**Lungwena Antenatal Intervention Study (LAIS)**


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1. SUMMARY OF THE STUDY

1.1 Funding, monitoring, and research sites. Sponsor: Investigator initiated study, funded by a research grant from the Academy of Finland (grant 79 787)

Monitor: No external monitor besides the review board

Review board: An independent body, overseeing the progress of the study and assessing the safety of the intervention

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1.2 Executive summary. Maternal anemia, preterm deliveries, and low birth weight are common in sub-Saharan Africa and contribute significantly to the ill-health of pregnant women and infants. The present study is based on the assumption that these adverse outcomes can be prevented by improved antimicrobial management of malaria and sexually transmitted infections (STIs) among pregnant women. To test the hypothesis, a randomized clinical trial following Good Clinical Practice is planned to be carried out in Malawi in southeastern Africa.

A total of 1,320 consenting women who present at a rural antenatal clinic after 14 but before 26 completed gestation weeks will be enrolled. One-third of the women will receive antenatal care according to national recommendations, including regular visits to health center, screening for pregnancy complications, hematin and vitamin A supplementation and two doses of presumptive malaria treatment with sulfadoxine-pyrimethamine. Another third will receive otherwise the same care, but sulfadoxine-pyrimethamine treatment is given at monthly intervals. The final third receives standard antenatal care, sulfadoxine-pyrimethamine treatment at monthly intervals and two doses of presumptive STI treatment with azithromycin. Women are monitored throughout pregnancy and delivery and the newborns will be followed-up for five years.

The primary outcome measure is proportion of preterm births in the three study groups. Secondary maternal outcomes include anemia and malaria parasitemia during pregnancy, at delivery and at one, three, and six months after delivery, gestational weight gain and morbidity and STI prevalence after delivery. Secondary child outcomes consist of proportion of babies with low birth weight, mean birth weight, growth in infancy and childhood, incidence of malnutrition in infancy and childhood, and mortality. Additionally, information is collected on the development of malaria-specific humoral immunity in pregnancy and participant experiences from the study. Participant safety is systematically monitored throughout the intervention.

The study is planned to be started in June 2003. Enrollment will take approximately 2.5 years and the total duration of the study is four years. The research team consists of researchers from Finland and Malawi and post-graduate students. Funding comes mainly from the Academy of Finland.

2. BACKGROUND

2.1 Introduction. Pre-term birth, low birth weight, and infant mortality are common in Malawi and many other countries in sub-Saharan Africa. Recent community-based studies from Malawi and Mozambique suggest that up to 20% of all deliveries occur preterm (before 37 completed gestation weeks) (Schultz and others, 1994; Kulmala and others, 2000; Osman and others, 2001; N. van den Broek, unpublished data). Approximately 15% of all newborns in sub-Saharan Africa have a low birth weight (<2,500 g) and 11% die before the age of one year (UNICEF, 2001). Although the three entities are all multifactorial, they are also interrelated, i.e., pre-term births contribute to low birth weights, that in turn predispose the subjects for perinatal and infant death, often through malnutrition.

Several lines of evidence suggest that maternal malaria parasitemias and STIs contribute to increased risk of maternal anemia, preterm delivery and low birth weight (Meuris and others, 1993; Bloland and others, 1996; Schulman and others, 1999; Verhoeft and others, 1999; Schulman and others, 2001). Among pregnant women enrolling for antenatal care in Malawi, the prevalence of malaria parasitemia and maternal anemia has typically been reported at 20–45% and 49–91%, respectively (Steketee and others, 1996; Verhoeft and others,
In the same areas, the prevalence of active syphilis has been approximately 10%, human immunodeficiency virus (HIV) 16–19%, gonorrhea 8%, *Chlamydia trachomatis* infection 6%, trichomoniasis 25%, and bacterial vaginosis 15% (Kulmala and others, 2001; National AIDS Control Commission Malawi, 2001; Tsui and others, 2001; van den Broek N, unpublished data). Thus, STIs and malaria parasitemia are common among pregnant women in Malawi, making an antimicrobial management strategy an apparently feasible option to prevent preterm birth and low birth weight.

### 2.2 Justification for making the study now

A wealth of data have documented improved malaria clearance among expectant mothers receiving two presumptive treatment doses of sulfadoxine-pyrimethamine during pregnancy (Schultz and others, 1994; Parise and others, 1998; Shultman and others, 1999). As a result, the national guidelines for malaria control in Malawi have since 1993 recommended this antimicrobial intervention; one treatment dose to be given at antenatal care enrollment and another one at 28–34 gestation weeks (Government of Malawi, 2002). Whereas this approach has undoubtedly been safe and beneficial, there have been a number of problems related to this approach, limiting the public health impact of the strategy.

The first problem related to the two-dose sulfadoxine-pyrimethamine treatment is poor customer compliance, possibly because it is difficult for the mothers to remember when they need the second dose (Verhoeff and others, 1998; Rogerson and others, 2000A; National Statistical Office (Malawi) and ORC Macro, 2001). Although suitable for directly observed treatment (DOT), sulfadoxine-pyrimethamine is often prescribed to be taken at home, which further decreases compliance. Additionally, the dosing interval appears too long because one dose will be effective against malaria only for approximately four weeks (Sullivan and others, 1999). Finally, the emerging sulfadoxine-pyrimethamine resistance of *Plasmodium falciparum* strains threatens to make these antibiotics unsuitable for malaria treatment in sub-Saharan Africa (Nzila and others, 2000; Sibley and others, 2001; Government of Malawi, 2002). New, more efficient therapeutic regimens are therefore needed.

Apart from the possible drug resistance, monthly dosing with sulfadoxine-pyrimethamine seems a logical choice for improved malaria control in pregnancy (Verhoeff and others, 1998). In Kenya, it was shown to be safe and more effective than fewer-dose regimens, and economic analysis suggested cost-effectiveness in populations with high HIV prevalence (Parise and others, 1998; Wolfe and others, 2001). Whereas other controlled trials have not been published, the World Health Organization already promotes this type of a frequent-dose intervention in the new guidelines for managing malaria in pregnancy (B. Nahlen, unpublished data).

Considering the frequency of STIs, their known association with preterm birth and low birth weight and the potential of antimicrobial treatment to prevent these outcomes when targeted to infected persons (Hauth and others, 1995; Regan and others, 1996; Lamont, 1999), presumptive STI treatment of all pregnant women provides another feasible antenatal intervention in sub-Saharan Africa. However, only a few trials have been reported. In Kenya (n = 400), a single intramuscular dose of ceftriaxone reduced the prevalence of gonorrhea from 4.2% to 1.8%, postpartum endometritis from 10.4% to 3.8%, and low birth weight rate from 9.2% to 4.0% (Temmerman and others, 1995). In Uganda (n = 4036), a single oral treatment consisting of 1 g of azithromycin, 400 mg of cefixime, and 2 g of metronidazole cleared various STIs and reduced the incidence of preterm delivery by 23%, low birth weight by 32% and neonatal mortality by 17% compared with placebo treatment (Gray and others, 2001). No major adverse reactions were reported from either study.

