Diagnosis of malaria in pregnancy: evaluation, new developments and implications
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Chapter 5

Antigen persistence and the implications for the diagnosis of malaria in pregnancy


Keywords
Malaria; pregnancy; Plasmodium falciparum; diagnostic tests; antigen persistence; treatment follow up.

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CHAPTER 5. ANTIGEN PERSISTENCE AND THE IMPLICATIONS FOR THE DIAGNOSIS OF MALARIA IN PREGNANCY

5.1 Abstract

Objectives

To evaluate persistence of several Plasmodium antigens in pregnant women after treatment and compare diagnostics during treatment follow-up.

Methods

Thirty-two pregnant women (N = 32) with confirmed malaria infection by a histidine-rich protein 2 (HRP2)-based rapid diagnostic test (RDT) and microscopy were followed for 28 days after artemisinin-based combination therapy (ACT). A Plasmodium lactate dehydrogenase (pLDH)-based RDT and two ELISAs based on the detection of dihydrofolate reductase-thymidylate synthase (DHFR-TS) and heme detoxification protein (HDP) were compared with each other and to RT-PCR at each visit.

Results

The mean visit number (95% confidence interval) on which the HRP2-based RDT was still positive after treatment was 3.4 (2.7-4.1) visits with some patients still positive at day 28. This is significantly later than the pLDH-based RDT [0.84 (0.55-1.1)], microscopy (median 1, range 1-3), DHFR-TS-ELISA [1.7 (1.1-2.3)] and RT-PCR (median 2, range 1-5) (p < 0.05), but not significantly later than HDP-ELISA [2.1 (1.6-2.7)]. Lower gravidity and higher parasite density at day 0 resulted in significantly longer positive results with most tests (p < 0.05).

Conclusions

HRP2 can persist up to 28 days after ACT treatment; therefore, this test is not suitable for treatment follow-up in pregnant women and can generate problems when using this test during intermittent preventive treatment (IPTp). DHFR-TS is less persistent than HRP2, making it a potentially interesting target for diagnosis.
5.2 Introduction

Malaria infection during pregnancy is related to adverse maternal health and poor birth outcomes [3, 4]. Diagnosis of malaria during pregnancy is often complicated by the absence of parasites in peripheral blood or parasitemia below the detection limit of microscopy, due to sequestration in the placenta [5, 33, 57]. Therefore, there is a need for different diagnostic methods, such as detection of circulating Plasmodium antigens by rapid diagnostic tests (RDTs). These RDTs detect circulating antigen, even when parasites are sequestered and not visible by microscopy [79]. Rapid diagnostic tests for malaria can detect histidine rich protein 2 (HRP2), Plasmodium lactate dehydrogenase (pLDH) and/or aldolase. Rapid diagnostic tests, especially those detecting HRP2, have the potential to accurately diagnose malaria in non-pregnant and pregnant individuals [5, 62, 63, 78]. HRP2-based RDTs are most commonly used because of their lower cost, better stability and lower detection threshold compared to pLDH-based tests [62, 63, 76, 77]. In non-pregnant individuals the HRP2 antigen has been reported to persist in the blood for several weeks after treatment even though parasites were cleared [67–74]. This was observed for pLDH-based tests as well, although to a much smaller extent, and often related to the presence of gametocytes [67, 69, 75]. It is reasonable to assume that antigen persistence also occurs in pregnant women, but to our knowledge, no studies have yet reported this issue.

Persisting antigen detected by RDTs poses particular problems for pregnant women. Intermittent preventive treatment (IPTp) is applied in high-transmission areas, where women have acquired immunity to malaria, and infection during the pregnancy is often asymptomatic, and needs to be confirmed by parasitological diagnosis. The use of IPTp, however, might lead to false-positive RDT results due to persisting antigen after an IPTp dose which could have cleared a recent malaria infection.

