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Diagnosing malaria in pregnancy: an update

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Abstract

Pregnancy malaria (PM) due to *Plasmodium falciparum* is a major cause of morbidity and mortality for women and their offspring, but is difficult to recognize and diagnose. During PM, parasites typically sequester in the placenta, whereas peripheral blood smears often appear negative. In addition, many infected women remain asymptomatic, especially in areas of high transmission where systemic immunity is high, although sequelae including maternal anemia and intrauterine growth retardation develop insidiously and increase mortality. New rapid diagnostic tests (RDTs) have shown promise for malaria diagnosis in nonpregnant individuals, including a product recently approved by the US FDA for use in the USA. However, the sensitivity and specificity of RDTs for diagnosis of PM may be suboptimal. Here, we review the methods that are used to detect or diagnose PM, including blood smear microscopy, RDTs, PCR-based methods, and finally placental histology, which is often cited as the gold standard for use in research studies and clinical trials.

Keywords

diagnosis; PCR; placenta histology; *Plasmodium falciparum*; pregnancy malaria; RDT

Introduction

Malaria is a deadly threat to pregnant women, but diagnostic tools often fail to accurately define either infected or uninfected women. Pregnancy malaria (PM) causes severe anemia in the mother and low birthweight (LBW) in the child, and these sequelae alone result in an estimated 10,000 maternal deaths and 200,000 infant deaths annually in Africa [1,2]. In

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Ethical conduct Human subjects: the manuscript includes original data obtained from pregnant women who were recruited between September 2002 and October 2005 into a longitudinal cohort conducted by the Mother–Offspring Malaria Studies Project in Muheza district, Tanzania. Pregnant women 18 years or older without clinical evidence of chronic or debilitating illness were asked to participate in the study and gave signed informed consent after receiving a study explanation form and oral explanation from a nurse in their native language. The protocol and study procedures were approved by the International Clinical Studies Review Committee of the Division of Microbiology and Infectious Diseases at the US NIH. Ethical clearance was obtained from the institutional review boards of Seattle Biomedical Research Institute and the National Institute for Medical Research in Tanzania.

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malaria endemic areas, pregnant women are more susceptible to malaria infection than their nonpregnant counterparts[3,4]. Susceptibility diminishes with successive pregnancies, and this pattern is most prominent in high transmission areas where primigravidae are significantly more susceptible to *Plasmodium falciparum* infection and disease than multigravidae [3,4]. This parity-dependent epidemiological signature distinguishes *P. falciparum* from several other infectious agents that can afflict pregnant women. In low transmission areas, women of all parities have increased susceptibility to malaria, although infection rates may still be highest in primigravidae [5]. Women in low transmission areas lack strong systemic immunity and are more likely to develop severe syndromes like respiratory distress and cerebral malaria [5].

PM is difficult to recognize and diagnose. During *P. falciparum* infections, parasites sequester in the placenta but are often undetectable in peripheral blood smears (BS), especially in high transmission areas [4]. Women in zones of high malaria transmission are often asymptomatic, leading to chronic untreated PM with insidious consequences that can include severe anemia, hypertension and LBW newborns. Other factors add complexity to the presentation, detection and outcome of PM. Mixed infections of *P. falciparum* and *Plasmodium vivax* might alter PM outcomes, but many mixed infections appear as mono-infections by peripheral BS. *P. vivax*, like *P. falciparum*, is associated with poor pregnancy outcomes [6], but unlike *P. falciparum*, the clinical sequelae may be more common in multigravid pregnancies (reviewed in [2,7]).

Paradoxically, although PM is difficult to recognize and diagnose, many women in endemic areas unnecessarily receive antimalarial treatments in the absence of infection. In Mozambique, over 70% of pregnant women with clinical symptoms of malaria (fever, headache and joint pain) have negative BS [8]. Because antimalarials are often prescribed on the basis of clinical and not laboratory criteria, many pregnant women receive unnecessary treatment with drugs that have an unclear safety profile, particularly during the first trimester when teratogenic effects are most likely.

