Malaria and HIV in pregnancy, and effects on the infant in western Kenya
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Introduction
1) Background

Diseases due to malaria and HIV-1 (HIV) infection have in common that they both pose a burden to millions of individuals, and each year cause millions of deaths. The World Health Organization (WHO) estimated that malaria caused 300-500 million cases worldwide and 1.5-2.7 million deaths in 1997. Over 90% of cases and deaths were estimated to occur in sub-Saharan Africa, in particular among children < 5 years of age (WHO 1998). By the end of 1999, an estimated 34.3 million people were living with HIV/AIDS, of whom 15.7 million were women and 1.3 million were children; 70% of the cases were in Sub-Saharan Africa (UNAIDS 2000). In Kenya, over 4 million cases of malaria were reported in 1995 (WHO 1998), and an estimated 2.1 million persons were living with HIV/AIDS by the end of 1999, of whom over half were estimated to be women (UNAIDS 2000).

There are some similarities between both diseases: Although in sub-Saharan Africa, the primary transmission routes are sexual contact for HIV and vector-borne for malaria, both can also be transmitted by blood transfusions and the infection rates can be reduced by behavior change and decreased exposure through use of barriers (condoms for HIV and insecticide treated nets for malaria) (Huff 2001). Both diseases are characterized by an immune response in which antibody production does not necessarily confer protection, and cell mediated immune responses (in particular Th1 cell responses) may constitute the most important part of immunity (Mossman 1994; Kuhn et al, 2001; Clerici et al, 1996; Cruz Cubas et al, 1994). An increased risk with lower socioeconomic status has been established for both diseases and both pose their largest burden in sub-Saharan Africa. In forty percent of the 39 sub-Saharan countries where malaria is endemic an antenatal clinic HIV seroprevalence of more than 10% has been reported. There is also some overlap in therapeutic arsenal; some drugs used for treatment of opportunistic infections in HIV have antimalarial activity and some drugs used for malaria have, at least in vitro, reported effects on HIV replication.

Although there are some similarities, the dissimilarities are pronounced. Malaria, a mosquito-borne protozoan disease, is caused by four members of the genus Plasmodium, of which P. falciparum is the most dangerous and the most common in sub-Saharan Africa. It has accompanied humans throughout recorded history (White 1996). The most vulnerable group in areas with high transmission of malaria are children under 5 and pregnant women. There are a variety of clinical manifestations of malaria, depending on the transmission intensity in the area, the age and immunological experience with malaria of the individual, and genetic factors. The most common symptoms are fever and anemia, and the course of disease can be rapid, resulting in death within a few days, or more protracted, resulting in chronic anemia. Although malaria parasites can harbor in the placentae of pregnant women, congenital malaria in newborns is not common. Most episodes of malaria, if treated promptly, can be cured, but drug resistance is a major problem. Affordable drugs, such as chloroquine and sulfadoxine-pyrimethamine (SP) have traditionally been the mainstay of therapy in sub-Saharan Africa, but as parasite resistance to these drugs becomes increasingly widespread, they will need to be replaced by more expensive drugs, most likely to be delivered in combination therapy.

HIV-1 (HIV) is a retrovirus; the predominant mode of transmission in developing countries is through heterosexual contact. The disease process is generally slow, resulting in progressive immune deficiency, and resultant opportunistic infections. The earliest reports of AIDS, the clinical end-stage of HIV infection, date from the late seventies, with the first
description in the United States in 1981 (Gottlieb et al., 1981). Pregnant women can transmit the virus to their children during pregnancy, labor, or breastfeeding. Although vertical transmission is the most common route for children to become infected, treatment of malaria-related anemia with blood transfusion can be an important infection route in areas where no reliable blood supply is available (Greenberg et al., 1988). Opportunistic infections can be prevented and treated and antiretroviral drugs are available to slow down disease progression. However, these drugs can have considerable side effects, and are expensive for most persons living in countries in sub-Saharan Africa.

Pregnancy

The normal course of pregnancy has been associated with a variety of changes in immunity, as a presumed adaptation of the host to the foreign genetic material of the fetus. An increased risk of acquisition and severity of several diseases during pregnancy has been reported, in particular for conditions in which cell-mediated immunity plays an important defense role, such as polio, influenza A, herpes genitalis, Epstein Barr virus, variola, cytomegalovirus, toxoplasmosis, measles, and malaria (Weinberg 1984). However, it is observed that pregnant women maintain essentially normal levels of humoral immunity, and gestation has been associated with depression of selective rather than general aspects of cellular immunity. Specific alterations in T cell subpopulations have been documented. However, as Miotti et al. (1992) and Landers et al (1997) pointed out, results of studies conducted in industrialized and developing countries to investigate T cell phenotype changes during pregnancies differed widely.