In areas with low transmission or high sulfadoxine-pyrimethamine (SP) resistance, screening and subsequent treatment of women during antenatal care are being used instead [44, 56]. This strategy might be more effective than a preventive approach in these areas and limits the number of women receiving drugs unnecessarily [44, 56]. In these screen-and-treat strategies, antigen persistence is a problem and leads to unnecessary treatment as well, especially if the screening is performed within a short-enough time window.

Regardless of the above mentioned problems, RDTs are currently advocated and implemented in large-scale malaria control programmes. Therefore, it is essential to know how long antigen-detecting tests remain positive after treatment. New antibodies have been developed detecting dihydrofolate reductase-thymidylate synthase (DHFR-TS) or heme detoxification protein
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(HDP), which can be used in ELISA to detect the presence of these antigens, which are potential targets for malaria diagnosis [180]. Therefore, the persistence of the antigens, HRP2, pLDH, DHFR-TS and HDP, was evaluated in the framework of a trial that assesses the safety and efficacy of artemisinin-based combination therapy (ACTs) for the treatment of malaria in pregnant women in Burkina Faso and compared with the clearance times of RT-PCR and microscopy. The effect of several factors such as parasitemia and gravidity on antigen persistence was evaluated.

5.3 Methods

Study area and population

The study was conducted in Nanoro and Nazoanga, in Boulkiemdé Province, Burkina Faso from November to December 2010. This area is considered holoendemic for malaria transmission with \textit{P. falciparum} as the main infecting species [1]. General incidence is high, and malaria prevalence in pregnant women in the country has been reported to range from 20 to 50\% in the high-transmission season [1, 87, 182–184]. Malaria transmission is perennial, with a seasonal peak during the rainy season (June to October). The study population was pregnant women older than 15 years with a gestational age of longer than 15 weeks and resident in the surrounding area of the healthcare facilities. This study was performed in conjunction with a study that assessed the safety and efficacy of three ACTs (artesunate-amodiaquine, arthemeter-lumefantrine & artesunate-mefloquine) for the treatment of malaria in pregnant women carried out at the district hospital of Nanoro: the results of which will be reported elsewhere (Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria (PREGACT); Institute of Tropical Medicine, Belgium; registered at clinicaltrials.gov; NCT00852423). Ethical approval to conduct this study in conjunction with the PREGACT-trial was obtained from the University Hospital in Antwerp (registration number ITG 10 30 2 732), and from the Institutional Ethics committee of Centre Murraz, Burkina Faso (registration number 019-2010/CE-CM).

Study design and sample collection

All pregnant women presenting for routine antenatal care were screened with an HRP2-based RDT, SD Bioline Malaria Antigen P.f. (Standard Diagnostics Inc.). The first 41 women, who were positive in the SD Bioline RDT, had malaria infection confirmed by microscopy and provided informed consent, were enrolled for follow-up. Clinical and obstetric data were collected and
women were allocated to one of three treatment-groups and followed actively for 28 days. At days 0, 1, 2, 3, 7, 14, 21 and 28 finger-prick blood (250-500 µL) was collected in an EDTA tube (microvette, Sarstedt or capiject, Terumo), which was used to prepare thick and thin blood smears, and blood was spotted on filter paper for real-time PCR (RT-PCR). The EDTA tubes were stored at 4°C, because of prior unavailability of the second RDT, Advantage Malcard Pf and PAN (J. Mitra & Co) detecting pLDH, and ELISAs detecting DHFR-TS and HDP. After a maximum of three months storage, the blood was tested with the pLDH-RDT and ELISAs. Blood was collected until the HRP2-RDT was negative on at least one visit or till day 28, since it was expected that this test was positive longer than the others. In the ELISAs, samples from day 0 to day 7 were tested.

**Laboratory procedures**

Blood was applied to both RDTs according to the manufacturers’ procedures. Briefly, blood and buffer were added to appropriate wells and after 15-20 min, depending on the test, the result was read. Test operators were blinded from each other’s test result, although for all tests except microscopy, they were aware of the patients’ visit number.