The optimal dose of azithromycin in the treatment of STIs is unknown because no direct dose-comparison studies have been conducted. Most STIs can be treated with a single 1 g oral dose, but some studies have questioned its adequacy in the treatment of gonorrhea because of which the U.S. guidelines recommend a 2 g dose for this illness (Centers for Disease Control and Prevention, 2002). However, many other studies have also documented a favorable result with the 1 g treatment (Odugbemi and others, 1993; Waugh 1993; Gruber and others, 1997; Swanston and others, 2001), and in Europe this lower dose is indicated also for gonorrhea (Pfizer Pharmaceuticals Group, 2002). Adverse effects (mainly abdominal) are less common with the lower 1 g dose (Pfizer Inc., 2002).

If presumptive treatment of STIs is considered during pregnancy, azithromycin has several advantages. It has a broad antimicrobial spectrum, covering e.g., gonorrhea, *Chlamydia trachomatis*, chancroid, and possibly syphilis. It is safe during pregnancy and can be administered under direct observation as a single oral dose or as two doses, one at the beginning of antenatal care and one later during pregnancy (Gray and others, 2001; Centers for Disease Control and Prevention, 2002). It also has antimalarial activity (Anderson and others, 1995; Taylor and others, 1999, Ohrt and others, 2002), which may become important in sub-Saharan Africa if parasite resistance against sulfadoxine-pyrimethamine continues to increase. Currently, the Kenya Medical Research Institute is conducting a phase II study to determine the antimalarial effects of azithromycin in combination with sulfadoxine-trimethoprim or with chloroquine in semi-immune pregnant women (Parise M, unpublished data).

In the light of above, it is justified to implement a clinical trial that tests the health effects of presumptive antimalarial treatment of pregnant women in sub-Saharan Africa with monthly doses of sulfadoxine-pyrimethamine, either alone or in combination with presumptive STI treatment with azithromycin.

### 2.3 Objectives of the study

In broad terms, the current study is set up to test the hypothesis that in rural Malawi maternal health could be improved and the prevalence of preterm births and low birth weight reduced by improved management of maternal malaria and STIs during pregnancy.

The specific objectives include detailed analysis of the health effects of two antenatal interventions (monthly sulfadoxine-pyrimethamine therapy and monthly sulfadoxine-pyrimethamine in combination with occasional azithromycin treatment). The analyses will focus on 1) the effect of antenatal interventions on the duration of pregnancy, birth size, and perinatal and neonatal mortality; 2) the effect of antenatal interventions on maternal weight gain and anemia during pregnancy and six months after delivery; 3) the effect of antenatal interventions on the prevalence of maternal malaria infection and febrile illnesses during pregnancy and six months after delivery; 4) the effect of antenatal interventions on childhood weight and length gain and the incidence of moderate or severe underweight (weight-for-age Z score [WAZ] < −2 or < −3).
and stunting (height-for-age Z score [HAZ] < -2 or < -3) during infancy and early childhood; 5) the effect of antenatal interventions on infant mortality; 6) the economic and cultural feasibility of the tested interventions; 7) the development of fundal height and fetal measurements (ultrasound assessed) in relation to the duration of pregnancy; 8) the value of fundal height measurement and Ballard score analysis in the assessment of the duration of gestation (a comparison to ultrasound assessment); 9) the prevalence and classification of main delivery complications; 10) the occurrence of antimicrobial resistance against sulfadoxine-pyrimethamine in malaria parasites found in the study area; 11) the development of humoral malaria-specific immunity during pregnancy and six months after delivery; 12) participant experiences from study participation and different antenatal interventions; and 13) changes in blood concentrations of selected growth-related hormones among a subgroup of infants growing poorly between 12 and 18 months of age, a delay in the onset of the so called childhood growth.

3. METHODS FOR THE STUDY

3.1 General design. A phase 3, randomized, parallel-group investigator-blinded clinical trial

3.2 Place of research. The study will be carried out in Lungwena, Mangochi District, Southern Malawi, where the current study group has had an ongoing research project on maternal and child health since 1994. The study site is rural and has an area of approximately 100 km². The nearest town (Mangochi) is approximately 30 km away. The population is approximately 20,000, most of whom are subsistence farmers, Muslims, and belong to the Yao tribe. The Yaos are typically organized matrilineally, which means that women and children reside near the extended maternal family, whereas men move to marry. The general educational level in Lungwena is low (only 41% of men and 14% of women are literate).

A government health center, which also serves as a center for a community health teaching and some primary health care interventions, is located in the middle of the area. This facility is equipped with solar electricity, research office and personnel, and a basic laboratory. It provides the local population with free preventive and curative modern health services such as family planning, antenatal and delivery care, and treatment of common illnesses.

3.3 Study population. 3.3.1 Participants. The crude birth rate in the study area is approximately 45 births/1,000 inhabitants. Almost all pregnant women attend the antenatal clinic at the health center several times during the pregnancy (Kulmala and others, 2000). The study population will be drawn from those pregnant women who come to the health center before they have completed their 26th gestation week.

The enrollment criteria for study participants are divided into inclusion and exclusion criteria. Inclusion criteria are signed informed consent, age ≥ 15.00 years, ultrasound-confirmed pregnancy, mother has felt the movements of the fetus (quickening), fetal age of at least 14 but not more than 26 completed gestation weeks (based on ultrasound assessment); maternal availability for follow-up during the entire period of the study. Exclusion criteria are known maternal tuberculosis, diabetes, kidney disease, or liver disease; any severe acute illness warranting hospital referral (judged by the attending nurse-midwife); mental disorder that may affect comprehension of the study or success of follow-up; twin pregnancy; pregnancy complications evident at enrollment visit (moderate to severe edema, blood hemoglobin concentration < 5 g/dL, systolic blood pressure (BP) > 160 mm of Hg or diastolic BP > 100 mm of Hg); prior receipt of azithromycin during this pregnancy; receipt of sulfadoxine or pyrimethamine within 28 days before enrollment; known allergy to drugs containing sulfonamides, macrolides, or pyrimethamine; history of anaphylaxis; history of any serious allergic reaction to any substance requiring emergency medical care; and concurrent participation in any other clinical trial.

