 (0.65–1.31)	0.65	67	0.92 (0.66–1.29)	0.63	73
Other causes	108	0.99 (0.76–1.30)	0.96	127	1.15 (0.88–1.50)	0.32	111
Smear positive	18	0.97 (0.50–1.87)	0.34	30	1.58 (0.88–2.86)	0.13	19
Smear negative	90	1.00 (0.74–1.35)	0.99	97	1.06 (0.79–1.42)	0.71	92

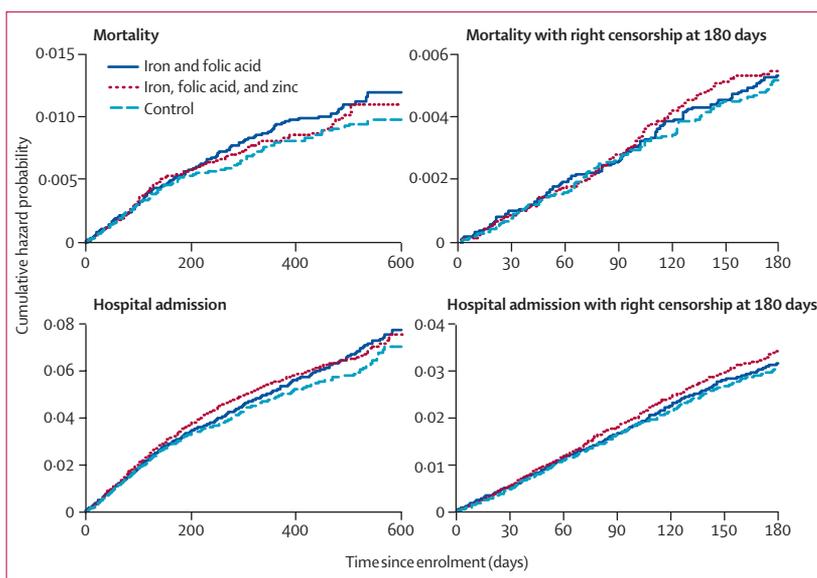
**Table 3:** Effects on overall adverse events, deaths, and admissions to hospital and on cause-specific adverse events in the two iron and folic acid groups compared with placebo

malaria, the RR in the iron and folic acid group was 1.32 (1.02–1.70;  $p=0.04$ ) and in the iron and folic acid with zinc group it was 1.14 (0.88–1.48;  $p=0.31$ ); when these two groups were combined, they had an RR of 1.22 (1.02–1.46,  $p=0.03$ ). This increased risk was not affected by changing the malaria definition to parasite count of more than 0 (1.20, 1.02–1.42;  $p=0.03$ ) or by exclusion of seizures from the definition (1.23, 0.98–1.54;  $p=0.07$ ). For cerebral malaria as a cause of death, there was increased risk in the iron and folic acid group (1.70, 1.08–2.68;  $p=0.02$ ), whereas in the group that received iron and folic acid with zinc the RR was 0.87 (0.51–1.47;  $p=0.60$ ).

Infection-related causes included confirmed febrile illness not meeting definitions for malaria—eg, pneumonia, sepsis, meningitis, measles, pertussis. Compared with placebo, the combined treatment groups had a significantly higher risk for serious adverse events (1.32, 1.10–1.59), deaths (1.61, 1.03–2.52), and admissions to hospital (1.28, 1.05–1.55) due to these causes. The results were similar in the groups that received iron and folic acid with and without zinc (table 3). To assess whether use of a conservative malaria definition was affecting this association, we divided the data for hospital admissions not attributed to malaria into malaria smear positive (parasite count  $>0$  in thick smear) and smear negative; the trend for higher risk of adverse events was noted in both smear positive and smear negative cases attributed to infection-related causes (table 3). In the combined treatment groups compared with the placebo recipients, there was a trend, not significant, for a smaller proportion of children with anaemia among all admissions: haemoglobin less than 5.0 (0.76, 0.52–1.11;  $p=0.13$ ); haemoglobin less than 5.0 (0.84, 0.68–1.03;  $p=0.09$ ); and haemoglobin less than 10.0 (0.93, 0.79–1.11;  $p=0.42$ ).

