**Child Health Outcomes after Presumptive Infection Treatment in Pregnant Women: A Randomized Trial**

Lotta Hallamaaa, MSc, Yin Bun Cheungb, PhD, Kenneth Maletac, MBBS, PhD, Mari Luntamoa, MD, MPH, Ulla Ashorna, PhD, Melissa Gladstoned, MBChB, MD, MRCPCH, Teija Kulmalaa, MD, PhD, Charles Manganic, MBBS, PhD, Per Ashorna,e, MD, PhD

aCenter for Child Health Research, Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland, bCenter for Quantitative Medicine, Duke-NUS Medical School, Singapore, cDepartment of Public Health, School of Public Health and Family Medicine, College of Medicine Malawi, Blantyre, Malawi, dNeurodevelopmental Paediatrics Department of Women and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom, eDepartment of Paediatrics, Tampere University Hospital, Tampere, Finland

**Address correspondence to:** Lotta Hallamaa, Center for Child Health Research, Faculty of Medicine and Life Sciences, University of Tampere, Arvo Ylpon katu 34, 33520 Tampere, Finland, [lotta.hallamaa@uta.fi](mailto:lotta.hallamaa@uta.fi), +358 40 778 0660

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**Clinical Trial Registration:** This trial has been registered at www.clinicaltrials.gov, trial identification NCT00131235 (<https://www.clinicaltrials.gov/ct2/show/NCT00131235?term=NCT00131235&rank=1> ).

**Abbreviations:**

AZI-SP - intervention group receiving monthly sulfadoxine-pyrimethamine and two doses of azithromycin

CI - confidence interval

GMDS-ER 2–8 - Griffith’s Mental Development Scales - Extended Revised: 2–8 years

HAZ - age- and sex-standardized height-for-age Z-scores

HIV - human immunodeficiency virus

HR - hazard ratio

IGF - insulin-like growth factor

IPTp - intermittent preventive treatment in pregnancy

LAIS - Lungwena Antenatal Intervention Study

RR - risk ratio

SD - standard deviation

SP - sulfadoxine-pyrimethamine

**Table of Contents Summary:**

Reduced prevalence of neonatal stunting of children whose mothers received presumptive infection treatment during pregnancy is sustained and reflected in childhood growth and development.

**What’s Known on This Subject:**

Stunting is a big global issue and associated with increased mortality and developmental delay. We showed earlier, that presumptive antenatal infection treatment during pregnancy against malaria and reproductive tract infections reduced prevalence of neonatal stunting in Malawi.

**What This Study Adds:**

Gains obtained among children whose mothers received monthly sulfadoxine-pyrimethamine with two doses of azithromycin rather than two doses of sulfadoxine-pyrimethamine during pregnancy were sustained for five years and reflected in the prevalence of childhood stunting, development and possibly neonatal mortality.

**Contributors' Statement Page**

Ms. Hallamaa planned the analyses, analyzed the data, drafted the initial manuscript and reviewed and revised the manuscript.

Prof. Cheung oversaw the data analysis and interpretation and critically reviewed the manuscript.

Dr. Maleta conceptualized the study, developed the data collection materials and critically reviewed the manuscript.

Dr. Luntamo and Dr. Kulmala conceptualized the study, developed the data collection materials, trained the data collection team, oversaw quality assurance and critically reviewed the manuscript.

Dr. U. Ashorn and Dr. Gladstone developed the data collection materials and critically reviewed the manuscript.

Dr. Mangani developed the data collection materials, trained the data collection team, oversaw quality assurance and critically reviewed the manuscript.

Prof. P. Ashorn conceptualized the study, developed the data collection materials, oversaw the study, data analysis, and interpretation, drafted the initial manuscript and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

**ABSTRACT**

**Background and objectives**

We showed earlier that presumptive infection treatment in pregnancy reduced prevalence of neonatal stunting in a rural low-income setting. We now assessed how these gains were sustained and reflected in childhood growth, development, and mortality.

**Methods**

We enrolled 1320 pregnant Malawian women in a randomized trial and treated them for malaria and other infections with either two doses of sulfadoxine-pyrimethamine (SP, control), monthly SP, or monthly SP and azithromycin twice (AZI-SP). Child height/length and mortality were recorded at one, six, 12, 24, 36, 48 and 60 months and Griffith’s Mental Development Scales at 60 months.