3.3.2 Study interventions. The study will compare the effect of two more active infection management strategies to that of the standard antenatal care. The control group (OR controls) will receive the standard Malawian antenatal care that includes regular antenatal clinic visits at four-week intervals until 36 completed gestation weeks and then at one-week intervals until delivery; screening for syphilis by using the Venereal Disease Research Laboratory (VDRL) test (if a test result is positive, treatment of the mother, her sexual partner, and the newborn baby with intramuscular injections of benzathine penicillin, adult dose = 2.4 mU, newborn dose = 50 kU/kg); gestational iron (ferrous sulfate, 200 mg/day) and folic acid (0.25 mg/day) supplementation starting at first antenatal visit and continuing until delivery; presumptive intermittent malaria treatment with sulfadoxine–pyrimethamine twice during pregnancy: at the enrollment visit and at a visit between 28–34 weeks of gestation. Each treatment dose contains sulfadoxine-pyrimethamine, 3 tablets orally, each containing 50 mg of sulfadoxine and 25 mg of pyrimethamine; two treatments with azithromycin-like placebo at the enrollment visit and at a visit between 28–34 weeks of gestation. Each treatment dose contains placebo that looks like Zithromax®, 2 tablets orally; vitamin A supplementation (200,000 IU oral capsule) immediately after delivery and at six months post-delivery; treatment of other maternal illnesses, pregnancy complications, infant malnutrition, and other conditions as medically indicated. In case of malaria, treatment with quinine (300 mg, 2 tablets orally three times a day for 7 days). In addition to the standard antenatal care, the control group is offered voluntary counseled testing for HIV infection. If test result is positive, the mother is advised to take the treatment at the onset of delivery and treatment of the newborn is given within 72 hours of delivery with nevirapine. The adult dose is Viramune®, 1 tablet orally, containing 200 mg of nevirapine. The newborn dose is a Viramune® suspension, 0.6 mL orally, containing 6 mg of nevirapine (newborn dose is approximately 2 mg/kg)

Intervention group A will receive more active presumptive treatment of malaria. This treatment includes standard antenatal and other health care as indicated above, including VDRL and HIV testing and iron, folate, and vitamin A supplementation, and appropriate medical treatment of health problems; presumptive intermittent malaria treatment with sulfadoxine–pyrimethamine at four-week intervals (more frequent dosing) starting at the enrollment visit and finishing latest at 37 completed gestation weeks. Each treatment dose contains sulfadoxine-pyrimethamine, 3 tablets orally, each containing 500 mg of sulfadoxine and 25 mg of pyrimethamine; and two treatments with azithromycin-like placebo: at the enrollment visit and at a visit between 28–34 weeks of gestation. Each treatment dose contains placebo looking like Zithromax®, 2 tablets orally.
Intervention group B will receive more active presumptive treatment of malaria and STIs. This treatment includes standard antenatal and other health care as indicated above, including VDRL and HIV testing and iron, folate, and vitamin A supplementation, and appropriate medical treatment of health problems; presumptive intermittent malaria treatment with sulfadoxine-pyrimethamine at four-week intervals (more frequent dosing) starting at the enrollment visit and finishing latest at 37 completed gestation weeks. Each treatment dose contains sulfadoxine-pyrimethamine, 3 tablets orally, each containing 500 mg of sulfadoxine and 25 mg of pyrimethamine; and two presumptive treatments for STIs with azithromycin at the enrollment visit and at a visit between 28–34 weeks of gestation. Each treatment dose contains Zithromax, 2 tablets orally, each containing 500 mg of azithromycin.

The study drugs are given under direct observation, together with fruit juice and biscuits. All participants will be informed about the benefits of pyrethrine-impregnated bet nets and encouraged to procure them for their own and their children’s use.

3.4 Study period. Data collection will last for 4–4.5 years. The planned detailed time schedule is finalizing the research plan during August–December 2002; approval by the ethics committee (COMRC); January–May 2003 consultation with community leaders; staff recruitment, and training; developing practical arrangements and standard operating procedures (SOPs); enrollment during June 2003–October 2005; interim analysis of safety in July 2004; data collection on maternal/newborn outcome during October 2005–May 2006; data collection on infant outcome during June 2006–May 2007; data analysis on maternal/newborn outcome; dissemination of results on analyzed data; data analysis on infant outcome during June–December 2007; and dissemination of results on analyzed data.

3.5 Sample size, its justification, and recruitment rate. 3.5.1 Sample size. The selected sample size is 440 women per group (total = 1,320) calculated from the expected numbers of pre-term deliveries (primary outcome).

Based on our own earlier results and those of others in Malawi and Mozambique, 20% of all deliveries are at present estimated to occur before 37 completed gestation weeks (Schultz and others, 1996; Kulma and others, 2000; Osman and others, 2001; van den Broek N, unpublished data). Because of a possible increase in the use of bed nets, this proportion is estimated to be 18% in the control group for the present study. A 40% decrease in the intervention group (from 18% to 10.8%) is considered clinically significant and achievable. A sample size of 400 per group will produce an 80% power and a 95% confidence to detect such a difference between the intervention and the control groups. Because of an estimated 10% loss to follow-up (see 3.14), 440 women are enrolled in each group.

For secondary outcomes, the chosen sample size provides 80% power and 95% confidence to detect a 0.5 gestation week difference in the mean duration of pregnancy, a 95 g difference in mean birth weight, and a decrease in the incidence of low birth weight from 20% to 12.5%.

3.5.2 Planned recruitment rate. The planned recruitment rate is 10 women/week or 535 women/year, i.e., recruitment will take approximately 2.5 years. This time is estimated from the number of people living in Lungwena Health Center catchment area (20,000) and a crude birth rate of approximately 45 births/1,000 inhabitants/year. Of the annual 900 pregnant women, 70% are estimated to begin antenatal care at the health center before 26 completed gestation weeks and 85% of them are assumed to enroll in the study.

The estimated enrollment rates at health center antenatal clinic and the clinical trial are based on an earlier cohort study and health center statistics in the same area, where 60–70% of all pregnant women came to the health center before 26 completed gestation weeks and 99% of them enrolled in the study (Kulma and others, 2000). For the present trial, the timely enrollment at health center is estimated to be at least as high as before (because of community sensitization), and enrollment is estimated to be a bit lower than the earlier study (because of more intensive follow-up).

3.6 Data collection. 3.6.1 Recruitment. All pregnant women attending the antenatal clinic at Lungwena Health Center are approached and briefed about the study (Appendix 1). Women wishing to participate and signing an informed consent form (Appendix 2) will undergo an interview on socioeconomic background and health history (Appendices 3 and 4), a medical examination (Appendix 5), an ultrasound assessment for duration of pregnancy (Appendix 6), and selected laboratory analyses (Appendix 7). Those meeting the predefined criteria will be enrolled and randomly allocated to different study groups (Appendices 8 and 9).

Before enrollment, the purpose and methods of the study will be discussed with community leaders and in larger village meetings. On these occasions, the importance of early antenatal care enrollment is emphasized.

3.6.2 Outcome measures and safety analysis. Primary outcome for efficacy: percentage of pre-term deliveries (birth before 37 completed gestation weeks).

