Novel biomarkers in the pathogenesis of placental malaria in sub-Saharan Africa
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Citation for published version (APA):
Owens, S. (2012). Novel biomarkers in the pathogenesis of placental malaria in sub-Saharan Africa Amsterdam: Rozenberg

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1. GENERAL INTRODUCTION
1.1 MALARIA IN PREGNANCY

1.1.1 The burden of malaria in pregnancy

Every year up to 125 million pregnant women are exposed to malaria, half of them in sub-Saharan Africa (1) where a quarter of mothers have evidence of malaria infection in the placenta at delivery (2). Four of the five human malaria species (Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi) may infect pregnant women, but only P. falciparum and P. vivax infections are strongly associated with adverse pregnancy outcomes. P. falciparum is responsible for the greatest burden of disease, estimated to cause 10000 maternal deaths annually through malaria anaemia in Africa alone (3), and the delivery of up to 200000 low birth weight (LBW) babies mediated through preterm delivery (PTD) and fetal growth restriction (FGR) (4). Maternal placental infection with P. falciparum is now recognised as a major contributing factor to perinatal and neonatal mortality in the Tropics (5-8).

Pregnant women are more susceptible to malaria infection than other adults and this susceptibility is greatest in first (primigravida) and second (secundogravida) pregnancies (9), peaking between 13 and 16 weeks gestation and declining toward term (10). As well as being apparently more attractive to mosquitoes than other adults (11), pregnant women tend to suffer heavier parasitaemias and more complex infections than non-pregnant adults (12), suggesting that pregnancy impairs the control of parasite replication.

Susceptibility to pregnancy-associated malaria (PAM) probably derives from a combination of immunological and hormonal changes in pregnancy, compounded by positive selection of a unique subset of parasites that can sequester in the placenta. Younger pregnant women have been found to be more susceptible to malaria infection in some settings, even after adjustment for parity (13) and this may reflect incomplete development of pre-pregnancy malaria immunity. Human immunodeficiency virus (HIV) infection also increases susceptibility to malaria and results in increased prevalence and density of parasitaemias and blunting of parity-specific immunity (14).

On average 9.2% of women in endemic areas have placental parasitaemia with negative peripheral blood films at delivery (9) and this may be as high as 68% in some settings (15). In highly seasonal transmission areas, the prevalence of placental malaria in the dry season often remains disproportionately high, compared to the low incidence of peripheral parasitaemia, suggesting that infections can persist in the placenta long after being cleared from the peripheral circulation (2).
1.1.1 The burden of malaria in pregnancy

Although the most sensitive method to detect malaria infection during pregnancy is to conduct weekly examinations of peripheral blood films from early pregnancy booking (16), this is not often practical in developing countries. Instead studies usually define infection on the basis of parasitaemia in placental blood smears or by the evaluation of placental histopathology, which is almost twice as sensitive for the detection of current parasitaemia and also reflects chronicity of infection (17). A simple classification of placental histological findings in malaria is outlined in table 1 and described below (15).

1.1.2 Pathology of malaria in pregnancy

The pathology of P. falciparum infection in pregnancy is characterised by the dense accumulation of infected erythrocytes (IE) in the blood-filled lacunae of the maternal side of the placenta – the intervillous spaces (IVS). Placental IE host parasites at all stages of development, including mature forms such as late trophozoites and schizonts, which are not usually seen in peripheral blood. In chronic and recently resolved infections, placental IE and associated macrophages are often observed to contain malaria pigment (haemozoin), a breakdown product of parasite digestion of haemoglobin, which is best visualised under polarised light (18).

The placental sequestration of IE stimulates maternal mononuclear cells to secrete β-chemokines, which are chemotactic for other monocytes and macrophages, and macrophage migration inhibitory factor, a cytokine that promotes retention and activation of macrophages. Induction of these chemokines is the basis of the infiltration of large numbers of monocytes into the IVS following parasitisation of the placenta, an histological finding known as massive intervillitis (19).