**Results**

Throughout follow-up, mean child length was 0.4–0.7 cm higher (P<0.05 at 1–12 months), prevalence of stunting 6–11 %-points lower (P<0.05 at 12–36 months) and five-year cumulative incidence of stunting 13 %-points lower (hazard ratio 0.70, 95% confidence interval [CI] 0.60–0.83, P<0.001) in AZI-SP than control group. Mean developmental score was 3.8 points higher in AZI-SP than control group (95% CI 1.1–6.4, P=0.005). There were no statistically significant between-group differences in cumulative five-year mortality (P=0.58). Total mortality during pregnancy and childhood was 15.3%, 15.1%, and 13.1% (P=0.60) in the control, monthly SP and AZI-SP group, respectively. Postneonatal mortality (secondary outcome) was 5.5%, 3.3%, and 1.9%, respectively (risk ratio AZI-SP vs control 0.34, 95% CI 0.15–0.76, P=0.008).

**Conclusion**

Provision of AZI-SP rather than two doses of SP during pregnancy reduced the incidence of stunting in childhood. AZI-SP during pregnancy also had a positive effect on child development and may have reduced postneonatal mortality.

This trial was registered with ClinicalTrials.gov, number NCT00131235

**INTRODUCTION**

Poor linear growth in childhood is common, especially in sub-Saharan Africa and in southern Asia. It is associated with increased mortality and developmental delay. Reducing its prevalence is a key global health target1,2. To date, most public health interventions to prevent growth failure have been based on promotion of a healthy diet and effective infection control in early childhood. This approach may, however, be insufficient as linear growth retardation often commences during the fetal period3,4. Maternal dietary supplementation with energy and/or nutrients during pregnancy has in some contexts resulted in modestly increased mean birth size, but gains in infant length have typically been lost within a year after birth5,6.

We have previously reported results from the “Lungwena Antenatal Intervention Study” (LAIS) in rural Malawi, in which pregnant women received intermittent preventive treatment in pregnancy (IPTp) either with two doses of sulfadoxine-pyrimethamine (SP, control group), monthly SP, or monthly SP and two doses of azithromycin (AZI-SP). SP is primarily an antimalarial with activity also on many bacteria7 and azithromycin is a broad-spectrum antibacterial drug that also has anti-inflammatory and anti-malarial activity8.

In the LAIS trial sample, incidence of preterm birth and low birth weight, and the prevalence of stunting at one month after delivery were 35–40% lower and mean length one month after delivery was 6 mm higher among babies born to women receiving AZI-SP than those born to women in the control group9,10. These results suggest that intensified infection and inflammation control during pregnancy can, in the Malawian context, promote fetal growth and increase length at one month after delivery. In this follow-up study, we aimed to assess the sustainability and consequences of these gains. Our primary hypothesis was that difference in mean length would be retained throughout first five years of life and reflected in a permanently lower incidence and prevalence of stunting among babies born to women treated with AZI-SP. We also hypothesized that these children would have a higher mean developmental score at the age of five years and lower mortality by five years than children born to women in the control group.

**METHODS**

**Background**

This study was a five-year follow-up to the LAIS trial, a single-center, randomized, partially placebo controlled, outcome assessor-blinded, three-arm clinical trial conducted in rural Malawi9. The main outcome of the original trial was the incidence of preterm delivery and the pre-defined secondary outcomes included birth weight and infant size at one month9,10.

**Participants and follow-up**

The LAIS trial enrolled women with uncomplicated second trimester pregnancies (gestational age 14–26 weeks by ultrasound assessment) who had felt movements of the fetus, had commenced antenatal care at Lungwena Health Centre, Southern Malawi and provided informed consent. Exclusion criteria included multiple pregnancy, severe illness, receipt of azithromycin during the current pregnancy or SP within preceding 28 days, allergy to study drugs, and any previous serious allergic reaction9.

Details of randomization are available in the original trial publication9 and in Supplemental Methods. In brief, we randomly allocated 1320 women to either a control group or to one of two intervention groups: monthly SP or AZI-SP. Women in the control group received standard Malawian antenatal care, which at the time of the study included IPTp with SP (three tablets orally, each containing 500 mg of sulfadoxine and 25 mg of pyrimethamine) twice: at enrollment and between 28–34 weeks of gestation. At these visits, they also received a placebo in lieu of azithromycin. Women in the monthly SP group received SP monthly from enrollment until 37 gestational weeks and a placebo in lieu of azithromycin. Women in the AZI-SP group received monthly SP and active azithromycin (two tablets orally, each containing 500 mg of azithromycin) twice: at enrollment and between 28–34 weeks of gestation. Active azithromycin and its placebo were manufactured and donated by Pfizer Inc. (New York, NY). SP tablets were obtained from Malawi Central Medical Stores that only made purchases from companies with Good Manufacturing Practice certification and quality assurance. We did not perform any pharmacological tests on the study drugs.

All children received standard Malawian care during follow-up; human immunodeficiency virus (HIV) positive mothers and their newborns received nevirapine for prevention of mother-to-child transmission of HIV.