With respect to the substudy, 2413 children were included in the analysis (figure 1). Baseline characteristics

did not differ between groups (table 1). At baseline, 26% ( $n=585$ ) of enrolled children were stunted, 6% ( $n=124$ ) wasted, and 2% ( $n=46$ ) were both stunted and wasted. Adherence to supplementation was 84% ( $n=23\ 6487$ ), 83% ( $n=22\ 1775$ ), and 86% ( $n=23\ 9445$ ) in the iron and folic acid groups without and with zinc and placebo group, respectively. At baseline, the prevalence of anaemia was 57% ( $n=1473$ ) and of severe anaemia 7% ( $n=243$ ; excluded from the trial); these prevalences did not differ between the groups. Prevalence of iron deficiency (zinc protoporphyrin  $>80.0\ \mu\text{mol/mol}$  haeme) was 75% ( $n=1813$ ) and did not differ between groups. In the substudy after 1 year of supplementation, blood samples were available for 308, 319, and 327 children in the iron and folic acid groups without and with zinc and placebo group, respectively. Prevalence of severe anaemia was 1%



**Figure 2:** Nelson-Aalen cumulative hazard estimates for mortality and hospital admission (main study)

	Iron and folic acid (with and without zinc)			p	Placebo	
	Children	Events (rate/ 100 child-years)	RR (95%CI)		Children	Events (rate/ 100 child-years)
Overall adverse events	1609	97 (6.46)	0.76 (0.52–1.09)	0.13	804	65 (8.55)
<b>Zinc protoporphyrin (<math>\mu\text{mol/mol}</math> of haeme)</b>						
<80	391	29 (7.86)	1.63 (0.72–3.66)	0.24	196	9 (4.83)
$\geq$ 80	1218	68 (6.00)	0.62 (0.41–0.93)	0.02	608	56 (9.76)
<b>Haemoglobin (g/L)</b>						
70–100	1018	52 (5.54)	0.59 (0.37–0.92)	0.02	455	40 (9.43)
>100	591	45 (8.00)	1.08 (0.58–1.98)	0.8	349	25 (7.44)
Iron replete and anaemic	127	10 (8.33)	2.00 (0.46–8.75)	0.36	52	2 (4.17)
Iron replete and non-anaemic	264	19 (7.63)	1.51 (0.57–3.98)	0.41	144	7 (5.06)
Iron deficient and anaemic	891	42 (5.13)	0.51 (0.31–0.83)	0.006	403	38 (10.11)
Iron deficient and non-anaemic	327	26 (8.29)	0.91 (0.42–1.98)	0.82	205	18 (9.09)

**Table 4: Effects of supplementation with iron and folic acid with or without zinc on adverse events overall and by iron status and anaemia (substudy)**

in the iron and folic acid groups with (n=4) and without (n=4) zinc, and 2% (n=7) in the placebo group. For combined treatment groups compared with placebo, the effect on severe anaemia had an RR of 0.58 (0.16–1.50; p=0.23).

The effect on outcomes in the iron and folic acid groups with and without zinc was similar for adverse events (0.76, 0.50–1.15, and 0.75, 0.48–1.17) and mortality (0.88, 0.34–2.28, and 0.58, 0.19–1.72). Compared with placebo, in the combined treatment groups there were non-significant trends toward fewer adverse events (table 4): deaths, RR 0.73 (0.31–1.71) and hospital admissions RR 0.76 (0.54–1.06). Baseline iron status seemed to modify this effect. In children who were iron-deficient by zinc protoporphyrin assessment or who had moderate anaemia at baseline, there was a significantly lower rate of adverse events than in those who were not (table 4).

When we further analysed the data by presence of anaemia and iron status at baseline, children with iron deficiency and anaemia had a significant protection from adverse events with iron and folic acid supplementation (table 4). In iron-deficient children who were not anaemic there was no effect. Iron-replete children with or without anaemia had similar trends for an increased risk of adverse events. With respect to malaria-related adverse effects, there was a reduced risk in the children with iron deficiency in the combined treatment groups compared with placebo (0.56, 0.32–0.97). In children aged younger than 12 months and at least 12 months old, those who were iron-deficient were at less risk and those who were iron-replete were at increased risk of adverse events if they were given iron and folic acid supplements (not significant; data not shown). There was no effect modification noted with anthropometric status (data not shown).