The rationale for histological classification of placental malaria is based on the different pathogenic significance of haemozoin and parasites and on the assumption that untreated infections will progress. Thus, the presence of parasites indicates active infection whereas haemozoin deposition, either free or in macrophages, indicates chronic haemolysis. However the absence of parasites and pigment on placental histopathology does not eliminate the possibility of resolved malaria infection earlier in pregnancy (16).

Excess of perivillous fibrinoid deposits, excessive syncytial knotting, and trophoblastic basement membrane thickening have also been reported in association with malaria infection of the placenta (15, 20-23). However these lesions are described only occasionally in chronic infections and rarely in past infections, suggesting that they do not play a significant role in the pathogenesis of placental malaria.
The most significant association with active malaria infection is massive chronic intervillositis (19). There is no increase in villous inflammatory cells and the infiltration is not seen in acute or past infections. Massive intervillositis is not seen when malaria is treated promptly (16).

Figure 1 illustrates the histological appearances of a malaria-infected placenta with parasitised red blood cells and infiltrates of pigmented monocytes, seen under non-polarised and polarized light (9).

Submicroscopic malaria infections during pregnancy, detectable by polymerase chain reaction (PCR) techniques are relatively common, but do not seem to be associated with maternal anaemia or diminished birth weight, nor are they more frequent in primiparae (24). This suggests that the clinical sequelae of malaria in pregnancy are a function of the density of placental parasitaemia and a failure to clear parasites from the placenta quickly. When infections are treated promptly, placental histology is often normal (16).

The binding characteristics of placental IE differ to those isolated from peripheral blood of non-pregnant adults. Placental IE bind to chondroitin sulphate A (CSA), a receptor not utilised by other IE (25), and do not bind to cluster of differentiation (CD)36 or Intercellular Adhesion Molecule (ICAM)1 (26, 27), receptors which usually are. This difference is considered to be the key feature of the pathogenesis of placental malaria (28). CSA is present in the placenta as a glycosaminoglycan sidechain to thrombomodulin, a tissue anticoagulant (29). It is also secreted as a low-sulphated aggrecan in the IVS, where it may bind reversibly to hormones and cytokines facilitating paracrine signalling in the placenta (30). *In vitro* binding of parasites to both forms of CSA has been observed (31).

Placental IE differ from other IE in additional ways that probably relate to their unique adhesion receptor specificity. They do not form rosettes with non-infected red cells as is commonly seen in cerebral malaria, for example (32, 33). They tend to sequester throughout the placental IVS in contrast to those observed in other organs, where they are usually found in close adherence to vascular walls (31). They do not commonly agglutinate in the presence of serum from individuals exposed to *P. falciparum*, a process which is mediated by cross-linking antibodies and is seen with IE from non-pregnant adults (34). Additionally, CSA-binding and placental IE can adsorb IgM unlike other IE (35) and this may play a role in pathogenesis which is as yet poorly defined.
1.1.3 Immunology of placental malaria

It is clear from the evidence summarised above that the parasite adhesion antigens expressed on placental IE are fundamentally different from those expressed on IE in non-pregnant infections. In non-pregnant individuals these variant specific antigens (VSA) are primary targets of the immunoglobulins that confer protective immunity to malaria (36). This immunity develops gradually in response to repeated infections over a number of years but appears to be lost when pregnant women living in high-transmission areas become pregnant for the first time (37). It was shown that serum immunoglobulins from multigravidae inhibit the adhesion of IE isolated from pregnant women to CSA, while those from primigravidae and men do not (38). None of the sera inhibited binding to CD36, which is a common adhesion receptor for non-placental IE and the gravidity effect was independent of the geographical origin of parasites and serum suggesting that the parasite antigen mediating adhesion are conserved among pregnancy-specific parasite lines (38).