Child length/height was assessed at the study clinic visits at one, six, 12, 24, 36, 48 and 60 months. Details of measuring equipment and inclusion criteria for length measurements are presented in Supplemental Methods. If the child did not come for a scheduled study clinic visit, the study team traced and interviewed the caretaker, and completed a structured verbal autopsy questionnaire if the child had died.

Child development was assessed at 60 months of age using the Griffith’s Mental Development Scales - Extended Revised: 2–8 years (GMDS-ER 2–8), which covers six domains: locomotor, personal-social, language, eye and hand co-ordination, performance and practical reasoning11.

Both the original trial and the follow-up were performed according to Good Clinical Practice and ethical standards of Declaration of Helsinki. The protocol was approved by the College of Medicine Research and Ethics Committee, Malawi and the Ethical Committee of Pirkanmaa Hospital District, Finland.

**Outcomes**

In the present analysis, primary outcomes were child length/height and stunting at six, 12, 24, 36, 48 and 60 months of age, total developmental score at five years, and the total number of abortions, stillbirths and child deaths combined. Secondary mortality outcomes included number of abortions and stillbirths, early neonatal deaths, late neonatal deaths, postneonatal deaths and child deaths until five years of age. Secondary developmental outcomes included the six individual subscale scores from the GMDS-ER 2–811.

We calculated age- and sex-standardized height-for-age Z-scores (HAZ), using the World Health Organization Child Growth Standards12,13. Values below -2 and -3 Z-scores were considered stunting and severe stunting, respectively.

We calculated a raw score for each subscale in GMDS-ER 2–8 as the sum of the passed items. We calculated a total developmental score as the sum of the subscale scores.

We defined spontaneous abortion as non-induced loss of pregnancy before 22.0 completed gestation weeks and stillbirth as fetal death at or after 22.0 gestation weeks, early neonatal death as death of a live-born baby within seven days of birth, late neonatal death as death within 8–28 days of birth, postneonatal death as death between 29 to 365 days, and child death as death between 366 days to five years of age. Mortality rates were calculated with standard definitions14,15.

Because of delays in starting the developmental assessments, some children were older than five years when assessed (range 59–74 months, except one at 48 months). We did not exclude any participants from the analyses but included child age at time of assessment and child sex as covariates in the statistical analyses. International guidelines16,17 highlight the importance of presenting the main results as per predefined plan. Hence all main results, besides developmental outcomes, are shown without covariate adjustment, as per predefined statistical analysis plan.

**Statistical analysis**

The sample size of 440 pregnant women per group was planned to give 80% power at a 5% level of significance to detect a 40% reduction in the rate of preterm delivery which was the trial’s main hypothesis9.

Group codes for the study were broken for the analysis of trial’s main hypothesis. Statistician for this follow-up was different than the one doing the analyses for the main hypothesis. For this follow-up study the statistician (LH) obtained and merged the intervention code with follow-up data only after data was cleaned, analysis plan written and the syntax for the analysis done with a mock code. The analysis was based on the principle of intention-to-treat. We conducted statistical analyses with Stata 13.1 (StataCorp, College Station, USA).

For prevalence of stunting and mortality outcomes, we calculated percentages and used a log-binomial regression model to estimate risk ratios (RR), or Poisson regression in case the log-binomial regression did not converge18. For absolute length/height, HAZ and developmental scores we calculated group means and used least squares regression to estimate differences between groups. We used Cox regression to estimate hazard ratios (HR) for mortality and competing-risks regression19 to estimate cumulative incidence of stunting under the competing risk of death. The main analysis for development was done with multiple imputed subscale data (details in Supplemental Methods).

We took intragroup correlation due to twin pregnancies into account by using robust standard errors for clustered data20. To prevent inflated type I errors due to testing between multiple groups, we began hypothesis testing with a global null hypothesis of no difference between any groups21. Pairwise null hypotheses were rejected only if the global null hypothesis was also rejected. We rejected a null hypothesis if two-sided P<0.05. We thus controlled familywise error rate at 5%. For developmental assessment we considered the differences in total score between groups the primary hypothesis and did not apply multiplicity adjustment for multiple subscales.

A number of sensitivity analyses were performed: an analysis with multiple imputation for missing data for growth outcomes22; an analysis of raw data without imputations for developmental outcomes; an analysis with adjustment for covariates selected based on predefined criteria as per statistical analysis plan (covariates listed in Supplemental Methods); and a post-hoc analysis stratified by maternal HIV status. Finally, we built Heckman’s selection models to estimate whether differences between groups in child growth at 60 months might have been affected by higher mortality of stunted children in some groups23,24 (Supplemental Methods).