Microscopy was performed in double reading according to international guidelines by local expert microscopists [185]. Briefly, thick and thin blood smears were Giemsa-stained, and parasites were counted against 200 leukocytes, with parasite negative results based on screening of 100 microscopic fields at 1000× magnification. In case of lower parasitemia (<10 parasites/200 leukocytes) parasites were counted against 500 leukocytes. A leukocyte count of 8000/µL was assumed to calculate the parasite density per microlitre.

Three spots of 30 µL blood were spotted on Whatman 903 protein saver cards, air dried and stored at room temperature (RT) in sealed satchels with desiccant until further processing in the Netherlands. DNA was isolated with the Boom method [186]. Briefly, punched-out filter paper was mixed with guanidium isothiocyanate (GuSCN) lysis buffer and incubated overnight at RT. Silica dioxide was added and subsequently, nucleic acids bound to silica were washed twice with wash buffer (10 M GuSCN, 100 mM Tris-HCl pH 6.4), twice with 70% ethanol, and once with acetone and then eluted in 10 mM Tris-HCl, 1 mM EDTA buffer, pH 8.0 and stored at -20°C.

A *P. falciparum*-specific 18s rDNA RT-PCR was performed on a CFX967M RT-PCR detection system (BioRad) with a FAM-labelled Taqman probe as described before, with a minor adjustment in primer concentrations (0.4 nM) (Biolegio) [83] and probe (5’ FAM-aacaattggagggcaag NFQ MGB-3’) (Biolegio) [187]. The reaction was performed in a final volume of 25 µL containing
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2.5 µL blood, 0.5 U HotStar Taq Polymerase (Qiagen) with Hotstar Buffer (Qiagen) and additional 2.5 mM MgCl2 (Qiagen) and 0.25 mM dNTPs (Qiagen). Each sample was performed in duplicate and a dilution series of *P. falciparum* 3D7 culture (4×10^4 - 4×10^2 parasites/µL) and negative controls (uninfected erythrocytes and Milli-Q water) were taken along. Amplification curves were analyzed with CFX Manager Software (BioRad) with a set threshold of 100. If there were discordant results in the duplicates or day 0 samples were negative, the PCR reaction was repeated. Samples were not declared positive until amplification was present in at least two of the reactions. Quantification was performed with a standard curve that was linear till 4 parasites/µl. The RT-PCR can detect up to 0.1 parasite/µl. Amplification results >4 parasites/µl were considered positive, but the parasitemia was not quantified in these cases.

For the ELISA, 37.5 µL blood was lysed with 75 µL of distilled water, mixed with an equal amount of 50 mM sodium carbonate pH 9.6 and coated in duplicate on two ELISA plates (medium binding, Greiner) for 2.5 h at RT. Wells were washed three times with PBS, 0.1% Tween-20 (PBST). Subsequently, 50 µL of α-HDP H16 (10 µg/mL) or α-DHFR-TS D20 (5 µg/mL) in 1% BSA in PBST was added and incubated for 1 h at RT. Wells were washed three times with PBST and incubated 1 h at 37°C with peroxidase conjugated goat-anti-mouse (Jackson Immunoresearch) 1:5000 diluted in 1% BSA in PBST. Wells were washed with PBST three times and the presence of antibody was measured by adding 0.04% 3,3′,5,5′-Tetramethylbenzidine, 0.04% urea peroxide in 0.1 M sodium acetate citrate pH 4 and the reaction was stopped with 0.5 M sulfuric acid. Colour development was measured at 450 nm with a Multiskan FC ELISA reader (Thermo Scientific).