T-cells mediate many of their effects via secretions of cytokines, and several patterns of responses to infection have been recognized; T helper cells 1 (Th1) cells uniquely secrete interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor-beta (TNF-β), and are helper cells for cell-mediated immunity. Th2 cells uniquely secrete interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-10 (IL-10) and are associated with antibody and allergic responses. It has been postulated that the maternal immune system during pregnancy preferentially mounts Th2-biased responses, resulting in an increased susceptibility to certain autoimmune diseases and intracellular infections (Wegmann et al., 1993).

HIV and pregnancy

Several studies suggest that CD4 cell function and phenotype are altered in pregnancy, and return to baseline postpartum in HIV-uninfected women but not in HIV-infected women (Landers et al., 1997; Rich et al., 1995). The adverse pregnancy outcomes associated with maternal HIV infections have been delineated in systematic reviews and meta-analyses by Brocklehurst & French (1998); maternal HIV-infection was associated with spontaneous abortion, stillbirths, perinatal mortality, infant mortality, intra-uterine growth retardation, low birth weight and preterm delivery. HIV progression during pregnancy was more common in developing countries (OR 3.71, 95% CI 1.82-7.75) than in developed countries (OR 0.55, 95% CI 0.27-1.11) (French & Brocklehurst 1998). A higher mortality among breastfeeding HIV-infected mothers compared to HIV-infected mothers who were not breastfeeding has been reported (Nduati et al., 2001). Although HIV-infected pregnant women may experience more pregnancy complications, including intrapartum infection, postpartum hemorrhage and postpartum endometritis, the effect may apply primarily to individuals who are severely immunocompromised (Landers et al., 1997; Rich et al., 1995). Anemia in pregnancy was common in sub-Saharan Africa before the introduction of HIV, but HIV infection adds yet another cause
to the long list of etiological factors (Fleming 1989; Van Den Broek et al, 1998).

**Malaria and pregnancy**

The epidemiology of malaria in pregnancy is intriguing. The already discussed immunomodulation observed during pregnancy induces an increase in the frequency and density of malaria parasitemia in pregnant compared to non-pregnant women in endemic areas. This is more prominent during the second trimester compared to the third trimester, more common in primigravidae than in women with higher gravidity number, and in younger women compared to older pregnant women (Brabin 1983; Menendez 1995). However, severe malaria-associated disease in pregnancy is mainly reported from areas with low to moderate transmission; in hyper- and holo-endemic areas most infections remain asymptomatic, undetected, and untreated, with the major manifestations being maternal anemia and low birth weight in the newborn, which is a major risk factor for subsequent infant mortality (Mc Cormick 1985). McGregor (1984) postulated that the malaria parasite may find a temporary place to sequester in the placenta, an organ that could be considered malaria-naive. During the pregnancy, organ specific immunity can build up and may explain the lower frequency of malaria at the end of pregnancy, and in each subsequent pregnancy. An immunological mechanism for this was recently proposed (Moore et al, 2000a). Recent studies showed that women up to their fourth pregnancy had an increased risk on malaria compared to gravidae 5 or more, and that this also translated into a higher risk for low birth weight, in particular in combination with severe anemia (Shulman et al, 2001). In an urban area in Malawi, young age was a more important predictor of malaria than gravidity (Rogerson et al, 2000).

In the Gambia, the changed cellular response in pregnancy was demonstrated in pregnant women, who had depressed lymphoproliferative responses to *P. falciparum* antigens compared to parity matched non-pregnant women; this was most marked in primigravidae (Riley et al, 1989). Fried et al reported that placental blood samples (obtained by compressing fresh tissue in a tissue grinder from a non-endemic area in Kenya) predominantly showed a bias towards type 2 cytokines, whereas samples from malaria-endemic areas showed predominantly a type 1 response (Fried et al, 1998). In this study, elevated IFN-γ and TNF-α levels were associated with a low birth weight. Moore et al (1999) postulated that IFN-γ is important in the control of placental malaria, because production of this cytokine by placental inter villous blood mononuclear cells, obtained by a placental perfusion technique, was elevated in multigravidae without placental malaria compared with primigravidae and multigravidae with placental malaria. The presence of antibodies to the ligand chondroitin sulphate A (CSA), a molecule present in the placenta, correlated with the absence of, or low placental parasitemias, and therefore CSA may be a molecular site for the sequestration of parasites in the placenta. This may indicate that parasites found sequestered in the placentas of pregnant women may differ from parasites derived from nonpregnant hosts by their ability to cytoadherence to CSA (Maubert et al, 2000; Ricke et al, 2000; Duffy & Fried 1999).