**RESULTS**

Of the 3358 pregnant women invited to participate in the study, 1320 (39.3%) were enrolled between December 1, 2003 and October 11, 2006 and randomized to control (436), monthly SP (441) and AZI-SP group (443) (Figure 1). Enrolled and non-enrolled women were approximately of the same mean age (25 and 26 years, respectively) and had the same mean number of previous pregnancies (2.3 and 2.5, respectively). At enrollment the intervention groups were similar except for small differences in the prevalence of malaria parasitemia and number of previous pregnancies (Table 1). We inadvertently enrolled three, two and two twin pregnancies to the control, monthly SP and AZI-SP groups, respectively, resulting in 1327 fetuses for follow-up (Figure 1).

The mean (standard deviation, [SD]) number of scheduled SP treatments received was 2.0 (0.2) in the control group, 4.0 (1.0) in the monthly SP group, and 4.0 (0.9) in the AZI-SP group. Women in the AZI-SP group received a mean (SD) of 2.0 (0.2) azithromycin doses.

The last follow-up visit was completed June 6, 2012 with data from 1323 children (99.7%) at five years for mortality outcomes and from 949 (71.5%) for development. Growth data at two and five years of age was obtained from 1039 (78.3%) and 952 (71.7%) participants, respectively (Figure 1). Success of follow-up was similar between the groups (P=0.72 and P=0.69 for growth at two and five years; P=0.60 for development). Maternal background characteristics were mostly similar for children lost to follow-up by five years compared to those who remained in the study, except for higher number of HIV (23.4% vs 10.3%, P<0.001) and malaria (12.2% vs 7.9%, P=0.017) positive mothers.

The mean (SD) length/height of children in the study cohort was 63.8 cm (2.8) at 6 months, 80.6 cm (3.3) at 24 months, and 101.9 cm (4.4) at 60 months of age, corresponding to a mean (SD) HAZ of -1.38 (1.22), -2.11 (1.05), and -1.68 (0.94), respectively (Supplemental Figure S1). The mean difference in absolute length/height between AZI-SP and the control group varied between 0.4–0.7 cm between one and 60 months of age (Figure 2, Supplemental Table S1). After covariate adjustment the mean difference varied between 0.3–0.6 cm (Supplemental Table S1). The difference in mean HAZ between AZI-SP and the control group was 0.25 Z-scores at six and 12 months, 0.17 at 24, 0.13 at 36, and 0.15 Z-scores at 48 and 60 months with statistically significant differences at six and 12 months (each P<0.05) (Supplemental Table S2). After covariate adjustment the mean difference in HAZ varied between 0.11–0.21 with statistically significant differences at 12 months (P=0.008) (Supplemental Table S1).

The cumulative incidence of stunting with competing risk of death by 60 months was 76.9% (control), 74.0% (monthly SP) and 64.2% (AZI-SP), with statistically significant differences between the AZI-SP and control group (HR 0.70, 95% CI 0.60–0.83, P<0.001) (Figure 3, panel A). Similarly, incidence of severe stunting was significantly lower in AZI-SP compared to the control group (HR 0.64, 95% CI 0.50–0.81, P<0.001) (Figure 3, panel A). Covariate adjustment did not markedly change the results (HR for stunting between AZI-SP and control 0.74, 95% CI 0.62–0.88, P=0.001, global P=0.003; severe stunting between AZI-SP and control 0.68; 95% CI 0.52–0.88, P=0.004, global P=0.01).

The prevalence of stunting varied between 27.4% (at six months) and 55.3% (at 24 months), and prevalence of severe stunting between 7.5% (at 60 months) and 18.3% (at 24 months). Compared to the control group, prevalence of stunting in the AZI-SP group was 5.5–10.9 %-points and severe stunting 0.5–4.5 %-points lower throughout the follow-up period (Figure 3, panel B; Supplemental Table S3), corresponding to a RR between 0.75–0.84 for stunting and 0.73–0.93 for severe stunting (Supplemental Table S3). Differences in the prevalence of stunting between AZI-SP and the control group were statistically significant at 12, 24 and 36 months (each P<0.05) (Supplemental Table S3). The differences in the prevalence of severe stunting between AZI-SP and the control group were not statistically significant at any time point (Supplemental Table S3). Covariate adjusted RR varied between 0.76–0.86 (P<0.05 at 12 and 24 months) for stunting and 0.76–1.09 (P>0.05 at all time points) for severe stunting (Supplemental Table S3).

Detailed description of growth in AZI-SP group compared to the monthly SP group and monthly SP group compared to the control group is reported in Supplemental Results. In general, AZI-SP group had higher mean length/height and lower incidence and prevalence of stunting at all time points compared to the monthly SP group (Figures 2, 3; Supplemental Results and Tables S2, S3). Monthly SP group had higher mean length/height and lower incidence and prevalence of stunting at almost all time points compared to the control group. However, the differences between groups were smaller than between AZI-SP and control group (Figures 2, 3; Supplemental Results and Tables S2, S3).