Secondary outcomes, maternal health: mean maternal blood hemoglobin concentration during pregnancy (separately for each antenatal visit and for the visits at one, three, and six months after delivery); percentage of women with mild, moderate or severe anemia (hemoglobin < 11, 8, and 5 g/dL, respectively) at every antenatal visit and at one, three, and six months after delivery; percentage of women with peripheral blood malaria parasitemia and mean parasite density at first visit, at approximately 32 gestational weeks and at delivery (only for those delivering at Lungwena Health Center); percentage of women with cord blood or placental malaria parasitemia and mean parasite density at delivery (only for those delivering at Lungwena Health Center); maternal weight gain during pregnancy; mean number of illness days during pregnancy (self-reported symptoms of malaria and other illnesses); prevalence of maternal Chlamydia trachomatis, Neisseria gonorhoea, and Trichomonas vaginalis infection at four weeks after delivery.

Secondary outcomes, child health: percentage of low birth weight babies (< 2,500 g); mean birth weight; mean duration of gestation; percentage of low chest circumference (< 30 cm, indicator of small birth size); percentage of low chest or head circumference (chest < 30 cm, head < 31 cm); incidence of moderate or severe underweight (WAZ < −2 or < −3) or stunting (HAZ < −2 or < −3) during infancy or early childhood. Age of onset of childhood growth; perinatal, neonatal, and infant mortality.

Primary outcome for safety: number of serious and any adverse reactions (especially rash, vomiting, abdominal cramps, and diarrhea).
We will also measure the development of malaria-specific humoral immunity towards the so-called variant surface antigens (VSA) of *P. falciparum*, that are believed to play an important role in the pathogenesis of malaria in pregnancy. Additionally, we will collect information from participant experiences from the study (especially about informed consent, information obtained during the study and perceived problems with different interventions).

A subgroup of approximately 100 participants, i.e., all those who grow poorly between 12 and 18 months of age (as assessed by plotting height gain in a specific growth chart) will be invited to participate in a growth velocity and a growth related hormone substudy. In this substudy, the participants’ weight and length are measured and plotted monthly on a specific velocity chart (Appendix 19). Visual inspection is used to determine the time point when the participant switches from infancy to childhood growth phase, i.e., when there is a rapid acceleration of growth velocity (infancy-childhood spurt). A venous blood sample is obtained at the 18-month visit and at the visit when a length gain velocity acceleration is observed. Serum is separated from both samples, stored at −20°C, and later analyzed (at Laboratory of Pediatric Endocrinology, University of Gothenburg, Gothenburg, Sweden) for concentrations of growth hormone (GH), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3 (IGF-BP3), insulin-like growth factor binding protein 1 (IGF-BP1), acid-labile subunit (ALS) of the IGF1–IGFBP3 complex, leptin, glucose, insulin, tumor necrosis factor (TNF), and interleukin 6 (IL-6) and for proteomics. The analyses investigate correlations between the change in growth velocity and blood levels of the indicated hormones and other substances. Frozen whole blood cells are stored for genomics.

### 3.6.3 Frequency and duration of follow-up.

The outline of the recruitment, group allocation and follow-up is shown in Figure 1. Briefly, all participants are seen at the health center at four-week intervals until 36 completed gestation weeks and...
weekly thereafter. At each visit, the mothers will undergo an interview, a routine antenatal investigation (Appendix 10) and blood sampling (normally 250–300 μL from finger tip, at first visit 5 mL from median antebrachial vein). Pelvic ultrasound (assessing only the fetal biparietal diameter, length of the femur, and pulsation of the heart, but not fetal morphology) is performed for all women at the first visit and for a subgroup of 300 women (100 for both interventions and 100 controls) at each visit. If the fetus is found dead (no heart pulsation), the mother is referred to Mangochi District Hospital for appropriate management. All women will be offered screening for syphilis and HIV infection at first antenatal clinic visit.

Within 48 hours of delivery, a research assistant will make a home-visit, during which she will interview the mother and (if possible) the person attending the delivery about delivery events (Appendices 11 and 12) and measure the birth measurements and assess the duration of pregnancy by modified Ballard score (Appendix 13). At four weeks after delivery, the mother and the child will undergo a medical examination at the health center (Appendix 14). Within two weeks after this four-week postnatal visit, a research assistant will make a home visit to interview mothers about their experiences from study participation (Appendix 18). A finger prick blood sample is obtained from mothers at one, three, and six months after delivery. For the assessment of growth and mortality in infancy, the babies are examined at the health center at 3, 6, 9, 12, 15, 18, 24, 30, 36, 48, and 60 months of age (Appendix 15). The subgroup of children growing poorly at 18 months will undergo additional examinations monthly until two repeated length/height measurements document acceleration in linear growth velocity (see chapter 3.6.2).

From the blood samples taken at each visit, 100 μL is stored on filter papers (2 spots, each 50 μL). These samples will be used to produce descriptive data on the incidence and prevalence of sulfadoxine-pyrimethamine–resistant malaria parasites in the study area.

A 10-mL urine sample and a vaginal swab with two cotton sticks will be collected from the participating women at four weeks after delivery. These samples will first be used to diagnose maternal vaginal trichomoniasis and then will be stored at −20°C and used within a month of sample collection to determine the impact of the intervention on the prevalence of *C. trachomatis* or *N. gonorrhoeae* infections. Women who are found infected (and their sexual partners) are offered appropriate antibiotic therapy (250 mg of siprofloxicin orally as a single dose for gonorrhea, 1 g of azithromycin orally as a single dose for *Chlamydia* infection, and 2 g of metronizole orally as a single dose for trichomoniasis).

Women who were treated for confirmed syphilis at enrollment (a positive *Treponema pallidum* hemagglutination [TPHA] test result) will be offered a possibility to have a follow-up blood test at four weeks after delivery. Additionally, they are offered to have their children tested for syphilis at six months of age (when passively transferred maternal antibodies have disappeared). All women and children found infected will be treated appropriately with antibiotics.

3.6.4 Methods used for data collection. Primary efficacy outcome: The duration of pregnancy at birth will be determined based on ultrasound assessment (by a research nurse) of fetal bi-parietal diameter and femur length at first antenatal visit.

Secondary maternal outcomes: Blood hemoglobin concentration is measured with a Hemo-Cue® instrument from a finger prick sample (on first visit from venous blood). At selected antenatal clinic visits, the same blood sample is used for the assessment of peripheral blood malaria parasitemia (Giemsa-stained thick and thin blood films). For women delivering at Lungwena health center, malaria parasitemia will also be assessed for peripheral and cord blood and the placenta.

Maternal weights are measured by a research assistant by using a digital bathroom scale (reading increment = 200 g). Maternal morbidity is determined by four-weekly interviews about the number of days, when the mother has had symptoms of malaria or other illnesses.

The prevalence of maternal *N. gonorrhoeae* and *C. trachomatis* infections will be determined by using a DNA amplification method from urine or vaginal swab samples obtained at the four-week post-delivery visit and stored at −20°C for a maximum of one month. Vaginal trichomoniasis is diagnosed four weeks after delivery by direct microscopy from a fresh vaginal mucous sample (taken with a cotton wool stick) smeared on an object glass.