**Sample size estimation**

To determine the difference between the mean visit numbers for the tests to remain positive, a sample size was calculated considering an expected difference of at least one visit number (7 days) and expected standard deviation of 1.4 visit number (10 days). With a desired level of significance, α of 0.05 and power of 80%, 30 cases were needed to be statistically significant (EpiCalc2000 v1.02). Because approximately 10% of study cases are expected to be excluded or drop out for any reason from the study, 34 cases should be included.

**Data collection and statistical analysis**

Demographic, clinical and obstetric data, including Hb measured by haemocue and axillary temperature as well as test outcome, were collected on case record
5.4. RESULTS

Forms and double-entered in an Access database (Microsoft 2003). Calculations were performed in STATA (version 9, StataCorp LP). Shapiro-Wilk test was used to test for normality ($W > 0.9$), and not-normal distributed variables were log-transformed for the analyses. A two-sample t-test was used to test the effects of several factors on the time a test remained positive. One-way ANOVA was used to determine whether there was a difference in mean temperature and Hb-value at each visit and to determine whether the mean number of visits that a test remained positive differed with the ACT-treatments. Kappa value was calculated to measure the level of agreement between the two ELISAs. For the purpose of comparing mean time for a test to remain positive, day numbers were transformed to visit numbers (i.e. Visit 1= day 0; visit 2= day 2; visit 3= day 3; visit 4= day 7, visit 5= day 14, etc.), because samples were only taken at the designated days, while an antigen could already have disappeared in between visits. A paired t-test was used to determine the difference between the mean visit numbers to remain positive of two tests or in case of not-normal distributions a Wilcoxon signed rank test. p-values below 0.05 were considered statistically significant.

### 5.4 Results

**Table 5.1 Baseline characteristics of the 32 pregnant women at inclusion.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age [yr]</td>
<td>25.5 (5.0)</td>
</tr>
<tr>
<td>Mean (SD) temperature [°C]</td>
<td>37.0 (0.5)</td>
</tr>
<tr>
<td>Mean (SD) hemoglobin [g/dL]</td>
<td>9.7 (1.4)</td>
</tr>
<tr>
<td>Mean (SD) gestational age [weeks]</td>
<td>25.7 (4.6)</td>
</tr>
<tr>
<td>Mean (SD) gravidity [# of pregnancies]</td>
<td>3.5 (2.0)</td>
</tr>
<tr>
<td>Geometric mean parasite density (95% confidence interval) by microscopy [parasites per µl]</td>
<td>611 (312 - 1198)</td>
</tr>
</tbody>
</table>

**Characteristics of study subjects**

A total of 41 pregnant women with positive results in microscopy and HRP2-RDT at day 0 were enrolled. Blood samples were taken on all visits until the RDT was negative at least one visit or up to day 28. Women that missed interim visits were not included in the analysis; therefore, nine patients were excluded. The majority of women (20/34) presented without symptoms. In the 14 women with symptoms, headache was reported most often (N = 6), followed by fever in the past 24 h (N = 4) and muscle and/or joint pains (N = 3). Women were treated with one of three ACTs at day 0, as determined by the protocol.
of the PREGACT-trial; 13 with mefloquine-artesunate, 11 with arthemether-
lumefantrine and 8 with amodiaquine-artesunate. Baseline characteristics at
day 0 of included women were summarized in Table 5.1 and were not different
from the excluded women, except that the mean age of excluded women was
lower (21.8 ± 1.3). Mean temperature of the patients decreased from 37.0 ±
0.49 at day 0 to 36.6 ± 0.40 at day 28 (one-way ANOVA, p = 0.0083). Hemoglobin was measured at day 0, 7, 14 and 28 and increases from a mean
of 9.7 ± 1.4 at inclusion to 10.5 ± 1.2 at day 28 (one-way ANOVA, p = 0.0241).