The mean (SD) total developmental score was 108.6 (17.1) in the control, 110.2 (17.0) in the monthly SP and 112.4 (17.7) in the AZI-SP group (P=0.02). The AZI-SP group had a 3.8 (95% CI 1.1–6.4, P=0.005) points higher mean total developmental score than the control group. After covariate adjustment the difference was 3.3 points (95% CI 0.7–5.9, P=0.01, global P=0.05) (Supplemental Table S4). Difference in means between monthly SP and control group was 1.6 (95% CI -1.1–4.3, P=0.24) points (covariate adjusted difference 1.4, 95% CI -1.2–4.1, P=0.28) (Supplemental Table S4). Difference between AZI-SP and the control group was mostly due to a higher mean score in the performance subscale in the AZI-SP group (P<0.001). There were no significant differences in the other subscales between groups (Table 2, Supplemental Table S4).

The proportion of abortions, stillbirths and children who died during the follow-up was 15.3%, 15.1%, and 13.1% in the control, monthly SP and AZI-SP group respectively (P=0.60) (Supplemental Table S5). There were no statistically significant differences between the groups in the cumulative five-year mortality using Cox regression (Figure 4). During the postneonatal period, the proportion of children who died was lower in the AZI-SP group (1.9%) than the control group (5.5%; RR 0.34, 95% CI 0.15–0.76, P=0.008; adjusted RR 0.31, 95% CI 0.13–0.74, P=0.009) (Supplemental Table S5). There were no statistically significant differences in the number of abortions and stillbirths, early or late neonatal or child deaths (Supplemental Table S5). Mortality rates are reported in Supplemental Results.

Results from the sensitivity analyses with imputed data for length/height, unimputed data for development, and covariate adjusted differences in means and risk ratios were consistent with those from the primary analyses (Supplemental Tables S1-S5). Among children born to HIV negative mothers, the results were consistent with those from the primary analyses. Among children born to HIV positive mothers, the intergroup differences were typically larger than in the full-group analyses but there was less statistical significance, presumably due to play of chance in the small number of HIV positive mothers (Supplemental Tables S6-S7). Finally, the point estimates for the difference in mean child length between AZI-SP and control group were not affected much by the higher mortality observed in the control group (Supplemental Results).

**DISCUSSION**

We tested hypotheses that rural Malawian children would on average be taller, have a lower incidence and prevalence of stunting throughout their first five years of life, and have a higher developmental score at five years if their mothers received monthly SP and two doses of azithromycin in pregnancy rather than two SP doses as preventive treatment. The study findings were consistent with the hypotheses. Children in the AZI-SP group also had a lower postneonatal mortality than children in the control group. It therefore appears that gains obtained among children whose mothers received intensified antenatal infection treatment were sustained for five years and reflected in the prevalence of childhood stunting and other health indicators. Differences between monthly SP and the control group were often to the same direction as AZI-SP versus control but smaller and not statistically significant.

The strengths of this trial include random group allocation, broad inclusion criteria, large sample size, comprehensive follow-up, and blinding of the outcome assessors. Internal validity could have been compromised by missed recordings of child deaths and missing data on anthropometric measurements and developmental items and we did not have comprehensive information on postnatal exposures that might affect the outcomes of interest. However, we implemented a very active tracing system for defaulters, verified mortality data from multiple sources, and the random allocation was likely to distribute the non-trial determinants of growth, development, and mortality evenly between the study groups. Furthermore, anthropometric and developmental results were robust to sensitivity analyses and the baseline characteristics of those lost to follow-up and those who remained in the study were similar. The point-estimates for the intergroup differences in mean length and stunting prevalence remained essentially constant throughout the follow-up and Heckman’s selection model suggested that differences in growth were not affected by higher mortality in the control group. The differences in mean length and stunting prevalence were not statistically significant at the older age groups, but this is to be expected if the absolute difference remains constant, because of the increasing population variance in length with age25. Because of the constant strength of association, the consistency between various indicators, and the biological plausibility of the findings, we consider our findings valid, representative, and indicative of a causal association26 between the antenatal AZI-SP intervention and the improved and sustained child growth and development outcomes. A positive impact on postneonatal mortality is also biologically plausible, but this finding arose from an exploratory analysis and hence needs further confirmation in future studies.

The positive impact of IPTp on birth weight in malaria-endemic areas has been well-documented27. Similarly, preventive antenatal provision of broad-spectrum antibiotics has been associated with increased mean duration of pregnancy and birth weight in sub-Saharan Africa, although opposite results have also been reported28. Few of these studies have, however, reported newborn or infant lengths and none have evaluated the intervention effect beyond infancy. Whilst some maternal dietary supplementation studies have suggested a favorable effect on birth or neonate length5,6,29,30, this difference disappears within 6–12 months after delivery in the few studies with postnatal follow-up5,6. This may be due to the fact that the intervention only affected duration of pregnancy, which in turn affected birth size, because longer gestational duration is negatively associated with early postnatal growth velocity. In our trial, approximately two thirds of the difference in birth weight was attributed to fetal growth velocity and only one third to pregnancy duration10.

