Diagnosis of malaria in pregnancy: evaluation, new developments and implications

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Malaria, a parasitic infection caused by *Plasmodium* parasites, can have severe effects among pregnant women and their (unborn) infants. Early detection and subsequent treatment are of great importance to prevent the development of severe disease. Even though there are control measures to prevent malaria infection during pregnancy, like the use of insecticide treated bed nets and preventive treatment, diagnosis remains essential in both low and high malaria transmission regions.

Diagnosis of malaria during pregnancy can be complicated by placental sequestration of the malaria parasites, resulting in the absence of parasites in the peripheral blood or in parasite densities that are below the detection limit of commonly used diagnostic methods. Accurate detection of parasite infection in the placenta requires examination of histological sections of fixed placental tissue, which is the gold standard for diagnosing placental malaria. Placental histology and microscopic examination of placental blood can only be performed after delivery. It would be beneficial for both mother and foetus if malaria could be diagnosed in the peripheral blood during pregnancy, followed by safe and adequate treatment. A blood test that would be evidence of malaria in the placenta would therefore be a great improvement of our diagnostic capacity. Currently available methods for the diagnosis of malaria in peripheral blood are parasite detection by microscopic examination of stained blood smears (microscopy), DNA or RNA detection methods (such as polymerase chain reaction, PCR, a method to amplify DNA) and detection of parasite antigens by rapid diagnostic tests (RDTs). RDTs are based on detection of parasite antigens circulating in the blood of patients, such as histidine rich protein 2 (HRP2) that is specific for *Plasmodium falciparum* and *Plasmodium lactate dehydrogenase* (pLDH) that is found in all human malaria parasites. These tests show presence or absence of the antigen within a few minutes.

In Chapter two a systematic review is presented of the diagnostic accuracy of microscopy, RDTs and molecular techniques (PCR) compared to histological examination of the placenta for the diagnosis of *Plasmodium* infections in pregnancy. This review shows that RDTs and PCR may have good perfor-
mance characteristics to serve as alternatives for the diagnosis of malaria in pregnancy. Due to the lack of data, accuracy could not be evaluated against placental histology. Both placental and peripheral blood microscopy, however, cannot reliably replace histology as a reference standard for placental *P. falciparum* infection, even though they were often used for this purpose. Therefore, more studies with placental histology as reference test are urgently required to reliably determine the accuracy of RDTs and PCR for the diagnosis of placental malaria.

There are concerns about test stability, about accuracy of RDTs in various climatological circumstances, about the inability to detect all species, antigen persistence and antigen genetic diversity, and this prompt the need for improvement of RDTs and RDT performance. Therefore monoclonal antibodies against three malaria antigens, glutamate rich protein (GLURP), dihydrofolate reductase-thymidylate synthase (DHFR-TS) and heme detoxification protein (HDP) were developed and characterised (Chapter three). It was demonstrated that the developed antibodies react with good affinity and sensitivity to the recombinant antigens and to *P. falciparum* and *Plasmodium vivax* isolates. An ELISA (enzyme-linked immunosorbent assay) was developed and in Chapter four the evaluation of the mentioned antibodies in this ELISA to detect several genetically divers *P. falciparum* cultured isolates and *P. vivax* from patient samples in comparison with two commercially available malaria antibodies is described. However, in this setup the ELISA did not have sufficient accuracy for the diagnosis of malaria.

A problem encountered with HRP2-RDTs in non-pregnant individuals is that the antigen that is to be detected can persist in the blood for several weeks even though parasites have been cleared after treatment. The persistence of several *Plasmodium* antigens in pregnant women after treatment was evaluated and different diagnostic tests were compared during follow up after treatment (Chapter five). This study demonstrated that the HRP2-RDT remains positive much longer after treatment in pregnant women than other commonly available diagnostic tests such as microscopy, the pLDH-RDT and PCR. Higher parasite densities at baseline correlated with a prolonged antigen persistence time.

Rapid diagnostic tests are being increasingly implemented for the diagnosis of malaria in pregnancy, whereas research on the diagnostic accuracy of these tests in this population is limited as shown in Chapter two. The performance of diagnostic tests including two RDTs for routine testing of pregnant women during their antenatal care visit was therefore evaluated (Chapter six). The same commercial RDTs were tested for their accuracy to diagnose placental malaria at delivery in comparison with microscopy and PCR of peripheral and placental blood (Chapter seven). These studies were performed in two ru-
ral villages in Burkina Faso. In these field evaluations relatively high malaria
dependence was seen in the women during pregnancy and at delivery. Both
PCR and the HRP2-RDT showed good accuracies in peripheral blood com-
pared to microscopy. The comparison with histology could unfortunately not
yet be completed. Placental blood microscopy failed to detect many cases that
were detected by the other tests, including peripheral microscopy and impres-
sion smears of placental tissue. The pLDH-RDT performed less well than PCR
and HRP2-RDTs, but much better than expected based on the evaluation of
pLDH-based tests presented in Chapter two.

The conclusions of the studies described in this thesis are mainly appli-
cable to areas with high malaria transmission in sub-Saharan Africa with
asymptomatic pregnant women with *P. falciparum* infections. Even though
this thesis has shown good performance of RDTs and PCR compared to mi-
croscopy of peripheral and placental blood to diagnose malaria in pregnant
women, more studies are needed that compare these tests to histological ex-
amination of the placenta to be able to make an evidence based conclusion on
their accuracy for placental malaria. This is especially true for pLDH-RDTs,
since such a large variation in accuracy has been found.

In high transmission areas a test should be used that can detect low par-
asite densities and sequestered parasites, such as PCR and HRP2-RDTs. In
low transmission areas, however, peripheral parasitaemia is more frequent
and parasite densities are higher, and pLDH-RDTs could be suitable in these
areas as well. The proportion of non-*falciparum* infections in Asia and Central
and South America is higher than in Africa, and in these circumstances, tests
that detect all parasite species might be more suitable, such as PCR detection
or RDTs with multiple species detection (PAN) by pLDH, combined with HRP2
detection for the low density *P. falciparum* infections. The persistence of HRP2
antigen is an important issue of malaria diagnosis in pregnancy, since this
can cause false-positive results. This can result in problems in the interval be-
tween doses of so called Intermittent Preventive Treatment (IPT), which means
intake of several therapeutic doses of antimalarial drugs during pregnancy,
irrespective of complaints or presence of parasites. IPT is widely applied in
sub-Saharan Africa. The finding of a positive HRP2-RDT test in these cases
can lead to unnecessary treatment and possible under-recognition of other
diseases. In low and seasonal transmission areas IPT is less often or not ap-
plied but “screen-and-treat” strategies are often implemented, meaning that
a diagnostic test will be used routinely at antenatal care visits, despite any
symptoms, and if positive the woman will receive treatment. If two screen-
ings follow each other within a small time-frame, antigen persistence will be
a problem as well.

In conclusion, there is an obvious need for new easy-to-use diagnostic tests
for the diagnosis of malaria in pregnancy with a better detection threshold and accuracy than pLDH-RDTs and microscopy, but with fewer problems in terms of antigen persistence as encountered with HRP2-RDTs.