Test outcome and agreement at inclusion

At day 0, all included women (32/32) were positive with HRP2-RDT and mi-
croscopy. One day 0 sample was not available for both RT-PCR and ELISA.
With the RT-PCR 100% (31/31) of the women tested positive and 62.5% (20/32), 64.5% (20/31) and 80.6% (25/31), respectively, with pLDH-RDT,
DHFR-TS- and HDP-ELISAs. The DHFR-TS-ELISA had a moderate agreement
with the HDP-ELISA (κ = 0.45 ± 0.17, p = 0.0032).
5.4. RESULTS

Persistence

In Figure 5.1, the number of positive patients at each visit number was plotted, showing that the HRP2-RDT stays positive much longer than other tests. The pLDH-RDT and microscopy become negative three days after treatment, followed by PCR with approximately one day delay. On day 7, however, five women were positive again in PCR after being negative at earlier visits. With the HRP2-RDT, 41% (13/32) of the women were still positive at day 7, which decreased slowly in the weeks after, and two women remained positive at day 28. With the ELISAs, the number of positive women decreases gradually till approximately 15% (4/31 and 5/31) at day 7. Gametocytes were encountered by microscopy in five patients, all on day 0, except for one woman, where they were found only on day 2. With the HRP2-RDT, the mean visit number [95% confidence interval] with the last positive test result was 3.4 [2.7-4.1] visits and median [range] of 4 [1-6]., which is significantly later than microscopy (median 1 [1-3]), pLDH-RDT (mean 0.84 [0.55-1.1]), RT-PCR (median 2 [1-5]) and DHFR-TS-ELISA (mean 1.7 [1.1-2.3]) (p < 0.05). The mean visit number [95% CI] with the last positive test result in the HDP-ELISA (2.1 [1.6-2.7]) was not significantly different from the HRP2-RDT (p = 0.053), but the HDP-ELISA was significantly different from microscopy (p = 0.0123). The PCR was significantly different from all tests (p < 0.05), except the ELISAs (DHFR-TS p = 0.8784 and HDP p = 0.0578). The mean visit number with the last positive test result of the pLDH-RDT is significantly smaller than all other tests (p < 0.05).

Factors affecting persistence

Parasite density by microscopy at day 0, gravidity and age had a significant effect on the mean visit number with the last positive test result, but not for all tests (Table 5.2). At above median parasite densities (≥500 parasites/µL), mean visit numbers were significantly higher for all tests, except the HDP-ELISA (two-sample t-test; p < 0.05) (Table 5.2). Presence of gametocytes (N = 5) did not have a significant effect on mean visit number with the last positive test result for any test. Primigravidae had a significantly higher parasite density (2963 [714-12301]) compared to multigravidae (393 [193-801]) (two-sample t-test; p = 0.0089). The significant effect of gravidity on mean visit number might be because of higher parasite densities seen in primigravidae. The same counts for patients with age below 25 years, which had a significantly higher parasite density (1401 [559-3514]) than patients with age above 25 (267 [110-646]) (p = 0.0095). There were four women with severe anaemia (Hb value < 8.0 g/dL) at day 0, which was too few to find a significant difference in persistence for any test compared to women with Hb value ≥ 8.0 g/dL. The type of ACT did
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not have an effect on the mean visit number with the last positive result for all tests (one-way ANOVA; p > 0.05).

### Tabel 5.2 Factors that have an effect on the mean visit number that has the last positive test result for the different tests.
Means [95% confidence interval (CI)] are listed for each test in each group, as well as p-values that were determined by two-sample t-test with equal variance (bold p-values are statistically significant).