2)Interaction of HIV and malaria: immunologic, clinical and epidemiological studies

**Overview**

There is a potential for interaction between malaria and HIV infection. Malaria is a powerful stimulator of the immune system during the acute phase of the illness and repeated attacks could theoretically lead to increased HIV viral load and an increased rate of progression
to AIDS (Chandramohan & Greenwood 1998). HIV infection might reduce immunity to malaria resulting in more frequent and severe malaria infections. It is unclear how the depletion of CD4 cells in HIV-infected persons affects the ability of the host to develop an effective immune response against malaria, and how immunity to malaria in children could develop effectively without CD4 cell involvement. However, early studies that examined how these two infections may affect each other did not find evidence for substantial interaction. Possible mechanisms suggested to explain this initial apparent absence of interaction between HIV and malaria included: the selective depletion of T cell subsets not implicated in malaria response (Migot et al., 1996), the T cell independent non-specific parasite killing by macrophages (Butcher 1992; Marussig et al., 1996), the development of compensatory mechanisms for the loss of subsets of lymphocytes, like monocytes or CD8 cells (Migot et al., 1996), or the role of HIV in increasing effector cell activation by increasing TNF levels, a cytokine generated by both malaria and HIV (Butcher 1992).

Immunologic studies

An interaction between malaria and HIV is evident in animal models or in-vitro studies: however, direction of the interaction and effect are not consistent. In an overview of observations in a rodent model, the murine acquired deficiency syndrome (MAIDS) affected T cell memory of Plasmodium but did not alter parasite killing by macrophages (Marussig et al., 1996). Viral infection protected against death from cerebral malaria in this model, and this effect increased with the severity of immunodeficiency. The effect of malaria infection on viral infection was opposite to what was expected: certain manifestations of MAIDS were reversed. Repetitive infections with parasites from the beginning of the viral infection slowed down the development of some of the MAIDS clinical and immunological manifestations. The viral load was unaffected, indicating that the effects of plasmodial infection on the development of the viral disease was not due to control of virus replication but more to interference with the immunopathogenesis of MAIDS (Marussig et al., 1996).

This is in contrast with results of Freitag et al (2001), where malaria increased viral replication transiently in a rodent model; the viral replication returned to baseline after 15 days, and reinfection and recrudescence of infection were not accompanied by increased viral replication. Neutralizing or blocking antibodies to TNF-α, IFN-γ and other cytokines thought to be stimulated by malaria infection, did not prevent the induction of increased HIV-expression (Freitag et al., 2001). In an in-vitro study, the presence of P. falciparum antigens significantly enhanced HIV replication by activating lymphocytes through the production of TNF-α, suggesting that P. falciparum modulates HIV pathogenesis (Xiao et al., 1998). This is supported by results of in-vivo studies of Hoffman et al (1999) who showed that HIV-1 proviral loads were approximately sevenfold higher in Malawian adult patients with malaria than in asymptomatic asexual HIV-infected blood donors; although the HIV-RNA concentrations decreased after treatment, they remained higher for at least 4 weeks in some participants. In this study, CD4 percentage and baseline RNA-levels were positive independent predictors of a reduction of RNA concentrations after antimalarial treatment, and not age, sex, malaria parasite density or plasma TNF-α concentration (Hoffman et al., 1999). The authors suggested that P. falciparum malaria may cause increased CD4 cell activation, and increase thereby the number of susceptible target cells for HIV-1 infection, potentially resulting in increased HIV-1 RNA levels and continued new infection of susceptible activated CD4 cells. However, it is difficult to assess if this hypothesis was the case or if, when selecting HIV-positive patients with malaria, HIV-positive...
persons with a more advanced HIV disease were selected (see further on). CD4 counts in patients were significantly lower than CD4 counts in asymptomatic blood donors.

The effect of HIV on the immune response to malaria has been examined in two studies. In a cross-sectional study in Uganda, AIDS patients (defined clinically) had significantly lower antibody levels to synthetic falciparum ring stage antigens (RESA 8) and other ring stages compared to HIV-uninfected patients (Wabwire-Mangen et al, 1989). Asymptomatic HIV-infected trauma patients had increased levels in comparison to HIV-uninfected trauma patients (controls) although this difference was not significant. The authors suggested that during HIV-1 infection, B cell activation may occur as noted in the HIV-1 seropositive trauma patients, but with increased immunosuppression in advanced clinical AIDS, B cell stimulation may be diminished, resulting in decreased production of malaria antibody (Wabwire-Mangen et al, 1989). In Burkina Faso, adult AIDS patients with a CD4 counts < 250 cells/μl, had higher total plasma IgG than did healthy subjects, indicating B cell polyclonal activation, but the mean levels of antibodies to P. falciparum were decreased in AIDS patients, suggesting that the B cell activation was selective (Migot et al, 1996). Compared with cells of healthy subjects, peripheral blood mononuclear cells of AIDS patients showed decreased levels of proliferation and of IFN-γ and IL-2 production in response to all malarial antigens except schizont extract, and IL-4 production was similar in both groups. Immune responses obtained after stimulation of whole blood by mitogens showed the same results. Thus, although some components of the specific human immune responses to falciparum parasites are modified during AIDS, other mechanisms of protection against malaria may be preserved.