Fetal length gain has been shown to be regulated by insulin-like growth factors (IGFs), secreted by maternal tissues, the placenta and the fetus upon various stimuli31,32. Downregulation of IGFs and their cellular receptor expression, increased concentrations of IGF-binding and inactivating proteins in fetal circulation, and reduced placental nutrient transfer have been associated with systemic inflammation elicited by maternal and placental infection33. The antenatal intervention that consisted of repeated doses of SP and azithromycin was designed to reduce the burden of malaria and reproductive tract infections in pregnant women9. In our trial, the prevalence of maternal peripheral malaria at 32 gestation weeks and at delivery, as well as postnatal prevalence of vaginal trichomoniasis was significantly lower in the AZI-SP than the control group9,34. Although we have little data on other infections and no data on maternal inflammation, it seems likely that the AZI-SP intervention affected fetal growth and the duration of pregnancy through its impact on infection-mediated inflammation. Besides malaria and trichomoniasis, azithromycin may have affected maternal bacterial vaginosis, oral infections and chorioamnionitis, all of which are common in Malawi and associated with reduced birth size35-37.

Compared to the control group, AZI-SP group had a 3.8 points higher total developmental score at five years. A recent study on extremely low birth weight children using GMDS-ER 2–8 found roughly the same difference at the age of five years when comparing children with and without learning disabilities38. This study, done in Italy, also showed that assessing children over the age of two was most effective in detecting children with learning disabilities. In our sample, most of the differences in the total developmental score were due to differences in the performance subscale which measures the visuospatial skills including speed of working and precision. This is consistent with the findings from the Italian study, the investigators of which suggest that lack of cognitive flexibility at preschool age might interfere with intellectual functioning and negatively affect academic attainment. Our results are promising but further follow-up assessments would be necessary to better understand the significance of the intervention effect on child development.

**CONCLUSION**

Taken together, the results from this study support the hypothesis that provision of AZI-SP rather than two doses of SP during pregnancy reduces the incidence and prevalence of childhood stunting, has a positive effect on child development and may reduce postneonatal mortality in Malawi. Monthly treatment with SP alone does not seem to have the same effect, although it does reduce the incidence of low birth weight by approximately 20%27. Whilst encouraging, these results should not be interpreted to promote wide spread use of broad-spectrum antibiotics as a routine antenatal treatment. Such practice could theoretically lead to problems with antibiotic resistance and cause detrimental long-term effects on the microbiota of both the mother and her offspring39.

There were no major health policy changes or secular trends in child health outcomes in the study area during the trial follow-up. However, after the implementation of our study, the recommendation for IPTp has changed from two doses to monthly dosing with SP40. Additionally, HIV-positive women nowadays receive antiretroviral therapy during pregnancy, delivery and lactation, as opposed to the single-dose nevirapine regimen used at the time of our study. Furthermore, childhood mortality rates in Malawi and elsewhere have significantly declined, at least partly due to improved diagnostics and management of malaria and severe acute malnutrition41. Although the results from our sample suggest that azithromycin combined with monthly SP would provide health benefits compared to the monthly SP alone, the recent health trends and policy changes might modify its impact on one or more outcomes. Hence, our results should mainly be considered indicative of a causal role of maternal infections and inflammation in fetal growth restriction and its sustained impact on child growth and development. Given the importance of these fetal exposures and the slow progress in stunting reduction in Sub-Saharan Africa and Southern Asia42-44, it seems warranted to conduct further trials on the effects of prevention and management of infections during pregnancy, and to address childhood stunting in low-income contexts also through interventions that reduce the prevalence of maternal infections in pregnancy.