Secondary child outcomes: Birth weight and chest and head circumference will be measured within 48 hours of delivery by research assistants using spring scales (100-g reading increment), and elastic tapes (1-mm reading increment). Child growth is monitored by quarterly measurement (at the health center) of infant weight (electronic scale, recorded to the nearest 10 g), length (infantometer, recorded to the nearest 1 mm), and head circumference (elastic tapes, recorded to the nearest 1 mm). Child survival is queried from the guardians at their quarterly visits to the health center.

Safety outcomes: Adverse reactions to study medications are queried with structured forms (Appendix 16) at every antenatal clinic visit and at any other visits of the mother to the health center. Data from all non-scheduled visits to the health center, either at antenatal clinic or at out-patient department, are documented in structured forms (Appendix 17).

Other collected data: Socioeconomic and demographic background of the mother and her medical history are queried with a structured interview at enrollment visit. Routine antenatal assessment will include an interview about recent medical history, measurements of weight and blood pressure (with an electronic sphygmomanometer), inspection for edema, measurement of urine protein (chemical screening with dipsticks) if indicated by other findings (high/increasing blood pressure or edema), auscultation for fetal heart sounds, external abdominal palpation, and measurement of fundal height (with elastic tapes, reading increment 1 mm). Pelvic ultrasound will be conducted with a portable analyzer by a research nurse.

Delivery complications will be queried with structured forms from the mother and the person attending the delivery (if possible).

For comparison with the ultrasound-obtained data, the duration of gestation will be calculated from fundal height at first antenatal visit (measured by a research nurse) and the modified Ballard score within 48 hours of birth (by a research assistant, Verhoeff and others, 1997).

Maternal HIV status is determined (after counseling and informed consent) at enrollment from separated serum samples by using two antibody enzyme-linked immunosorbent assays (ELISAs) (Determine; Abbott Laboratories, Abbott Park, IL, and Uni-Gold; Trinity Biotech Plc., Bray, Ireland). The same serum samples are used for syphilis screening (Determine; Abbott Laboratories, Tokyo, Japan).
All screening-positive women will receive penicillin treatment, but the screening results are later confirmed with a TPHA assay (Lorne Laboratories Ltd., Reading, United Kingdom). The prevalence and pattern of sulfadoxine-pyrimethamine resistance in *P. falciparum* strains is assessed by using DNA technology with filter paper–impregnated blood samples obtained from malaria-positive women at various antenatal clinic visits.

Malaria-specific humoral immunity is investigated from stored serum samples by using immunofluorescent assays and ELISAs and various VSAs from *P. falciparum*-infected erythrocytes. These analyses will be done by Dr. Stephen Rogers at the University of Melbourne (Melbourne, Victoria, Australia).

Maternal experiences from study participation and antenatal care are queried by using a structured interview at 4–6 weeks after delivery. The interviews will be carried out by a trained research assistant at the participant’s home. Bed net use (frequency of use, age of net, and its impregnation history) will be queried and documented at each antenatal clinic visit and at four weeks after delivery.

For the substudy on length velocity change and hormonal changes, the following hormones are measured in obtained serum samples and cells: proteomics, GH, IGF-1, IGFBP3, IGFBP1, ALS, leptin, glucose, insulin (HOMA-IR is calculated), TNFα, and IL-6. The analyses will be conducted by using specific radioimmunoassay at the Laboratory of Pediatric Endocrinology, University of Gothenburg (Gothenburg, Sweden).

### 3.6.5 Practical arrangements for allocating participants to groups

The study participants will be randomized (by using a computer-generated random number list) in blocks of 90 into control and intervention groups. From all groups, 100 women will further be randomized into a more intensive ultrasound follow-up. The randomization list will be placed in three separate sealed opaque envelopes that will be stored by the principal investigator (University of Tampere, Finland), a member of the review board (Blantyre, Malawi), and a coordinating investigator at the study site (Lungwena Training Health Center).

Based on the randomization list, a series of 1,350 sealed opaque envelopes will be made and stored in groups of 10 (Lungwena Antenatal Intervention Study [LAIS] randomization group 1–135) in a locked cabinet at Lungwena research office and labeled LAIS randomization group 1–135. At any one point, the randomization group with smallest number and any remaining envelopes is used for randomization. When a woman is willing to participate and she meets all the enrollment criteria, the LAIS study coordinator pulls out the group of LAIS randomization envelopes being used at that point and asks the study participant to choose and open one of them. The envelope contains a paper indicating the LAIS identification code for that particular participant. The study coordinator then pulls out a study drug container having a comparable study number. This container, which is stored at the research office throughout the study, includes seven small drug envelopes prepacked for all individual study visits.

### 3.6.6 Methods for protecting against other sources of bias

Randomization, group allocation, and distribution of study drugs will be conducted by a research assistant not participating in the outcome evaluation. The researchers and the staff measuring the outcome variables will remain blinded to the group allocation until the end of data collection.

### 3.6.7 Likely rate of loss to follow-up and participant tracing

Ten percent of the women and their offspring are expected to be lost to follow-up. This is a conservative estimate based on the potential problems mentioned in the previous paragraph (3.14) and the fact that the corresponding rate was 4.8% in an earlier study from the same area. If a pregnant woman misses a planned visit by more than two weeks, the study coordinator will send a research assistant to make a home visit and see if the baby has already been born. If the baby has been born, the research assistant interviews the mother and delivery attendant about delivery events, takes the anthropometric measurements from the newborn, and post-natal follow-up will continue as planned. If the baby has not been born, the mother is reminded about the trial and the importance of follow-up visits. If the participant still fails to attend the antenatal clinic, the reminder home visits are repeated at four-week intervals until delivery.

If an infant misses a planned visit by more than two weeks, the study coordinator will send a research assistant or other member of the research team to make a home visit and remind the guardian about the trial and the importance of follow-up visits. If the participant still fails to attend the antenatal clinic within the next two weeks, the study team will make another home visit and interview the guardian and measure the child’s growth at home.

### 3.6.8 Management of the study. Day-to-day management

The study team on the research site will have a three-member core group (a coordinating investigator, a study nurse, and an office assistant) responsible for smooth implementation of the study. The core group will meet weekly to review progress of enrollment and follow-up, discuss any problems encountered, and accept a general plan of activities for the subsequent week.

An office assistant will keep records and produce weekly graphic summaries of the success of enrollment and follow-up. She will also create a master chart of visits for the enrolled participants. Based on this chart, she will make a weekly duty plan for other members of the team. The office assistant will distribute these plans to members of the study team at the end of each week. Once a month, the study team will have a larger staff meeting to discuss the progress and any acute issues related to the study.

A systematic participant flow will be designed for the health center to ensure smooth antenatal visits for the participants. The participants will report to the office assistant, obtain all necessary forms for source data, visit appropriate study stations (information about the study, background interview, antenatal examination, ultrasound, HIV counseling, laboratory), and finally return to the office assistant, give her the source data, obtain planned drug treatment, and agree on the date of the subsequent appointment. The office assistant, but no other members of the study team, will know the group allocation of each participant.

At each study station, research assistants will document their findings on the source data forms carried by the participant. Additionally, the research assistants will keep log books of all participant contacts at their study stations.