These and similar findings were interpreted as evidence of distinct pregnancy-specific VSA (VSA\textsubscript{PAM}) (39). VS\textsubscript{PAM} - specific immunoglobulin concentrations correlate with parity and with inhibition of adhesion of IE to CSA \textit{in vitro} (39-41). Such immunoglobulins are not detectable until around 20-weeks' gestation in primigravidae but appear earlier and rise faster in concentration among multigravidae (37) (42). Immunoglobulin (Ig) G1 is the main subclass of VSA\textsubscript{PAM} - specific immunoglobulin (41). Crucially, high concentrations of anti-VSA\textsubscript{PAM} IgG are protective against LBW and PTD (43) and are positively correlated with maternal haemoglobin concentrations, while concentrations of antibodies to other VSA found on isogenic parasites in non-pregnancy malaria are not (44).

Thus VSA\textsubscript{PAM} appears to elicit a different Ig response to that elicited by the VSA of non-placental parasites, a response which matures and becomes more effective over a number of pregnancies. This model largely explains the re-emergence of susceptibility to malaria among primigravidae in high-transmission areas.

However the level of pre-existing immunity to other blood-stage parasite antigens has also been associated with protection against placental malaria and offers an explanation for the importance of young maternal age as an independent risk factor for PAM and the rarity of severe maternal disease in areas of intense pre-pregnancy exposure to infection (13, 45, 46).

Molecular techniques have identified \textit{var2csa} as the gene encoding VSA\textsubscript{PAM}, which is now known to be a variant form of the well-described parasite antigen \textit{P. falciparum} erythrocyte membrane protein 1 ( PfEMP1), called VAR2CSA (47). The gene is selectively transcribed by CSA-binding parasites and is conserved between clones (48, 49), while VAR2CSA has binding sites for CSA (50) and is a target for
pregnancy-malaria specific IgG (51). Antibodies to VAR2CSA are found in women only, their concentration correlating with parity (52).

Although VAR2CSA expression appears to be the key factor in placental sequestration of malaria parasites and is an important target of pregnancy-associated malaria immunity, other factors and protective mechanisms are also likely to exist and require investigation.

Placental malaria is associated with substantial production of tumour necrosis factor (TNF)α, interferon (IF)γ, interleukin (IL)1β and IL2, characterising a robust Th1 response to infection (53, 54). While this pro-inflammatory response facilitates parasite clearance from the placenta by enhancing phagocytosis, generating reactive oxygen intermediates and stimulating T-cell proliferation (55-57), it also threatens pregnancy outcome, which is ordinarily dependent on a shift in cytokine balance toward a Th2-type, anti-inflammatory response (58-60). Understanding how the balance of pro- and anti-inflammatory cytokines is maintained in placental malaria is important since it affects several other immune responses and ultimately pregnancy outcome. A schematic illustration of the basic mechanisms of this balance from a previously published paper is given in figure 2 (9).

There are few human studies on the role of innate immunity in pregnancy-associated malaria. Production of immunoregulatory cytokines early in the course of malaria by cells of the innate immune system probably shapes the adaptive immune response to the infection. Macrophages phagocytose parasites and release reactive oxygen species (61); IE adhere to natural killer (NK) cells, provoking production of IFγ (62), and to dendritic cells (DC), modulating their function (63). Asexual stages of P. falciparum are recognised by innate cells via toll-like receptors (TLR). In women with chronic placental infection, polymorphisms in TLR4 were associated with fetal growth restriction and maternal anaemia (64). Concurrent antiviral responses may be impaired because systemic activation of dendritic cells via TLR by malaria antigens impairs cross-presentation (65). Further human studies on the role of these cell types in regulating adaptive immunity to malaria in pregnancy are warranted.

It is thought that cell-mediated immune responses are suppressed in pregnancy but data on the impact of such suppression on the risk of pregnancy-associated malaria is scarce and open to interpretation. It has become clear that many apparently uninfected pregnant women actually have submicroscopic (slide negative but PCR-positive) parasitaemias. Thus reduction in T-cell proliferative responses with pregnancy malaria noted in early studies (66) could reflect the absence of memory T-cells from peripheral blood samples following their deep tissue sequestration in response to low levels of infection (67), rather than immunosuppression.
As previously outlined, high levels of VSA_PAM - specific IgG are produced in response to pregnancy-associated malaria. It has recently also been shown that the frequency of VSA_PAM - specific memory B-cells in postnatal multigravidae can be as high as 1:4000 B-cells and these cells produce conformation-dependent antibodies to surface-exposed epitopes in VAR2CSA (68, 69).