<table>
<thead>
<tr>
<th></th>
<th>Mean visit number [95% CI] with the last positive test result</th>
<th>Parasite density</th>
<th>Parasite density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>&lt; 500 p/µl (N=16)</td>
<td>≥ 500 p/µl(N=16)</td>
</tr>
<tr>
<td>SD Bioline (HRP2)</td>
<td>29</td>
<td>2.2 [1.5 - 2.9]</td>
<td>4.9 [4.3 - 5.5]</td>
</tr>
<tr>
<td>Microscopy</td>
<td>32</td>
<td>1.0 [1.0 - 1.0]</td>
<td>1.4 [1.1 - 1.8]</td>
</tr>
<tr>
<td>Advantage Malcard (pLDH)</td>
<td>32</td>
<td>0.31 [0.057 - 0.57]</td>
<td>1.4 [0.99 - 1.8]</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>30</td>
<td>1.4 [1.0 - 1.8]</td>
<td>2.2 [1.7 - 2.7]</td>
</tr>
<tr>
<td>DHFR-TS-ELISA</td>
<td>27</td>
<td>1.1 [0.14 - 2.0]</td>
<td>2.3 [1.5 - 3.1]</td>
</tr>
<tr>
<td>HDP-ELISA</td>
<td>27</td>
<td>2.4 [1.5 - 3.2]</td>
<td>1.9 [1.1 - 2.8]</td>
</tr>
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<td>≥ 500 p/µl(N=16)</td>
</tr>
<tr>
<td>SD Bioline (HRP2)</td>
<td>29</td>
<td>5.6 [4.9 - 6.3]</td>
<td>3.0 [2.3 - 3.7]</td>
</tr>
<tr>
<td>Microscopy</td>
<td>32</td>
<td>1.4 [0.70 - 2.2]</td>
<td>1.2 [1.0 - 1.3]</td>
</tr>
<tr>
<td>Advantage Malcard (pLDH)</td>
<td>32</td>
<td>1.7 [1.0 - 2.4]</td>
<td>0.6 [0.33 - 0.87]</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>30</td>
<td>2.4 [1.3 - 3.6]</td>
<td>1.6 [1.3 - 1.9]</td>
</tr>
<tr>
<td>DHFR-TS-ELISA</td>
<td>27</td>
<td>3.0 [2.1 - 3.9]</td>
<td>1.3 [0.64 - 2.0]</td>
</tr>
<tr>
<td>HDP-ELISA</td>
<td>27</td>
<td>1.8 [-0.24 - 3.8]</td>
<td>2.2 [1.6 - 2.9]</td>
</tr>
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</tr>
<tr>
<td>SD Bioline (HRP2)</td>
<td>29</td>
<td>4.3 [3.2 - 5.4]</td>
<td>2.7 [1.9 - 3.5]</td>
</tr>
<tr>
<td>Microscopy</td>
<td>32</td>
<td>1.3 [0.99 - 1.6]</td>
<td>1.1 [0.94 - 1.3]</td>
</tr>
<tr>
<td>Advantage Malcard (pLDH)</td>
<td>32</td>
<td>1.2 [0.70 - 1.7]</td>
<td>0.5 [0.22 - 0.78]</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>30</td>
<td>2.0 [1.4 - 2.6]</td>
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</tr>
<tr>
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<td>2.3 [1.4 - 3.2]</td>
<td>1.1 [0.30 - 2.0]</td>
</tr>
<tr>
<td>HDP-ELISA</td>
<td>27</td>
<td>2.3 [1.4 - 3.3]</td>
<td>2.0 [1.2 - 2.8]</td>
</tr>
</tbody>
</table>

### 5.5 Discussion

This study demonstrated that the HRP2-RDT stays positive longer after treatment in pregnant women (3.4 ± 1.8 visits) than other commonly available diagnostic tests such as microscopy and a pLDH-RDT. This can be attributed to persistence of HRP2 antigen in the circulation as has been reported with non-pregnant individuals, where HRP2 persistence also occurred up to 28 days even when there was rapid clearance of microscopically detectable parasites [67–71, 73, 74]. Higher parasite densities at baseline have been correlated with a prolonged antigen persistence time, which is confirmed by this study [67,69–71].