To assist with interpretation of differences in results between the main trial and the substudy, we calculated adverse event rates for both. In the placebo group, the rate of adverse events (hospital admissions and deaths) per 1000 child-years in the main trial was 112.5 and in the substudy group was 85.4. Although we do not have a record of all the

treatments provided at the clinics, 487 courses of sulfadoxine/pyrimethamine were provided by the study group for confirmed malaria to substudy children during the study.

## Discussion

Our trial was originally designed to assess the effects of supplementation with iron and folic acid, with or without zinc, on hospital admission for severe illness and mortality. Midway through the trial, the iron and folic acid-containing groups had to be stopped because they were associated with higher rates of adverse events (hospital admissions and deaths) than the zinc and placebo groups, which have been continued. The 12% higher rate of serious adverse events noted after supplementation with iron and folic acid calls into question the current global recommendations on the use of these supplements to prevent iron deficiency. We identified these adverse effects in a setting where suboptimum care seeking and treatment in the routine public-health system results in high mortality from infectious diseases. This scenario is repeated over most of sub-Saharan Africa and some parts of Asia that are covered by current recommendations for routine iron supplementation in children.

These recommendations are that for populations with a prevalence of anaemia of 40% or more in children aged 6–24 months, which would include Pemba, children of normal birthweight should receive iron and folic acid (12.5 mg iron and 50  $\mu\text{g}$  folic acid) daily between the ages of 6 months and 24 months, and that children with low birthweight should receive these amounts from ages 2 months to 24 months.<sup>21</sup> Our findings do not lend support to these recommendations. We enrolled children aged 1–36 months and gave them supplements until they were 48 months old. We halved the doses of iron and folic acid given to infants younger than 12 months old. Thus, the trial followed the recommendations exactly for children in the second year of life, provided a reduced dose of iron and folic acid in infants, and provided a reduced dose of iron in children older than age 24 months

(recommended dose being 20–30 mg daily or 2 mg/kg bodyweight).<sup>21</sup> Although children were meant to receive supplements continuously between enrolment and age 48 months, possibly receiving supplements for several years, the premature termination of the groups of the study that were taking iron and folic acid resulted in all children being supplemented for only about 1 year.

We began supplementation from 1 month of age, because of the possibility that infants younger than 6 months could build iron stores that would help reduce mortality in the second half of infancy<sup>14</sup> and because of the possible benefits of zinc supplementation in this age group. Age was not associated with adverse effects. Children older than 12 months who were taking the recommended doses or less of iron and folic acid had significantly more adverse events than those on placebo, most directly challenging the current recommendations for supplementation.

There has been some debate about the potential for folic acid supplementation to interact with sulfadoxine-pyrimethamine treatment of malaria. Such an interaction would not affect malarial incidence, but could potentially affect the outcome of infection. However, published work indicates that combination sulfadoxine/pyrimethamine prevents use of host folate by blocking uptake in the parasite,<sup>22</sup> and we did not find any association of recovery from sulfadoxine/pyrimethamine-treated malaria episodes or subsequent recrudescence with iron and folic acid versus placebo (unpublished data).

Previous data from clinical trials of the effect of iron supplementation on infectious-disease morbidity, including malaria, have been conflicting. There have been two attempts to summarise trial data. A review by Oppenheimer<sup>2</sup> included controlled trials in all age groups of parenteral and oral iron supplements or fortified foods in which groups differed only in provision of iron. He concluded that iron supplementation increased episodes of clinical malaria in six of seven trials, respiratory infections in one of three trials (in that study 89% of pneumonia cases also had malaria), and diarrhoea in one of five trials. A meta-analysis by Gera and Sachdev<sup>11</sup> used the same inclusion criteria and calculated a small, non-significant increase in clinical malaria episodes (five studies). They also calculated that there was an 11% (95% CI 1–23) increase in diarrhoea with iron supplementation. These reviews did not assess separately studies from malaria-endemic areas or studies in different age groups. Furthermore, the authors did not differentiate between studies in which iron was given as therapy for anaemia and for prevention of iron deficiency.