Interestingly there is some evidence to suggest that prevention of malaria in pregnancy may adversely affect protective immunity to pregnancy-specific parasite variants. Primigravidae receiving sulphadoxine pyrimethamine (SP) intermittent preventative treatment in pregnancy (ITPp) developed less antibody to CSA-binding isolates than did matched placebo recipients (70). Insecticide treated nets (ITN) use throughout pregnancy was associated with decreased IgG antibody to liver stage antigen 1 and merozoite surface protein 119 but increased antibody to circumsporozoite protein; antibodies to CSA were not measured (71). Further studies should explore immunological correlates of malaria chemoprophylaxis and the impact that malaria prevention in first pregnancies, including possible effects of vaccination, might have on subsequent susceptibility to placental malaria.

1.1.4 Consequences of malaria in pregnancy

The majority of studies on pathogenesis and immunity in pregnancy-associated malaria (PAM) have been conducted in areas of high-transmission. In areas of low-transmission women of all gravidities are at risk of symptomatic and severe disease which may be complicated by miscarriage, stillbirth, LBW and congenital malaria (2). The more severe manifestations of PAM in low-transmission settings are thought to be the result of delayed acquisition of malaria immunity rather than to differences in parasite virulence.

Fetal growth restriction and preterm delivery

LBW is a function of PTD and FGR. In highly endemic areas of sub-Saharan Africa malaria in pregnancy is thought to account for approximately 20% of LBW deliveries, directly contributing to the deaths of approximately 100000 infants each year (72). A large meta-analysis demonstrated that the correlation between LBW and placental parasite prevalence is significant for primigravidae (correlation coefficient 0.57; p=0.04; 13 studies) and multigravidae (correlation coefficient 0.84; p<0.01; 10 studies), although LBW and placental parasite prevalences are much lower in multigravidae (9). The graphical summary presented in figure 3 supports the conclusion that placental malaria is associated with reduced birthweight; the regression slope indicates that for every 10 per cent increase in placental malaria prevalence there is a 9.0 per cent increase in the risk of LBW.
Acute malaria parasitaemia is associated with PTD (73) but although a causative ‘fever-pathway’ linking infection and early parturition may be surmised from known effects of malaria on cytokine production and an increased risk of maternal anaemia, the specific mechanisms are unknown. On the other hand FGR is strongly associated with chronic malaria and the evidence behind the potential pathogenic mechanisms to explain this association has been recently reviewed (74). The timing and duration of infection, modulated by concurrent maternal factors and fetal genotype, probably determine the severity these effects.

The IVS of the early placenta open up to maternal blood cells from the end of the first trimester, coinciding with the peak period for maternal malaria infections, between 13 and 16-weeks gestation (10). It is hypothesised that infections during this period contribute to placental insufficiency, through effects on placental vascular development, causing FGR (74). This hypothesis is supported by observations from recent studies of fetal growth, which demonstrated that most neonates born to women with malaria infections at delivery are symmetrically growth restricted, a form of FGR classically described with feto-placental insults sustained in early pregnancy (75, 76). A doppler study at 32-35 weeks of pregnancy showed a relationship between malaria infection and increased resistance to utero-placental blood flow (77), which is a marker of impaired placental trophoblast invasion of the uterus and poor placental vascular development up to 20-weeks gestation. Hypertensive disorders of pregnancy, which are another marker of poor placental vascular development in early pregnancy, have also been associated with malaria in pregnancy (78, 79), although the significance of these findings in unclear. Such observations are underpinned by more recent work showing that malaria impacts on placental production of factors which regulate placental vascularisation, such angiopoietins (80), vascular endothelial growth factor (VEGF) (78) and soluble VEGF receptor-1 (78, 81), while also contributing to over-activation of the complement system (81), which causes placental vascular dysfunction in experimental models (82).