To date there is limited information on the effect of the combination of malaria and HIV in pregnant women and the placenta. Placental transfer of specific IgG antibodies to Streptococcus Pneumonia was reduced in the presence of HIV infection or placental malaria in a Malawian study (De Moraes-Pinto et al, 1998), potentially increasing the risk of pneumococcal infection in early infancy. In a study of placental malaria in Kenya, cytokine production by intervillous blood mononuclear cells obtained by placental perfusion was reduced in placentas from HIV-infected women with a low CD4 count (< 500/μl), particularly in response to malaria antigens (Moore et al, 2000b). Similar to what has been described for peripheral blood mononuclear cells in adults, intervillous blood mononuclear cells of HIV-positive asymptomatic women showed impaired IFN-γ responses to malarial antigens. TNF-α was generally enhanced in both HIV-positive women and women with placental malaria (Moore et al, 2000b). IL-4 and IL-10 responses to malarial antigen were reduced in HIV-positive women; IL-10 was particularly reduced when placental malaria had been present. An increased IL-10 production has been associated with HIV-infection as part of cytokine-induced immune dysregulation (Clerici et al, 1994), and this may suggest that malaria disrupts this dysregulation.

Clinical and epidemiological studies in children

Several hospital based retrospective and prospective cross-sectional and autopsy studies showed no association between HIV infection and malaria in children (Muller & Moser 1990; Muller et al, 1990; Nguyen-Dinh et al, 1987; Lucas et al, 1996; Bell et al, 1997).

Four hospital based cohort studies addressed the potential interaction between HIV and malaria in children. In a study in Zaire among children infected with HIV through vertical transmission, malaria blood smears were obtained whenever the child had documented fever or a history of fever in the past two days; a slight but significantly higher prevalence of peripheral parasitemia and fever was seen in HIV-positive children who had progressed to AIDS compared
to HIV-negative children of HIV-positive mothers, and HIV-negative children of HIV-negative mothers (Greenberg et al, 1991). In children with AIDS, the geometric mean parasite density was higher, compared to the other groups, but this was of borderline significance. Malaria did not contribute significantly to deaths among HIV-positive children and did not appear to accelerate the rate of progression to AIDS in HIV-positive children; to the contrary, there was a suggestion that malaria was more likely to be protective (OR 0.6, 95% CI 0.2-2.2).

In a cohort of transfused children in Kinshasa, Zaire, those who had been infected with HIV through blood transfusions presented more often with episodes of fever and malaria positive thick blood smears per person month observation than HIV-negative patients ($P = 0.04$ and $P = 0.003$, respectively, Colebunders et al, 1990). However, percentages of positive thick films per episode of fever were the same in both groups (46%).

In a birth cohort in Malawi, blood smears were obtained every three months; no significant difference was found in frequency of malaria parasitemia by maternal or infant HIV sero-status after controlling for child's age; unscheduled visits also showed no difference after controlling for malaria treatment (Taha et al, 1994). In this last study, no information was given on clinical malaria and HIV-disease progression, and data was not presented by person month of observation.

In a prospective birth cohort in Kampala, Uganda, malaria blood smears were obtained when a child had documented fever or a history of fever in the past two days during scheduled (every three months) and unscheduled visits; both types of visits were combined in the analysis (Kalyesubula et al, 1997). There was an increase in smears positive for malaria parasitemia among seroreverters (risk ratio, 1.5; 95% CI 1.1 to 1.9) and control infants (risk ratio, 1.6; 95% CI 1.2 to 2.2) compared with HIV-positive infants. Even in older age groups, the risk of malaria was reduced in HIV-infected children compared to seroreverters and control infants, but the levels of parasitemia were similar. A greater proportion of malaria episodes among the HIV-positive group than among the control groups resulted in hospitalizations ($P = 0.001$) and blood transfusions ($P = 0.02$), suggesting that HIV-infected children may be more susceptible to the effects of malaria (Kalyesubula et al, 1997), or that they may be more ill due to the concomitant presence of other infections. However, because a positive association was seen between time to clinical AIDS and absence of malaria in HIV-infected children, the authors suggested that either malaria may offer some protection against HIV progression, or that chloroquine used to treat malaria may have a direct effect against HIV.

Clinical and epidemiological studies in adults

In cross-sectional studies in Zambia, Uganda, Rwanda and Malawi no association was found between HIV infection and malaria (Simooya et al, 1988; Simooya et al, 1991; Muller & Moser 1990; Allen et al, 1991; Nwanyanwu et al, 1997). Among male adults in Malawi, an association between HIV and fever, and not malaria, was reported; the authors suggested that this may lead to an overestimation of fever as presumably caused by malaria in areas with a high prevalence of both HIV and malaria (Nwanyanwu et al, 1997). In a cross-sectional study in rural Tanzania, malaria infection was more common in HIV-positive than in HIV-negative persons, but no association was seen with parasite densities (Atzori et al, 1993). In a study in Burundi of risk factors for poor prognosis in cerebral malaria in 31 adults, HIV infection did not affect the clinical or biological presentation of cerebral malaria, and did not appear to influence the outcome (Niyongabo et al, 1994). No difference in the prevalence of HIV was noted among patients admitted with cerebral malaria compared to the prevalence of HIV among ICU patients.
in Zambia (Leaver et al, 1990).