To assess the published work in the context of our trial results and the recommendations for routine preventive iron supplementation in malaria endemic areas, trials in preschool children in malaria endemic areas, using oral preventive iron supplementation (not treatment of selected anaemic children) need to be considered. In one small study<sup>14</sup> (411 children in iron or placebo groups), iron

(2 mg/kg) or placebo was given to an unselected population in a malaria endemic area, but only to children aged 8–24 weeks. Furthermore, morbidity was assessed only at 8–48 weeks. The prevalence of severe anaemia was lower in the iron-supplemented group and incidence of malaria did not differ between that and the placebo group; however, there were few serious episodes of malaria and admissions to hospital. Furthermore, because iron was only given from 8 weeks to 24 weeks of age, when the rates of severe malaria are relatively low because of maternally-derived immunity, the findings of this study cannot be used to assess the safety of the recommendations to give iron and folic acid to children aged 6–24 months who are at much higher risk of malaria. In another small trial<sup>15</sup> (163 children), iron (2–3 mg/kg per day) or placebo was given to children aged 6–36 months for 9 months. The investigators reported a benefit on anaemia and no adverse effects on diarrhoea or respiratory infections or on malaria parasitaemia. A third trial<sup>16</sup> assessed rates of malaria parasitaemia, but not infectious episodes. By comparison, our trial included 25 524 child-years and we present detailed data on 2675 admissions for serious illnesses, primarily malaria and other infections, and on 425 deaths. Only a study of sufficient size and design to identify serious adverse events has the power to assess the safety of universal supplementation with iron and folic acid, and our large trial provides significant and clinically important evidence of harm. The analyses of cause-specific admissions to hospital and deaths confirm that malaria and other infectious diseases are significantly increased in children given iron and folic acid.

The results of previous studies of iron supplementation in areas endemic for malaria show a reduction of severe anaemia of about half.<sup>11</sup> In our substudy, the prevalence of severe anaemia after 1 year of supplementation in all groups was low, presumably because children with severe anaemia at baseline and at 6 months were treated and excluded, and malarial and other infections were treated throughout the study. Yet the 42% drop (not significant) in prevalence of severe anaemia after 12 months in the children given iron and folic acid compared with placebo was of the magnitude noted in other studies.

In the substudy, the overall effect of supplementation with iron and folic acid was a non-significant reduction in adverse events. We believe that different results between the main study and the substudy are the result of more intensive diagnosis and management of children with malaria and other infections in the substudy. The substantially lower incidence of adverse events in the placebo group in the substudy than in the main trial also suggests that additional treatments at community level reduced hospital admissions and deaths. The lower infection rates (because of malaria treatment and prevention during a 3–4 week half-life of sulfadoxine/pyrimethamine) might have reduced the potential negative effects of iron. These overall findings are consistent with the results from small trials in which iron

supplementation had a beneficial effect when combined with either treatment of clinical malaria or intermittent preventive treatment for malaria.<sup>12,13,23,24</sup> Nevertheless, the substudy results suggest that only children with anaemia associated with iron deficiency benefit from supplementation with iron and folic acid with respect to hospital admissions and deaths. Some of this benefit could be in prevention of severe anaemia, a common cause of admission to hospital in this area. We certainly noted a sizable, albeit not significant, effect on this outcome in the substudy. Those with iron deficiency without anaemia are not adversely affected by supplementation with iron and folic acid, whereas children without iron deficiency might still have adverse effects, even in the presence of enhanced detection and management of malaria and other infections. These findings are consistent with data from studies in animals and people on increased susceptibility to infection with iron supplementation<sup>25-27</sup> and adverse effects on growth if iron is given to children without iron deficiency.<sup>28,29</sup> Thus, the identification and treatment of anaemia caused by iron deficiency could prove to be a safer approach than universal supplementation with iron and folic acid in areas endemic for malaria.