Later infection of the mature placenta, associated with monocyte infiltration and severe inflammation, has been reported to alter cortisol metabolism (83, 84), placental gene expression (85) (86) and may alter amino-acid transport across the syncytiotrophoblast (ST), the unicellular membrane separating the maternal and fetal components of the placenta, through the induction of IL1β (53, 54, 87). These changes may lead to dysregulation of the insulin-like growth factors’ axis (88), leptin (89) and other hormone production, decrease the activity of placental glucose transporter systems (90) and result in asymmetrical growth restriction.

Adverse effects of malaria in pregnancy on fetal nutrition and growth are likely to be exacerbated by undernutrition of women, especially those living in poor rural communities. Like malaria, the intensity and specific effects of maternal undernutrition are often seasonally determined (91). Macro- (92) and micronutrient supplementation (93) has been shown to increase birth weight but combined interventions for maternal malaria and malnutrition have not been evaluated.
There is some evidence to suggest that the deleterious impact of malaria on fetal growth could lead to abnormal and persistent changes in the metabolism, growth and development of infants independently of its effects on birth weight (94, 95). It is not known if these effects influence the risk of chronic disease in later life – an idea known as fetal programming (96).

Infant morbidity and survival

FGR and PTD are major consequences of placental malaria and LBW delivery is a useful marker of either or both, as LBW babies have increased morbidity and mortality. But independently of birth weight, maternal placental infection with *P. falciparum* has also been positively associated with malaria morbidity during the first two years of life (97), infant anaemia (98), fetal anaemia (99-101) and cord malaria parasitaemia (102). There are also concerns about more insidious and deleterious effects on the early immune system, which may render infants susceptible to tetanus (103) and measles (104) but which are difficult to quantify and require further study.

Thus placental infection with *P. falciparum* appears to have a much more significant role in infant survival in Africa than has been previously assumed. A recent meta-analysis of three randomised studies calculated that malaria chemoprophylaxis during pregnancy reduced perinatal mortality of babies born to primigravidae (105). The relative risk for those given anti-malarials was 0.73 (95% confidence interval 0.53-0.99). In a previous analysis, it was estimated that adequate malaria control during pregnancy could avert 3-8% of all infant deaths associated with malaria-induced LBW delivery, or between 100000 and 250000 infant deaths in sub-Saharan Africa alone (4).

Although cord blood malaria parasitaemia is relatively common, especially when molecular techniques are used to detect it, symptomatic congenital malaria is an unusual event for babies born to semi-immune mothers living under holoendemic conditions (106). Accumulation of infected red cells at the interface between the maternal and fetal circulation does however result in a small number of cases of symptomatic congenital malaria and there are case reports of babies born to non-immune women who have developed symptomatic malaria parasitaemia and died (107-109). This suggests a central protective role for transplacental antibody transfer of malaria-specific antibodies from mother to baby (110). However placental malaria infection itself has been shown to reduce the transport of other pathogen-specific antibodies across the placenta (111-113) so the process requires further clarification. It has been argued that placental parasitaemia can be used as an marker to identify which infants need active screening and treatment of peripheral parasitaemia, to prevent death from malaria (16).
Placental malaria and anaemia have been associated with fetal immune priming to malaria antigens but whether this leads to the acquisition of malaria immunity by infants, or increases their susceptibility, is contentious (110, 114-117). In Tanzania, there appeared to be an important interaction between gravidity and infant susceptibility to malaria. Although primigravidae are at highest risk of parasitaemia, it was the infants of multigravid women with placental infection who were at highest risk of infection in the first year of life (118).

Other protective mechanisms against infant malaria infection in infancy are not well understood but may include innate factors such as the relative resistance of fetal haemoglobin to malaria digestion and low para-aminobenzoic acid concentration of human breast milk, as well as cultural practices such as swaddling which limit infant exposure to mosquitoes (119).