A cross-sectional study in Malawi enrolled 161 febrile adult patients and 18 healthy aperasitemic controls, and compared HIV viral RNA levels among HIV-positive participants (n=129) stratified by presence of malaria and positivity of a blood culture (Jason et al, 2001). HIV viral RNA levels were significantly lower among HIV-positive controls compared to HIV-positive parasitemic patients with a negative blood culture, aperasitemic patients with a negative blood culture and aperasitemic patients with a positive blood culture; between the last three groups no significant differences in HIV RNA-levels were observed. HIV-positive patients with parasitemia had less severe HIV immunosuppression, as represented by CD4 percentage, compared to aperasitemic HIV-positive febrile persons with or without a positive blood culture (Jason et al, 2001). In this study no association was seen between HIV serostatus, severity of HIV infection (as measured by HIV RNA viral load level and CD4 counts) and degree of parasitemia.

To date, the most comprehensive study is an 8-year prospective cohort study from Ugandan adults (Whitworth et al, 2000). In this study, parasitemia was more common in HIV-positive individuals compared to HIV-negative persons. In addition, clinical malaria was more common as well in HIV-positive individuals and the frequency of clinical malaria increased with increasing immunosuppression as measured by CD4 counts (Whitworth et al, 2000). Increasing incidence rates of fever in association with P. falciparum infection and decreasing CD4 cell counts were also reported among HIV-infected adults in another Ugandan cohort study with 14 months of follow up (French et al, 2001). In this study, parasite densities in asymptomatic malarial infections were significant lower across all CD4 count levels compared to parasite densities in symptomatic infection; the rates of recurrent disease were high irrespective of CD4 counts. Despite this increased susceptibility, there was no apparent effect on the severity of malarial fever and no cases of severe and complicated malaria were observed in this study. However, an HIV-negative control group was not included.

In a case-control study in Uganda, co-infection in adults with HIV and malaria parasites was associated with an increased occurrence of acute febrile episodes compared to those with single infection with HIV or malaria (Francesconi et al, 2001). This may indicate that the “anti-disease” immunity for malaria may be different in HIV-infected persons. In this last study, clinical malaria was associated with HIV infection as well.

Pregnant women

In three cohort studies, HIV-positive pregnant women had a higher prevalence of parasitemia at enrollment, delivery and in the placenta than HIV-negative women (Stekete et al, 1996; Paris et al, 1998; Verhoeff et al, 1999). When stratified by gravidity, this was only significant for HIV-positive multigravidae in the studies in Malawi; however, parasite densities were higher in HIV-positive women across all gravidities (Stekete et al, 1996). In the study of Paris et al (1998) in Kenya, women were enrolled in their first and second pregnancy only; a significantly higher proportion of HIV-positive pregnant women had peripheral parasitemia in the second trimester and placental parasitemia at delivery (Paris et al, 1998). One could speculate that the immune-modulation during pregnancy resulting in an increased frequency of parasitemia is augmented by HIV infection, but whereas HIV-negative women can develop an immune response important for malaria control during the last trimester or in the next pregnancy, this response may be diminished in HIV-positive women.

Conflicting reports exist on the importance of placental malaria for the vertical
transmission of HIV. In a large cohort study in Malawi, infants born to HIV-1-infected women with placental malaria infection had a 3.4-fold increased risk of post-neonatal death compared to infants born to HIV-infected women without placental malaria infection (Bloland et al, 1995). The levels of immunosuppression in the HIV-infected mothers and HIV infection status of infants were not determined (Bloland et al, 1995). In a large community based cohort in The Gambia, birth in the rainy season, when generally higher transmission of malaria occurs, was a risk factor for vertical transmission of HIV-1 infection (O'Donovan et al, 1999). However, placental malaria was not a risk factor for perinatal HIV transmission in a hospital based cohort in Kinshasa, Zaire (St Louis et al, 1993).

HIV-infection and antimalarial treatment

There are some major concerns on efficacy and toxicity of antimalarial treatment in HIV-positive persons, the effect of antimalarial treatment on progress of HIV infection, and of the effect of prophylaxis against opportunist infections for HIV-positive individuals in Africa on malaria treatment.

I: Toxicity

Side-effects to sulfonamides, pyrimethamine and dapsone are reported to be more frequent in HIV-infected persons (Bayard et al, 1992). However, in two studies in pregnant women where presumptive antimalarial treatment with SP was given, there was no evidence of an enhanced risk of side-effects after treatment with SP (Parise et al, 1998; Verhoeff et al, 1998). It is likely that the use of SP will increase after introduction of presumptive treatment of SP as national policy for malaria in pregnancy. Continued surveillance is needed.