Iron excess is harmful to the host and favours the pathogen for many postulated reasons. Iron is thought to inhibit absorption of zinc, which can compromise the immune response to infection.<sup>30</sup> Limitation of metabolically active iron in pathogen-invaded cells inhibits pathogen growth; free iron is essential for multiplication of bacteria, including *Escherichia coli*, *Mycobacteria* sp, *Pasteurella* sp, *Shigella* sp, and staphylococcus,<sup>31</sup> and parasites like plasmodia.<sup>32</sup> Iron can also have indirect nitric oxide-mediated effects. Iron blocks the synthesis of nitric oxide by transcriptional inhibition of inducible nitric oxide synthase (iNOS).<sup>33</sup> In the absence of iron and after interferon stimulation, synthesis of nitric oxide, which reacts with enzymes needed for DNA synthesis and electron transfer, is increased, resulting in death of pathogens.<sup>34</sup> Addition of free iron reverses this inhibition of viral replication induced by chelation.<sup>35</sup> Excess iron deposits in the liver are detrimental to interferon therapy responsiveness in patients infected with hepatitis C,<sup>36</sup> and iron overload results in progression of hepatitis C.<sup>37</sup>

Limitations of our study with respect to cause-specific effects were that we could not do lumbar puncture, comascoring, blood cultures, or blood gas analysis in the hospitals, resulting in possible misclassification of meningitis, septicaemia with acidosis, and cerebral malaria. We did, however, impose stringent conditions for confirmed malaria to reduce to a minimum the overestimation of the diagnosis of cerebral malaria. Because malaria is the predominant severe pathology in young children in this setting, the increase in adverse effects must involve malaria. The findings with respect to other infections could be a result of undetected malarial infections or other infections that are secondary to

malaria. That supplementation with iron and folic acid has an adverse effect on pneumonia and other infections independent of malaria is possible.

Further research is needed to ascertain which indicator of anaemia associated with iron deficiency is most efficient for screening of children who will benefit from iron supplements. In a previous study<sup>16</sup> of children from Zanzibar, zinc protoporphyrin was strongly associated with haemoglobin concentration, and less perturbed by malarial infection than serum ferritin or transferrin receptors; however, malarial infection and recent fever were weakly related to zinc protoporphyrin, suggesting that it might be slightly affected by the acute phase response to infection.<sup>16</sup> Our findings emphasise that iron-deficient and anaemic children are at a higher risk of morbidity and mortality and that provision of iron supplementation confers a substantial benefit, a finding that would have implications for settings where iron deficiency can be identified. That this benefit was similar in the groups taking iron and folic acid with and without zinc indicates that there was no significant negative effect of zinc on the absorption or effect of supplementary iron.

Our findings indicate a potential risk of routine supplementation with iron and folic acid in preschool children in a population with high rates of malaria and other infections. These findings are different from effects of such supplementation in settings without malaria.<sup>38</sup> A risk-benefit analysis needs to be undertaken to ascertain whether the current guidelines of universal supplementation with iron and folic acid to all children in such populations are appropriate. Our results should not be extrapolated to treatment of anaemia, iron supplementation along with malaria treatment, or iron supplementation during pregnancy. The possible benefit noted in the substudy suggests that the recommendations for prevention of anaemia in children who live in areas with high malaria transmission should stress the need for a combination of iron supplementation and improved management of malaria. These approaches need to be assessed to ensure that the supplementation is not causing harm to a segment of the population.

#### Contributors

S Sazawal and R E Black coordinated the trial and made a primary contribution to its development, rationale, design, and undertaking, analysis of data, and writing of the report. R J Stoltzfus contributed to rationale and design of the study, analysis and interpretation of the data, and writing of the report. A Dutta contributed to implementation of the trial, design of surveillance systems, quality control, and, along with U Dhingra, was responsible for programming, data management, and analysis. M Ramsan, H M Chwaya, I Kabole, M K Othman, and F M Kabole contributed to the implementation of the trial, design of data collection instruments and surveillance systems, and clinical care of patients. S Deb was responsible for the management and quality control of all laboratory procedures.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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