**Malaria and HIV infection**

Malaria increases HIV viral load in pregnant women (120) and has been associated with increased transcription of C-C chemokine receptor type 5 (CCR5) messenger ribonucleic acid (mRNA), a co-receptor for HIV cells entry, in macrophages in the IVS and by Hofbauer cells (fetal villous macrophages) (121). Despite these findings, the effect of malaria on the risk of MTCT of HIV is controversial (122, 123). There are surprisingly few data on which to assess this effect; however a recent study of an historical cohort in Rwanda reported that placental malaria was associated with increased risk of mother-to-child-transmission (MTCT) of HIV-1 (adjusted odds ratio [aOR] = 6.3; 95% confidence interval [CI] = 1.4–29.1), especially among primigravidae (aOR = 12.0; 95% CI = 1.0–150; p < 0.05). In the other direction, HIV infection increases susceptibility to malaria in pregnancy (122, 124) by suppressing variant-specific immunity (125, 126) and by impairing cytokine responses, especially the production of IL12 (127) and IFγ (128). Maternal HIV infection could also have negative implications for the transplacental transfer of maternal anti-malaria antibodies to the fetus, increasing the risk of infant malaria in high-transmission settings.

**Malaria anaemia**

Malaria anaemia is caused by bone marrow dysfunction induced by the infection, in conjunction with increased destruction of infected and uninfected erythrocytes. Pigmented monocytes accumulate in the chronically infected placenta, releasing inflammatory mediators, including especially TNFα, which suppress erythropoiesis (129) and lead to increased erythrocyte destruction in the presence of heightened oxidative stress (61). For women living in poor countries, these processes are frequently superimposed on micronutrients deficiencies, HIV infection or hookworm infestation which are independent factors in the aetiology of maternal anaemia (130-
Malaria anaemia is estimated to cause 10000 maternal deaths every year (3). In holoendemic malarious areas with a 5% severe anaemia prevalence (hemoglobin 70 g/L), it was estimated that in primigravidae, there would be 9 severe-malaria anaemia-related deaths per 100,000 live births (133).

Malaria and hypertensive disorders of pregnancy

Placental malaria and pre-eclampsia are characterised by reduced placental perfusion, loss of placental integrity and endothelial cell dysfunction and there is striking commonality in their epidemiology, immunology, haematology and biochemistry (134). It has been suggested that more than a quarter of cases of pre-eclampsia in African primigravidae may be a consequence of malaria in pregnancy, implying that the malaria-attributable fraction of maternal mortality is much higher than is currently recognised (78, 135).

1.1.5 Treatment and prevention of malaria in pregnancy

Effective control measures for malaria in pregnancy currently include the distribution of ITN, which have been shown to decrease parasite prevalence, reduce the risk of LBW and stillbirth and to improve trends for reduced risks from maternal anaemia and clinical malaria (136). Secondly IPTp using regular doses of SP from the end of the first trimester decreased peripheral and placental parasitaemia and increased maternal haemoglobin and infant birth weight, especially among primigravidae and secundigravidae (137-141).

However in addition to concerns about the impact of treatment of placental malaria on maternal and infant malaria immunity, there are also issues around drug resistance especially to SP and the optimal timing of ITPp (142-145). In 2006 the World Health Organization (WHO) recommended the use of artemisinin-based combination treatments for malaria during the second or third trimesters, but data on efficacy and safety in Africa are scarce. In a small trial conducted in Uganda, artemisinin derivatives were reported to be “not inferior to oral quinine for the treatment of uncomplicated malaria in pregnancy” (146). It was concluded that such drugs might be preferable on the basis of safety and efficacy. Several artemesinin combinations have been proposed for the purposes ITPp in the future (147). In any case intensive coverage with existing interventions is presently incomplete (145) and a better understanding of the pathogenesis of malaria in pregnancy underpins the development, evaluation and improvement of programs to control the infection and its sequelae in the future.

Nutrient-malaria interactions have been little studied in pregnant women. There is evidence that iron deficiency may reduce malaria risk in pregnancy (148) and that
iron supplementation may increase risk in this population (149). Further data are required from women living under different levels of malaria transmission in order to determine the magnitude of these effects in subjects with different levels of malaria immunity, and how ITPp interacts with routine antenatal iron and folate supplementation especially in high-risk groups such as adolescents.