One of the limitations in the study was that not all patients could be followed until they were negative in all tests, because of to the setup of the study. Two patients were positive in the HRP2-RDT up to day 28, and one patient was positive till day 14, after which no more samples were collected. In the
ELISA, samples till day 7 were tested, and for three patients each, the ELISAs remained positive till this day. For these patients, the exact visit when the tests became negative could not be determined and could not be included in the analyses. Another limitation is that the pLDH-RDT and the ELISAs were performed on stored blood, which might have negatively influenced the accuracy of these tests due to degradation of antigen. A group of approximately 400 women were screened at antenatal care visits (of which the patients included in this study are the first 41 positive women). These women were part of a larger study that will be reported separately. No significant difference in accuracy of the pLDH test between stored blood and fresh blood samples was found (unpublished data).

WHO has reported which RDTs are more likely to provide higher sensitivity in the field [62, 63]. The performance characteristics of the Advantage Malcard RDT is superior to the other PAN + P. falciparum detecting pLDH-based RDTs, and the SD Bioline RDT was one of the 10 best-performing tests. In general, pLDH-based RDTs perform less well than HRP2-based tests, most likely due to the poor sensitivity of pLDH tests at lower parasite densities [5, 63, 78].

Although RDTs are a practical and accurate alternative to microscopy for the diagnosis of pregnant women, the persistence of the antigens can have clinical implications. Especially, HRP2 can persist for a long period after ACT treatment: therefore, this type of test is not suitable for treatment follow-up in pregnant women. No difference was found in duration of persistence between the three ACT combinations, and there is no reason to expect that the persistence will be shorter with other drugs; especially as in non-pregnant patients, longer persistence was reported after treatment with non-ACT drugs [67, 74]. This poses particular problems for pregnant women, first of all as SP clears parasites more slowly and induces more gametocytes than ACTs [55, 188]. A positive HRP2-based RDT result between IPTp doses can mean the presence of a true infection, the presence of only gametocytes or or it maybe false-positive result because of HRP2 persistence. This has consequences for countries where besides IPTp, HRP2-RDTs are used or considered as routine diagnostics for malaria in pregnancy, such as Burkina Faso, as this can lead to unnecessary treatment and possible under-recognition of other diseases. Secondly, in screen-and-treat strategies, where women are regularly screened during antenatal care visits, antigen persistence will be a problem, especially if the screening is performed within a short time window. In the case of monthly screening, the problems will be limited, as only 2/32 women were positive in the HRP2-RDT after 28 days. However, in the case of weekly screening many women will have persistent HRP2 seven days after treatment. Microscopy and pLDH-based RDTs are more accurate at showing parasite clearance after treatment, but insufficient sensitivity has been reported for
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the diagnosis of placental malaria [5]. This highlights an obvious need for new easy-to-use diagnostics with a better detection threshold and accuracy than pLDH-based RDTs, but fewer problems in terms of antigen persistence. If pLDH or possibly antigens such as DHFR-TS which are less persistent than HRP2 could be optimized to more sensitive assays they could be a good alternative for malaria diagnosis in pregnant and non-pregnant individuals.

5.6 Acknowledgements

We acknowledge the work of Anneke Taal and Yao Mnimou for the collection of the blood samples and for the performance of the RDTs, preparation of slides and PCR spots and Anneke Taal and Janneke Zoeten for performing RT-PCR. We thank the clinical and laboratory staff at the Clinical Research Unit of Nanoro (CRUN) for their contribution to the work and all patients for participating in the study. We acknowledge Umberto D’Alessandro (Institute of Tropical Medicine, Antwerp, Belgium) as coordinator of the PREGACT-trial, and thank him for allowing us to perform this study in conjunction and for his contributions in the preparation of the study protocol. The EDCTP, the Belgian Cooperation and SANOFI gave financial support for the PREGACT-trial in Burkina Faso. The work of JH Kattenberg is supported by the Foundation for Innovative New Diagnostics (FIND) in a collaborative effort for the improvement of malaria diagnosis.