II: Efficacy

In two studies, one in children infected perinatally with HIV, and the other in children and adults infected with HIV through blood transfusion, antimalarial treatment with oral quinine showed no significant difference in the proportion of treatment failures (defines as parasitemia on day 7 following treatment) by HIV status (Colebunders et al, 1990; Greenberg et al, 1991). Moreover, no difference by HIV-infection status in response to treatment with chloroquine or SP for children or adults was reported in a retrospective hospital based study in Uganda (Muller & Moser 1990). In a Ugandan study, HIV-positive children under 5 years of age were perhaps more likely to fail chloroquine treatment than HIV-negative children in the same age-group (Fisher's exact test \( P = 0.096 \); no difference was seen in the subjects 5 years and older. However, the numbers of HIV-positive children were small, and this observation needs confirmation in larger studies (Kamya et al, 2001).

In a study in 27 HIV-positive Malawian adults, followed for 4 weeks after SP treatment for symptomatic malaria with SP, all patients were aparasitemic and afebrile after one week, and all but one remained aparasitemic for the remainder of the study (Hoffman et al, 1999).

The previously cited study by Parise et al (1998) is the first to suggests a difference in efficacy of treatment for malaria in HIV-positive compared to HIV-negative pregnant women. Although SP was efficacious in clearing peripheral parasitemia in both HIV-infected and uninfected women, the two dose regimen of presumptive treatment with SP (once in the second and once early in the third trimester) was not as efficacious in clearing placental malaria infection in HIV-positive compared to HIV-negative women; a monthly dosing interval increased the efficacy in HIV-positive women. The study was conducted among women in their
first and second pregnancy who are more prone to asymptomatic malaria; the results need to be confirmed in an other study.

Trimethoprim-sulfamethoxazole is recommended by UNAIDS for prophylaxis in Africa against opportunistic infections for HIV-infected adults with CD4 counts < 500/μl. This recommendation was based on two studies in Abidjan, Cote d'Ivoire, which showed that daily prophylaxis with trimethoprim-sulfamethoxazole decreased morbidity in HIV-positive individuals (with stage 2 and 3 AIDS) and mortality in HIV-infected individuals with tuberculosis (Anglaret et al, 1999; Wiktor et al, 1999). For *P. falciparum*, cross-resistance between sulfonamides is known (Triglia et al, 1997) and cross-resistance between trimethoprim and pyrimethamine has been described (Petersen 1987; Iyer et al, 2001). Iyer et al (2001) expressed concern that the daily prolonged use of trimethoprim-sulfamethoxazole prophylaxis “may shorten the useful life of sulfadoxine-pyrimethamine where it is already in use, introduce sulfadoxine-pyrimethamine resistance where these drugs are currently held in reservoir; or introduce resistance to other antifolate antimalarials under development”. In malaria endemic areas, HIV-infected persons using trimethoprim-sulfamethoxazole prophylaxis and who suffer *P. falciparum* infection may increase the prevalence of antifolate mutations in the *P. falciparum* population, thus increasing the prevalence of SP-resistant malaria. In countries, like Kenya, where the chloroquine resistance is high and the first line treatment is SP, treatment options for malaria in HIV-positive persons on cotrimoxazole prophylaxis or the general population are limited, and expensive.

SP, azithromycin and atovaquone are all drugs that are used in the therapeutic arsenal for both diseases, and effects of the drugs on both diseases and resistance patterns may need to be considered before using them.

III: Effect of antimalarial medication on progression of HIV

Due to the increasing resistance of *P. falciparum* to chloroquine, the importance of this antimalarial for treatment of malaria in sub-Saharan Africa is declining; however, recent studies have suggested that chloroquine has interesting antiretroviral qualities.

The effects of chloroquine on HIV-infection are not consistent; reports are conflicting as to whether chloroquine can inhibit or enhance viral replication. In one murine study, replication of viruses (Semliki forest Virus and encephalomyocadritis virus) was enhanced by antimalarials such as chloroquine, primaquine, pyrimethamine, sulfadoxine and quinine (Maheshwari et al, 1991). In vitro experiments demonstrated that chloroquine enhanced cellular uptake of Tat protein, a promoter of HIV replication in activated T cells (Frankel & Pabo 1988).