1.1.6 Future directions

Based on our existing knowledge of the epidemiology, pathology and immunology of this disorder, prevention by vaccination represents an attractive and potentially achievable public health strategy. The aim of vaccination would be to use recombinant proteins to elicit functional antibodies against IE that bind to CSA in the placenta by targeting certain domains of VAR2CSA and other conserved antigens (135). The recognition of conformational epitopes is therefore a likely requirement of a VAR2CSA-based vaccine that induces adhesion-blocking antibodies (150, 151). Since it is not yet technically possible to manufacture whole VAR2CSA proteins, it will be necessary to assemble smaller parts of the molecule with the desired characteristics. To induce antibodies which are effective against a high proportion of placental parasites, a vaccine should target the domains of the molecule that do not vary and investigations will need to determine how sequence variation affects CSA and antibody binding. Meeting these technical criteria is still some way off and the unanticipated effects of a comprehensive vaccination strategy for malaria on maternal and infant immunity yet require evaluation.

1.2 STUDY OBJECTIVES

In summary malaria in pregnancy is a major if often insidious cause of maternal and child morbidity and mortality in poor countries, which arises from a distinct subset of parasites with the uncommon ability to bind to CSA and thereby to sequester in the placenta. Immunity to malaria in pregnancy correlates with the development of antibodies to the exported surface proteins on the surface of IE infected with this form of the parasite, antibodies which interfere with parasite adhesion to CSA and perhaps other receptors on syncytiotrophoblast.

Although a vaccine that prevents *P. falciparum* malaria in pregnant mothers is feasible and would potentially save hundreds of thousands of lives each year, this goal has yet to be achieved. There is still a pressing need for research on the pathogenesis and immunity of malaria in pregnancy, especially on the interactions between malaria, HIV, maternal nutrition and anaemia in determining birth weight and on the effects of malaria on placental function, pregnancy outcome and infant immunology. Emerging knowledge of the special characteristics of parasites that cause placental infection or other forms of complicated malaria may lead to new treatments and the better
application of existing ones in order to attenuate the enormous burden of this infection in pregnancy.

The aim of the current research was to examine hitherto poorly explored aspects of placental malarial pathogenesis including binding characteristics of the ST and parasitised red blood cells, effects on markers of placental function and infant immunity and maternal endocrine interactions. Specific objectives were as follows:

1. To estimate the prevalence of placental malaria among women delivering in Kumasi, Ghana and its impact on maternal and newborn malaria parameters
2. To describe maternal DHEAS levels in relation to placental malaria histology and functional parameters
3. To describe the occurrence and co-factor dependence of anti-phospholipid antibodies
4. To analyse ABO blood group phenotypes in relation to placental malaria pathology
5. To examine the role of sialylated glyconjugates in parasite adhesion in the placenta
6. To determine rates for transplacental transfer of measles maternal immunoglobulin in relation to placental malaria
Table 1: Classification of placental histology in malaria. Adapted from (15).

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Figure 1: (A) Massive malarial infection involving the placenta. Many maternal erythrocytes in the intervillous space are parasitised. (B) The same field under polarised light. Note that no parasites are detected in the villi. (Haematoxylin and eosin, 200x). From (9) with permission.
Figure 2: Placenta-related mechanisms of malaria parasite clearance, suppression and adhesion. (PfEMP P. falciparum erythrocyte membrane protein; DBL Duffy binding like domain; CSA Chondroitin sulphate A; NK Natural killer cells; IFN Interferon; TGF Transforming growth factor; TC T cell (cytotoxic); THO T helper (precursor). From (9) with permission.

Figure 3: Regression plot of placental parasite prevalence (per cent) and low birthweight incidence in primigravidae (0) and multigravidae (+) for 23 cross-sectional studies with available data. $y=8.089+1.113X$. R$^2=42.6$ per cent. Correlation coefficient ($r$)=0.653. Stippled line: 95% confidence interval. From (9) with permission.
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