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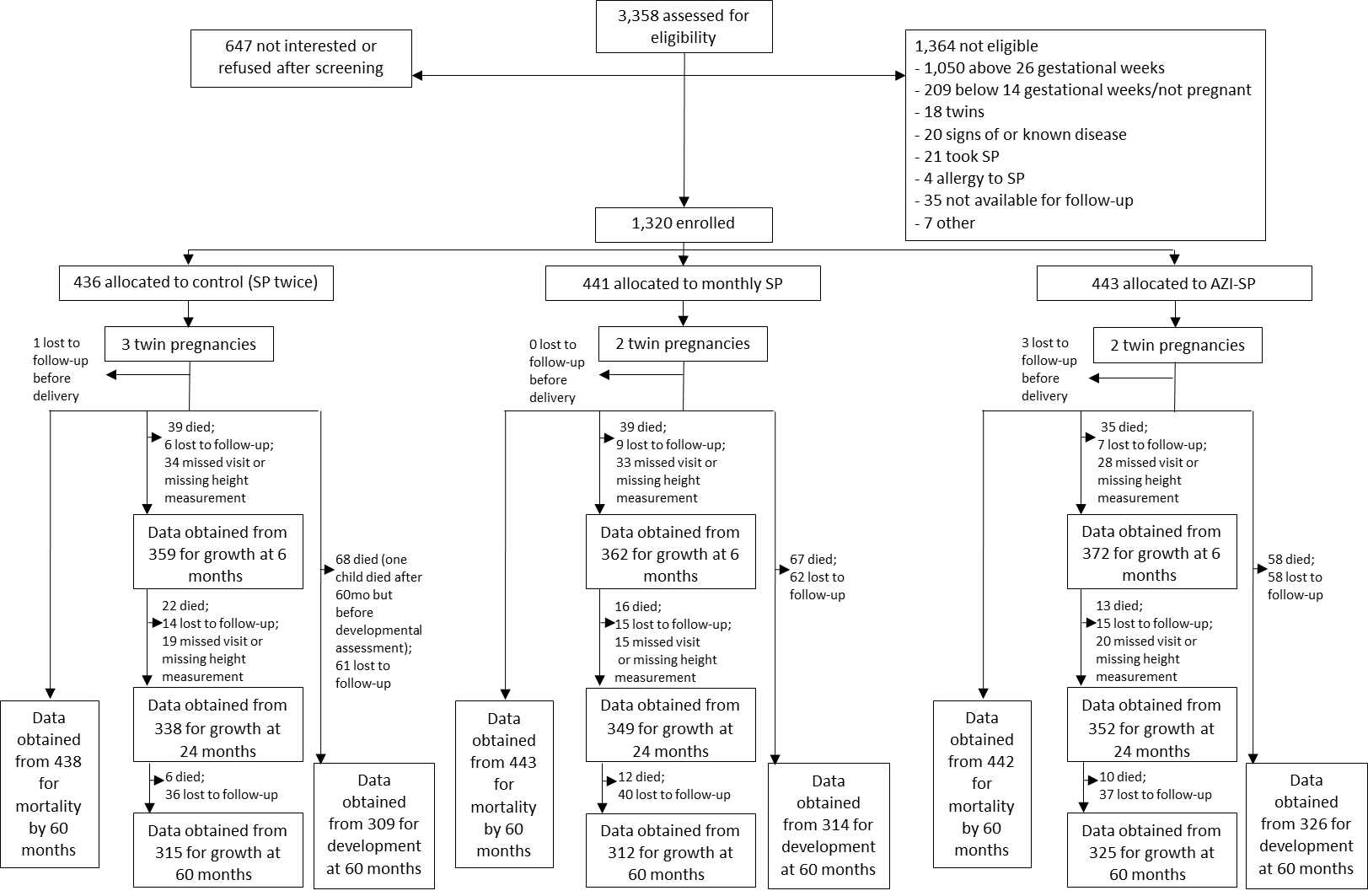


Figure 1. Enrollment, randomization and follow-up. SP = sulfadoxine-pyrimethamine; AZI-SP = intervention group with monthly SP and two doses of azithromycin.



Figure 2. Differences between groups and 95% confidence interval for AZI-SP vs control in height (cm) at one, six, 12, 24, 36, 48, and 60 months. Statistical significance between AZI-SP and the control group at P<0.05 marked with \*. SP=sulfadoxine-pyrimethamine; AZI-SP=intervention group with monthly SP and two doses of azithromycin.

Figure 3. Cumulative incidence of stunting and severe stunting (panel A) by 60 months of age by intervention group. Prevalence of stunting and severe stunting with 95% confidence interval by intervention group (panel B). SP=sulfadoxine-pyrimethamine; AZI-SP=intervention group with monthly SP and two doses of azithromycin.



Figure 4. Mortality survival curve by intervention groups. Time to death during pregnancy = time between enrollment and abortion/stillbirth. All pregnancies resulting in live birth were coded to have lasted 25 weeks and time in study after birth was added to that. SP=sulfadoxine-pyrimethamine; AZI-SP=intervention group with monthly SP and two doses of azithromycin.