On the other hand, chloroquine inhibited HIV-1 and HIV-2 replication in HIV-infected cells exposed to chloroquine concentrations in-vitro, and it decreased the infectivity of the newly produced virus (Savarino et al, 2001). An in vivo study comparing hydroxychloroquine (800 mg/day) to zidovudine (500 mg/day) for 16 weeks in asymptomatic HIV-1 infected patients with CD4 counts between 200 and 500 cells/mm³ showed reduced levels of HIV-1 RNA load in plasma in both groups. There was no difference in CD4 count at the end of the study between the two groups (Sperber et al, 1997). Boelaert et al reported that chloroquine can accumulate in breast-milk cells, and suggested this may have a potential role on HIV-transmission through breastfeeding (Boelaert et al, 2001a). Other benefits of chloroquine may include an inhibitory effect on opportunistic infections like Cryptococcus neoformans (Levitz et al, 1997), an inhibitory effect on the synthesis of several pro-inflammatory cytokines that may play a role in the progression of HIV infection, and a potential to restrict tissue iron accumulation that may
possibly affect HIV in a negative way (Boelaert et al, 2001b). Chloroquine is an attractive drug because it is inexpensive, not stigmatizing, and widely distributed in developing countries with malaria. The principal mechanism of HIV-1 inhibition by chloroquine seems to be an effect on gp120 on a post-transcriptional level (Tsai et al, 1990; Savarin et al, 2001). Because this is a different mechanism than known from other anti-retrovirals, it has been proposed as a potential useful drug in combination therapy (Boelaert et al, 2001b; Savarin et al, 2001).

Several countries with a high prevalence of HIV-infection, like Kenya and Malawi, have changed to the use of SP as first line treatment for malaria. Although no report of an effect of SP on HIV-replication could be identified, in vitro studies suggest that pyrimethamine in concentrations corresponding to clinical practice suppresses the stimulation of human blood mononuclear cells (Bygbjerg et al, 1986). SP has been successfully used for the prevention of opportunistic infections in HIV, in particular infections with *Pneumocystis carinii* and toxoplasmosis, and may be more likely to decrease viral load indirectly through its effect on opportunistic infections.

For amodiaquine and quinine, no effects on HIV-infection are known, except for the enhanced replication of viruses as described above (Maheswari et al, 1991). Primaquine has been reported to be an inhibitor of HIV integrase, an enzyme used for viral replication, in an in-vitro model (Fesen et al, 1993). No publication on an interaction between artemesinin derivatives and HIV or AIDS could be identified.

**Conclusion**

Although theoretically an interaction was expected between HIV and malaria, initial studies showed conflicting results. Several studies had potential for bias (case-control studies, retrospective studies) and/or an inadequate sample size. More recent studies suggest that there is an interaction between malaria and HIV, and that this may be influenced by the “native” state of immunity before HIV-infection was contracted:

**Pregnancy:** HIV-positive pregnant women have a higher prevalence of peripheral and placental parasitemia, and higher parasite densities in comparison to HIV-negative pregnant women. HIV-infection may act as an effect modifier in the intermittent preventive treatment of placental malaria in pregnancy. The role of malaria in pregnancy on transmission of HIV is not clear.

**Adults:** HIV-infected adults appear to have an increased risk of *P. falciparum* parasitemia, higher density infection and symptomatic infections compared to HIV-uninfected adults. The prevalence of parasitemia and clinical malaria increases with decreasing CD4 counts in HIV-infected adults. However, no association has been demonstrated between HIV infection and presentation with cerebral malaria.

**Children:** Although data are somewhat conflicting, overall there is not strong evidence that HIV infection is associated with an increase in the prevalence of malaria. Children may be more susceptible to the pathogenesis of malaria (increased morbidity), once they have the infection. Malaria in infants may be associated with a less rapid progression of HIV.

Because malaria is such a common disease likely to be encountered by HIV-infected inhabitants
in sub-Saharan Africa, it is important to assess possible interactions between HIV and malaria through carefully designed studies.

3) Placental malaria infection and perinatal transmission of human immunodeficiency virus type 1 infection in Kenya

This study was designed to assess whether placental malaria is a cofactor for perinatal HIV transmission and/or for earlier progression of HIV-related illness during the first year of life. Effective strategies to prevent placental parasitemia are available and would have the potential for decreasing the incidence of mother-to-infant transmission of HIV during pregnancy and/or in the peripartum period or possibly to slow the rate of progression of HIV-related disease. The primary objectives were:

A. To determine whether children born to HIV-infected women who have placental malaria parasitemia are at increased risk of congenital HIV infection and/or earlier progression of HIV-related disease.

B. To determine whether HIV-infected women are at increased risk of placental malaria infection and/or to have higher placental parasite densities compared to women who are not HIV-infected.

C. To assess whether HIV-infected women with low CD4-lymphocyte counts (<200 cells/ul) are more likely to have placental malaria infection and/or higher placental parasite densities than HIV-infected women with higher CD4+-lymphocyte counts.

The “vertical transmission study” (VT-study) was a unique opportunity to address other issues as well. This thesis describes results of studies of malaria and HIV in pregnancy, their effects, and consequences for the infant.