The intervention drugs needed in the trial are either provided by the manufacturer of Zithromax® or purchased from the Malawi Central Medical Stores (sulfadoxine-pyrimethamine). Other drugs needed in the antenatal clinic are purchased from the Central Medical Stores or obtained from a drug donation

...
program (Viramune®). The drugs are prepacked for individual participants and visits (each participant will have a separate drug envelope containing seven smaller envelopes with study drugs for each individual visit), and stored in safe containers under appropriate conditions, and accounted for by the study office assistant.

3.6.9 Scientists and their responsibilities. Dr. Per Ashorn, MD, is a pediatrician and pediatric infectious disease specialist who currently holds a position as a Senior Scientist at the University of Tampere (Tampere, Finland). During 1993–1995, Dr. Ashorn worked as a Medical Officer and Senior Clinical Lecturer in Malawi. Since then, he has coordinated studies on maternal and child health at the proposed study site. Dr. Ashorn is one of the two principal investigators of the present study, and he coordinates its planning and analysis and bears the overall responsibility for its implementation and financing.

Dr. Chiwoza Bandawe is the head of Community Health Department, College of Medicine (COM) (Blantyre, Malawi). He provides on site supervision for PhD students involved in the study, especially in relation to behavioral and cultural questions.

Dr. Teija Kulmala, MD, is a physician who works as a medical advisor for a non-government organization in Finland, the Family Federation of Finland. She did her doctoral thesis on maternal health in Malawi, and she has analyzed much of the data used in the planning of the present study. Dr. Kulmala is a co-principal investigator and she participates in the design, implementation, and analysis of the study.

Dr. Mari Luntamo, MD, is a general practitioner who will act as a field coordinator of the proposed study for the first one and a half years. Her responsibilities include the practical implementation and data analysis of the study with the support of the study group. During the study, Dr. Luntamo will check all data and verify and complete case report forms (CRFs). The work will form Dr. Luntamo’s postgraduate studies (Doctorate in Medical Science at the University of Tampere, Tampere, Finland), and she will be the responsible author of the first block of eventual publications.

Dr. Kenneth Maleta, MBBS, is a physician who has specialized in rural health problems and who will complete his doctoral studies (at the University of Tampere, Tampere, Finland) before the onset of the proposed study. Dr. Maleta has a position as assistant lecturer in Community Health at the College of Medicine, University of Malawi (Blantyre, Malawi), and he will act as a local supervisor for the field coordinators of the study.

Dr. Bernard Mbewe is the vice-head of Community Health Department (COM). He will coordinate the study after Dr. Luntamo’s field work. His responsibilities will be the same as those of Dr. Luntamo. The work will form his postgraduate studies (Doctorate in Medical Science at the University of Tampere, Tampere, Finland), and he will be the responsible author of the second block of eventual publications.

The substudy on malaria immunity will be conducted in collaboration with Dr. Stephen Rogerson, University of Melbourne (Melbourne, Victoria, Australia). Dr. Rogerson will be responsible for the assessment of malaria-specific immunity from serum samples collected by the LAIS team.

The substudy on delayed onset of childhood growth phase and serum levels of growth-related hormones and other factors is conducted in collaboration with Professors Karlberg, Albertsson-Wikland, and Hochberg. Professor Johan Karlberg, University of Hong Kong, will be responsible for the postnatal growth analyses, and Professor Kerstin Albertsson-Wikland, Göteborg University, Professor Zeév Hochberg, Göteborg University and Meyer Children’s Hospital (Haifa, Israel) will be responsible for growth-related hormone analysis, proteomics, genomics, and metabolism.

3.6.10 Responsibilities of the research assistants. The team will consist of one study nurse/midwife, three office assistants, one laboratory assistant, and three field assistants. Additionally, two nurse/midwives working at the Lungwena Training Health Center will work part time for the study. Apart from one of the health center nurses, all members of the team have several years of research experience from the proposed site.

The study nurse/midwife will act as a chief of staff and provide the participants with information about the study, counsel them about HIV before and after antibody testing, and perform ultrasound assessments. One office assistant will allocate the participants into treatment groups; coordinate study visits; collect, record, and store all source data; and distribute study drugs. The other office assistants and the field assistants will interview mothers, collect information on participants’ background and delivery events, and measure birth size and growth of babies. The health center nurse/midwives will be responsible for antenatal medical examinations.

3.7 Data management and analysis. 3.7.1 Data handling and record keeping. Written records (in the form of structured questionnaires) will be made from each data-producing contact between the study participants and the study team. These source data are identified (each sheet containing date, participant number, and signature of the data collector) and stored in individual participant folders.

Based on the source data, CRFs will be provided for each subject. All data on the CRF will be legibly recorded in ink. Any corrections will be made by striking through the incorrect entry with a single line and entering the correct information adjacent to it. The corrections will be initialed and dated by the investigator or a designated, qualified person. Any requested information that is not obtained as specified in the protocol or changes in medication will have an explanation noted on the CRF.

All CRFs will be filled out by an office assistant, then reviewed and signed by an investigator to indicate their correctness.

All documentation regarding the participants, including the laboratory samples, source data, and the CRFs, will be identified with appropriate participant codes on paper and in computer files. The names will only appear on informed consent forms and a separate coding list.

The investigators shall maintain the records of disposition of drug receipts and drug inventory logs and their copies of the CRFs and regulatory documents (informed consents, ethical approval) for 15 years after the end of the study. All records will be kept in a secure place. Clinical information will not be released without written permission of the study participant, except as necessary for monitoring.

No external data monitoring is pre-planned for the study. The quality assurance of LAIS is performed through the use of detailed standard operating procedures (SOPs) and weekly internal monitoring rounds. The investigators will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection only to those national or international authorities.
who have a legal right to monitor proper conduct of clinical trials.

3.7.2 Analysis strategy. The analysis will compare the preselected outcomes (chapter 3.6.2) in the intervention and control groups. All women and children will be analyzed in the group where they were initially randomized, i.e., on an intention to treat basis. For most outcomes, the analysis will compare group means or proportions at a single time-point and results are expressed as absolute differences and their 95% confidence intervals. The statistical significance of observed group differences is calculated by using the chi-square test (categorical variables), Student’s t-test (quantitative variables with normal distribution) or with a rank score test (quantitative variables with non-normal distribution). For secondary outcomes, Bonferroni corrections will be made before the interpretation of calculated P values. For single time-point analyses, persons lost to follow-up (with no outcome data) will be excluded.

Comparison of infant malnutrition and mortality in the three groups will also be performed by using a survival analysis, i.e., using a time-dependent outcome variable. All enrolled participants are included in these analyses.

3.7.3 Frequency of analyses. The randomization code is broken (but not given to research assistants measuring the growth of infants) after the last woman and child have attended the four-week follow-up visit, i.e., data on pregnancy and puerperal period are complete. The main analysis will be conducted at this point. The analysis of infant growth will be done a year later when all children have become one year of age.