Table 1. Baseline characteristics of the participating women at enrollment, by study group

| **Characteristic** | **Control (SP twice) (N=436), n (%)** | **Monthly SP, (N=441), n (%)** | **AZI-SP (N=443), n (%)** |
| --- | --- | --- | --- |
| Age, years, mean (SD) | 25 (7) | 25 (7) | 25 (6) |
| Height, cm, mean (SD) | 155.0 (5.5) | 154.8 (5.4) | 155.3 (5.6)a |
| BMI, kg/m2, mean (SD) | 21.7 (2.2) | 21.8 (2.1) | 21.9 (2.1)a |
| Gestational age at enrollment, weeks, mean (SD) | 20.3 (3.0) | 20.0 (3.2) | 20.0 (3.0) |
| Primiparous | 110 (25.2%) | 107 (24.3%) | 89 (20.1%) |
| HIV positive | 48/396 (12.1%) | 64/400 (16.0%) | 49/398 (12.3%) |
| Positive syphilis status | 18/433 (4.2%) | 27/435 (6.2%) | 21/440 (4.8%) |
| Blood Hb concentration, g/L, mean (SD) | 110 (19) | 111 (17) | 110 (20) |
| Moderate or severe anemia, Hb < 100 g/L | 116 (26.6%) | 106 (24.0%) | 129 (29.1%) |
| Severe anemia, Hb < 70 g/L | 9 (2.1%) | 2 (0.5%) | 9 (2.0%) |
| Microscopic peripheral blood malaria parasitemia | 49/435 (11.3%) | 41 (9.3%) | 27 (6.1%) |
| Literate participants | 116 (26.6%) | 129 (29.3%) | 139 (31.4%) |
| Years of schooling completed, mean (SD) | 2.1 (2.7)a | 2.2 (2.6) | 2.4 (2.8) |

SP = sulfadoxine-pyrimethamine. AZI-SP = intervention group with monthly SP and two doses of azithromycin. BMI = body-mass index. HIV = human immunodeficiency virus. Hb = hemoglobin.

a Value missing for one participant

Table 2. Total developmental score and six subscale scores (locomotor, personal-social, language, eye and hand coordination, performance, practical reasoning), mean (SD) by intervention group at 60 months of age (all models adjusted for child sex and age at the time of developmental assessment) using multiple imputed data.

| **Outcome** | **Control**  **(SP twice) (N=371), mean (SDa)** | **Monthly SP (N=376), mean (SDa)** | **AZI-SP (N=387), mean (SDa)** | **Global P-value** | **Comparison between AZI-SP and control group** | | **Comparison between AZI-SP and monthly SP group** | | **Comparison between monthly SP and control group** | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Difference in means  (95% CI)** | **P-value** | **Difference in means  (95% CI)** | **P-value** | **Difference in means  (95% CI)** | **P-value** |
| Total score | 108.6 (17.1) | 110.2 (17.0) | 112.4 (17.7) | 0.02 | 3.8 (1.1 to 6.4) | 0.005 | 2.2 (-0.4 to 4.8) | 0.10 | 1.6 (-1.1 to 4.3) | 0.24 |
| Locomotor score | 24.6 (3.8) | 24.8 (3.5) | 24.8 (3.9) | 0.88 | 0.1 (-0.5 to 0.7) | 0.67 | 0.0 (-0.6 to 0.6) | 0.97 | 0.1 (-0.5 to 0.7) | 0.64 |
| Personal-social score | 27.0 (3.9) | 27.4 (3.9) | 27.4 (3.8) | 0.27 | 0.4 (-0.2 to 1.0) | 0.17 | 0.0 (-0.6 to 0.6) | 0.95 | 0.4 (-0.2 to 1.0) | 0.16 |
| Language score | 14.7 (4.9) | 15.1 (5.1) | 15.6 (4.8) | 0.09 | 0.8 (0.1 to 1.6) | 0.03 | 0.5 (-0.3 to 1.2) | 0.24 | 0.4 (-0.4 to 1.2) | 0.34 |
| Eye and hand coordination score | 8.4 (3.1) | 8.9 (3.2) | 8.6 (3.0) | 0.14 | 0.3 (-0.2 to 0.7) | 0.28 | -0.2 (-0.7 to 0.2) | 0.32 | 0.5 (0.0 to 1.0) | 0.05 |
| Performance score | 15.1 (6.2) | 15.3 (5.9) | 16.9 (6.1) | <0.001 | 1.8 (0.8 to 2.8) | <0.001 | 1.5 (0.6 to 2.5) | 0.001 | 0.3 (-0.7 to 1.2) | 0.60 |
| Practical reasoning score | 18.8 (3.7) | 18.7 (3.7) | 19.2 (3.7) | 0.25 | 0.3 (-0.2 to 0.9) | 0.24 | 0.5 (-0.1 to 1.0) | 0.10 | -0.1 (-0.7 to 0.5) | 0.67 |

a SD for multiple imputed data calculated as an average SD from 50 imputations

SP = sulfadoxine-pyrimethamine; AZI-SP = intervention group with monthly SP and two doses of azithromycin