In chapter 1 we give an overview of malaria, and HIV in pregnancy, and the potential and evidence for interaction. It also gives an overview of the study site, population and the research unit. Chapter 2 reports on risk factors for HIV infection among asymptomatic pregnant women attending an antenatal clinic in Western Kenya, and chapter 3 reports on HIV and malaria as risk factors for anemia in late pregnancy. Chapter 4 reports on the effect of HIV on prevalence of malaria in pregnancy, and the interaction as was observed in the VT-study. In chapter 5 the effect of malaria in the presence of HIV infection on the outcome of pregnancy is described. Chapter 6 reports risk factors for malaria in pregnancy in the urban and periurban population of Kisumu, and chapter 7 reports on the effect of maternal and infant infection with malaria and/or HIV on the prevalence of anemia in the study population. In chapter 8, summaries of the findings are presented.

Study site and population

The studies were conducted in the city of Kisumu, located on the shores of lake Victoria with a population of approximately 300,000 inhabitants. In 1999 an estimated 17,696 pregnancies occurred, resulting in a crude birth rate of 54/1,000 inhabitants and “birth density” of 61/km² (Ministry of Finance and Planning, 1999). Most of the population belongs to the Luo tribe, but other tribes are represented (Luhya, Kisii, Kikuyu, Kalenjin, Nandi). Important sources
of income are trade, small business labor, subsistence farming, and fishing. Most people in the urban area have casual jobs.

In the areas surrounding Kisumu, estimated individual exposure to mosquito bites infective for malaria ranges from 90 to 400 per year (Beier et al, 1990; Githeko et al, 1993); however, vector densities and entomological inoculation rates within Kisumu are largely uncharacterized. Transmission is likely to be highest during peaks of rainfall in April-July and October-December. The majority of malaria infections in Kisumu are due to *P. falciparum*. Malaria is consistently the leading cause of outpatient and inpatient morbidity and mortality, accounting for 15.3% of inpatient admissions and 901 deaths in 1999, according to Kisumu District Medical Office of Health figures.

The Provincial Hospital is a 400-bed referral hospital, serving the local low-income population. Approximately 100 pregnant women attend the antenatal clinic per day, and 30 of them do so for the first time. There are approximately 2,200-2,500 deliveries per year, and an estimated 70% of the women who deliver in the hospital have visited the ANC of the same hospital.

Among women who visited the ANC of the Provincial Hospital and agreed to participate in the VT-study, 20% carried malaria parasites in their blood. The HIV prevalence among asymptomatic women participating in the study has been stable around 25% during the study period. An HIV prevalence of 31% among ANC attendees was reported by a different study using the same hospital, when consecutive ANC attendees were interviewed, indicating some selection bias in the VT-study enrollment (Glynn et al, 2001). This last study showed that the HIV prevalence among ANC attendees was very similar to that found in women in the general population in Kisumu.

**CDC/KEMRI Research Unit**

The Division of Parasitic Diseases (DPD), CDC, Research Station in Kenya was established in 1979 as part of the Kenya Medical Research Institute (KEMRI). Over the past 21 years, CDC’s investment in the Research Station has resulted in a well-trained staff of Kenyan scientists, clinicians, laboratory technicians, and field workers.

Since 1994, the Research Unit has been involved in research activities at the Provincial hospital. The VT-study, starting in 1996, was originally conducted in the outpatient department of the hospital, but the study soon outgrew its capacity. A study clinic was built on the hospital compound and was completed in 1999. The laboratory can perform basic tests such as assessment of hemoglobin level, rapid HIV testing, preparation, staining with Giemsa, and reading of blood smears, and preparation of blood samples. More complex procedures, like DNA HIV-PCR testing, are performed in the laboratories in Kisian, 16 kilometers out of Kisumu. Samples are transported daily.

**Ethical issues**

This study was carried out in a changing environment of perinatal HIV and malaria prevention. In 1998, the Kenyan Ministry of Health switched from chloroquine to SP for the first line treatment of malaria in pregnancy, and to intermittent preventive treatment with SP for the control of malaria in pregnancy. In cooperation with the Ministry of Health, intermittent treatment with SP was implemented in the Provincial Hospital.

Studies in 1998 and 1999 have shown the benefit of short courses of antiretrovirals in preventing perinatal HIV-transmission. During the yearly review process of the study protocol,
the Kenya Ministry of Health repeatedly determined that, in the absence of national consensus and policy, it was inappropriate to introduce provision of services outside of the research questions being addressed that were not standard of care and were not available to women outside of this study; IRB officials at participating institutions accepted this approach. However, since 2000, CDC’s global AIDS Program (GAP) has initiated AIDS prevention and treatment programs in Kenya. GAP, in cooperation with the Kenyan Government, has made nevirapine available in the Provincial Hospital for the prevention of vertical transmission in HIV-positive women who know their HIV-status.
References


Transmission according to maternal immunologic, virologic, and placental factors. JAMA 269: 2853-2859.


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**Fig. 2. Study site**