Besides the above efficacy assessment, an interim analysis for safety and efficacy is planned to take place when 100 women have been enrolled to the trial and followed-up to the primary end point (i.e., delivery). Further number of analyses will be determined after that analysis. If there are no points of concern raised by that analysis, one additional analysis will be undertaken half way through the study enrollment.

The interim analyses will investigate numbers and types of adverse events in the three intervention groups without indicating the actual treatment given to each group. Efficacy analysis will be focused to the primary outcome (proportion of preterm births) and one secondary outcome (percentage of women with mild [hemoglobin < 110 g/L], moderate [hemoglobin < 80 g/L] or severe [hemoglobin < 50 g/L] anemia at four weeks after delivery). If group-level differences in the incidence of serious adverse events exist, the code is broken and members of the Review Board become aware of the treatment given to each group.

3.7.4 Subgroup analyses. Three subgroup assessments are planned in advance. These assessments include analyses stratified either by maternal HIV status (seropositive/seronegative/not known) or by maternal parity (primipara/secondipara/multipara). Additionally, a subgroup analysis is planned for those who were enrolled before 24 completed gestation weeks (because ultrasound assessment of gestational age is believed to be most reliable before this cut-off value).

3.7.5 Co-enrollment guidelines. Infants who become malnourished (WAZ < −2) are withdrawn from the follow-up and they may after six months of age be enrolled into intervention trials for malnutrition rehabilitation.

3.7.6 Coordination with other studies. The study involves collaboration with Dr. Irving Hoffman (University of North Carolina) and the University of North Carolina Project Laboratories, Lilongwe Central Hospital (Lilongwe, Malawi), Dr. Hoffman will be responsible for the C. trachomatis and N. gonorrhoea testing from swab and urine samples obtained as part of the proposed study. Additional collaboration involves Dr. Steven Meshnick (University of North Carolina) and Dr. Stephen Rogerson (University of Melbourne), who are coordinating a malaria research project elsewhere in Malawi (Blantyre). Drs. Meshnick and Rogerson will coordinate the analysis on malaria parasite resistance against sulfadoxine-pyrimethamine (using samples from Lungwena).

4. PRESENTATION, DISSEMINATION, AND USE OF THE RESULTS

The results will be distributed and discussed with the local community and representatives of the Ministry of Health and Population and College of Medicine, Malawi. Main findings will be published in international peer-reviewed journals and the Malawi Medical Journal.

The results can be used in the planning and development of antenatal care in Malawi and other countries in which malaria is endemic and there is a high frequency of preterm deliveries. These results may have policy implications for the management of pregnant women at Malawian health centers. The study material will also be used for postgraduate training of students in Malawi and Finland.

5. ETHICAL CONSIDERATIONS

5.1 Informed consent and compensation to participants. All potential participants will receive structured information about the study during their antenatal clinic visit (Appendix 1). Those interested in participation, will be invited to a private discussion with study personnel, during which the potential participants may ask questions about the study. Those persons wishing to participate will then sign an informed consent form, indicating the voluntary nature of the study and the participants’ right to discontinue follow-up at any point (Appendix 2).

The participants are not paid for enrollment or follow-up, but they will be compensated for their time with a bar of soap at enrollment, at the 32-week antenatal visit, and after delivery. They will also be reimbursed with 50 kwacha (1 US $) for informing the study team rapidly about the delivery when it has taken place. All visits and study medications will be free to the participants, and the study team will support nutritional status of the babies by giving them a package of likhuni phala (maize/soy flour) at 6 and 12 months of age. For the health center visits at 15, 18, 24, 30, 36, 48, and 60 months, the guardians are compensated with 1 kg of rice, 1 kg of sugar, and 1 bar of soap.

5.2 Possible risks to the safety of participants involved in the study. Both drugs used in the study are registered and they have proven safe for use in pregnancy under conditions similar to the intended trial (Parise and others, 1998; Shulman and others, 1999; Gray and others, 2001). Two doses of SP as a presumptive intermittent treatment of malaria has been the national standard in Malawi since 1993 (Government of Malawi, 2002), and no problems have been observed in three studies (total = 2,533 persons) reporting a more frequent dosing (Parise and others, 1998, Verhoeven and others, 1998, and others, 1999).
Shulman and others, 1999). Azithromycin is recommended by the Centers for Disease Control and Prevention as an alternative regimen for the treatment of several different STIs among pregnant women (Centers for Disease Control and Prevention, 2002).

Despite these experiences, there are theoretical risks related to the administration of the two drugs to pregnant women. Sulfadoxine-pyrimethamine and azithromycin (Zithromax®) have been associated with occasional adverse effects; sulfadoxine-pyrimethamine most commonly with blood dyscrasias and various allergic, gastrointestinal, central nervous system, and respiratory reactions (Roche Pharmaceuticals, 2002) and Zithromax® with mild gastrointestinal symptoms and, less commonly, cardiovascular, genitourinary, nervous system and allergic reactions have been reported (Pfizer Inc., 2002). Sulfadoxine-pyrimethamine can cause hemolysis in glucose-6-phosphate dehydrogenase–deficient persons and kernicterus in the baby if given to the mother at term or during the nursing period. Severe but very rarely reported reactions include Stevens-Johnson syndrome and toxic epidermal necrolysis (sulfadoxine-pyrimethamine) and angioedema, anaphylaxis, or severe skin reactions (Zithromax®). However, because of the rarity and nature of expected adverse effects, the potential benefits of participating in the study outweigh the potential harmful effects.

The mechanism of action of azithromycin is different from that of anti-folate antibiotics (sulfadoxine-pyrimethamine). In phase 2/3 drug interaction studies conducted by Pfizer, Inc., administration of trimethoprim/sulfamethoxazole DS (160 mg/800 mg), a similar combination as sulfadoxine-pyrimethamine, for seven days to healthy persons with co-administration of 1,200 mg of azithromycin on the seventh day had no significant effects on peak concentrations or total exposure or urinary excretion of trimethoprim or sulfamethoxazole. Serum concentrations of azithromycin following administration of a single 1,200 mg dose after administration of trimethoprim/sulfamethoxazole DS for 7 days were similar to those produced following a 1,200 mg dose of azithromycin in other studies (Pfizer Inc., 2002). Therefore, synergistic toxicity or drug interaction are not likely to follow co-administration of azithromycin and sulfadoxine-pyrimethamine.

To avoid any teratogenic effects and kernicterus of the newborn, no study drugs are given before 14 or after 37 completed gestation weeks.

Any adverse reactions arising during the study are treated within the national health system, i.e., there is no special health insurance for the study participants. However, if needed, the study team will provide assistance in transportation to appropriate national health care facilities.

All adverse effects are documented according to standard operating procedures. Serious adverse effects are immediately reported to the principal investigators, who will notify the external reviewers about them. Persons experiencing such reactions are withdrawn from the study, and the external reviewers and principal investigators will jointly decide about the continuation of enrollment and follow-up for others.

5.3 Review board. Three external reviewers (Dr. George Kafulafula, Department of Obstetrics and Gynaecology, College of Medicine, Blantyre, Malawi; Dr. Sarah A White, Malawi-Liverpool-Wellcome Trust Clinical Research Program, Blantyre, Malawi; and Professor Elizabeth Molyneux, Department of Pediatrics, College of Medicine, Blantyre, Malawi) will oversee the progress of the study and assess the safety of the intervention (in interim analyses and in case of any serious adverse effects). All reviewers have extensive experience on malaria research in Malawi. The review board meetings may also be attended as observers by a representative of the research team and a representative of the pharmaceutical company manufacturing and donating azithromycin (Pfizer Inc.).

5.4 Study discontinuation criteria. 5.4.1 Discontinuation of individual participants. The participant can decide to discontinue the study at any point without giving a reason for her decision and without this having any negative impact on her continued medical care. To control for a potential bias caused by losses of follow-up, information on the duration of pregnancy at birth, birth weight, and infant size at six months of age shall also be sought (with permission) from those participants who otherwise discontinue follow-up.

Persons experiencing serious adverse effects, becoming seriously ill, meeting the criteria for infant malnutrition (WAZ < –2), or severely violating the protocol are withdrawn from the study by the investigator (but included in the analysis). In such occasions, the reason for withdrawal will be given and specified in the CRFs. Information on the duration of pregnancy at birth, birth weight, and infant size at six months of age shall also be sought (with permission) from these participants.

5.4.2 Discontinuation of the study. The principal investigator and/or the review board have the right to terminate the study if the incidence and/or severity of adverse events outweigh the benefits of the study.

5.5 Potential public health consequences of the study. 5.5.1 Cost of the intervention. Azithromycin is a rather expensive drug, which limits its current national use. However, the manufacturer of this drug, Pfizer Inc, has experience from special accessibility programs in low-income countries for selected indications (trachoma). Similar arrangements might be possible if the current intervention proves successful. This possibility, together with international activities, such as the development of the Global Fund, are believed to make the drug accessible to women in Malawi, if it proves medically indicated.

If the new antenatal intervention is beneficial compared with the standard antenatal care (as shown by health benefits without adverse effects to the mother or infant), and the regional health authorities wish to change the antimicrobial policy accordingly, the local population will be guaranteed free access to the same intervention for three years. To ensure this access, the manufacturer of azithromycin (Pfizer Inc.) will provide Lungwena Health Center with 7,500 doses (1 g each) of free Zithromax® (1,000–1,500 pregnancies/year, 2 doses/ pregnancy, 3 years). Free sulfadoxine-pyrimethamine will continue to be provided by the Malawian Ministry of Health and Population, but the study team is committed to assist it in temporary provision problems during the three years after the trial.

5.5.2 Potential induction of antimicrobial resistance. One further public health problem needs to be addressed if the intervention with azithromycin proves efficient on an individual level. This problem is the potential for development of macrolide (especially erythromycin)—resistant Streptococcus pneumoniae strains in azithromycin-treated women and the spread of these strains to children, whose respiratory infections are often treated with erythromycin in Malawi. Although
azithromycin therapy in children has been associated with detection of antimicrobial resistance in treated children (Leach and others, 1997), changes in microbial flora are minimal and antibiotic pressure does not seem to significantly increase population level prevalence of drug-resistant strains, even in conditions where azithromycin is widely used for a population-based control of endemic trachoma (Fry and others, 2002; Matute and others, 2002; Knirsh C, unpublished data). Furthermore, the spread of antibiotic-resistant bacterial strains from mothers to their children is unlikely unless the therapy is given to children. Finally, the respiratory carriage rate of *S. pneumoniae* among adults in Malawi is only 10% (Molyneux M, unpublished data), which further decreases the possibility of mother-to-child transmission of macrolide-resistant bacteria, even if all pregnant women received presumptive treatment of STIs with azithromycin.

### 6. POSSIBLE CONSTRAINTS

Possible problems with enrollment include hesitation over blood specimens and HIV testing. However, based on our earlier findings, neither factor is likely to produce a significant threat to enrollment, especially because the enrollment rate is already estimated to be much lower than in earlier studies from the same area. Compliance with study protocol may be affected by the high number of individual drug tablets the participants have to take at some antenatal clinic visits (maximum = 5 tablets) because of which they are taken under direct observation.

Heavy rains during January–March may prevent the participants from attending the antenatal clinic on the exact dates agreed, but the delays are expected to be not significant, and the research team can provide transportation in critical situations. In case of a serious famine, migration to Mozambique will become more common and loss to follow-up can double from the expected 10%.

Nevirapine tablets are given to HIV-infected persons during pregnancy by the study nurse offering the post-test counseling. The women are advised to take the tablet during delivery. To avoid stigmatization by HIV status, those women who are not infected with HIV will be given a placebo tablet (containing vitamin C). All deliveries are noted by traditional birth attendants to the study office within 48 hours, after which the study nurse allocates and sends (with research assistants) nevirapine suspension to the newborns of HIV-infected mothers and identical looking placebo suspension to the babies of HIV-negative women.

### 7. TRAINING PROVIDED

The project involves two post-graduate students, one from Malawi and one from Finland, who will work for a PhD degree at the University of Tampere (Finland). Both of these students have a two-year personal stipend included in the proposed budget, and additional funding will be sought during the study (estimated total duration of PhD studies is four years). The Malawan PhD student will analyze the economic feasibility of the planned intervention, which would strengthen the health economics knowledge base at the COM Department of Community Health.

Through the study, all researchers and research assistants will gain experience in running a randomized trial according to Good Clinical Practice. The health center nurses will learn to do gestational ultrasounds and the laboratory assistant will practice measuring haemoglobin levels of pregnant women.

Within the framework of the study, opportunities exist for COM medical students or MPH projects on KAP to malaria or STIs; evaluation of uptake and use of bed nets in the area; and contact tracing for patients with STIs among other topics. Such projects would be the subject of separate College of Medicine Research and Ethics Committee applications.

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### 8. REFERENCES


9. APPENDICES

APPENDIX 1. Participant information form.

APPENDIX 2. Identification and informed consent form.

APPENDIX 3. Socioeconomic background form.

APPENDIX 4. Mother’s health history form.

APPENDIX 5. First visit antenatal examination form.

APPENDIX 6. Ultrasound examination form.

APPENDIX 7. Laboratory measurements form.

APPENDIX 8. Inclusion criteria and randomization form.

APPENDIX 9. Treatments form.

APPENDIX 10. Follow-up antenatal examination form.

APPENDIX 11. Delivery information-mother form.

APPENDIX 12. Delivery information-attendant form.


APPENDIX 15. Child follow-up form.

APPENDIX 16. Adverse event report form.

APPENDIX 17. Non-scheduled visit information form.

APPENDIX 18. Participant experience questionnaire.

APPENDIX 19. Linear growth velocity chart.