A recent meta-analysis compared the performance of rapid diagnostic tests (RDTs) to peripheral and placental blood microscopy, PCR and placental histology [9]. Here, we review the methods available for the diagnosis of PM, and we relate diagnosis by the different methods to pregnancy outcomes. We also consider placental histology as a diagnostic tool, because it is the gold standard by which novel interventions, biomarkers and new diagnostics are frequently assessed.

Blood smears & rapid diagnostic tests

Pregnancy malaria detection in peripheral blood

Currently, two types of diagnostic tools for PM are available for clinical practice: BS microscopy, which is viewed as the gold standard owing to longstanding clinical practice; and RDTs that detect soluble *Plasmodium* antigens including HRP-2, aldolase or pLDH. Several studies compared the performance of RDT that detect soluble HRP2 or pLDH to other methods like peripheral BS, placental BS, placental histology, or PCR of placental blood (Table 1). The OptiMAL test, based on the detection of pLDH, gave varying results between studies when compared with peripheral BS. The sensitivity ranged from 15 to 96.6% and specificity from 90.8 to 98% [10–12]. The sensitivity of the OptiMAL test increases with parasite density. In one study, all samples with parasite density of <100/μl were missed [11]. In a larger study [12], OptiMal had 100% sensitivity and 93.3% specificity at parasite densities >50/μl blood, but a sensitivity of only 57.1% at lower parasite densities.

In general, RDT-HRP2 tests have a higher sensitivity compared with RDT-pLDH. When performed on peripheral blood samples for PM diagnoses, RDT-HRP2 sensitivity was more than 90% when compared with peripheral BS, and 80–95% when compared with placental BS, with specificity between 61 and 94% [13–16]. The sensitivity of RDT-HRP2 using peripheral blood samples was much lower when compared with PCR detection of parasite nucleic acids in peripheral or placental blood (Table 1)[15–17].

Several RDTs performed similarly as PM diagnostics in a recent WHO-coordinated evaluation that used peripheral blood microscopy as a reference [101]. In studies that used both peripheral and placental blood microscopy as references, RDT sensitivity was lower when compared with placenta blood microscopy [10,13,18]. Even under optimal conditions, a large proportion of PM cases are missed by peripheral blood microscopy ([13] and Table 1), which calls into question the use of peripheral BS as a reference for PM diagnosis. Compared to PCR performed on placental or peripheral blood [14,15,17], the sensitivity of RDT tests was low in two of three PM diagnostics studies. RDTs also demonstrated variable sensitivities compared with PCR for the diagnosis of malaria in nonpregnant symptomatic populations [19–22].

An important weakness of RDT-HRP2 tests is the prolonged half-life of the antigen. HRP-2 can be identified in plasma samples several weeks after parasite clearance, and therefore cannot be used to distinguish current from recent infection [23–25]. RDTs that detect pLDH are designed to detect only live parasites; however, the sensitivity for diagnosing PM is low. Gametocytemia in the absence of asexual blood-stage parasites can also produce positive results. These shortcomings hinder the use of existing RDT for managing malaria, and also for monitoring treatment efficacy [23–25]. In a recent study that followed pregnant women after treatment with artemisinin combination therapy [26], 2 out of 32 parasitemic women (diagnosed by BS and HRP2-RDT) continued to have detectable HRP2 antigen 28 days post-treatment [26].

Placental blood rapid diagnostic test

RDT performance using placental blood samples has also been evaluated. In these studies, RDT tests had a sensitivity of >87.5%, but a relatively large variation in the test specificity ranging between 68 and 97% (Table 2) in comparison to placental BS and placental PCR.

Parasite detection by DNA amplification & submicroscopic infection

PCR methods to detect malaria infection were described more than two decades ago, and are generally more sensitive for detecting parasite DNA than thick BS is for detecting parasites [27,28]. Real-time quantitative PCR (qPCR) methods have followed, with good sensitivity and range that allows monitoring parasitemia levels [29,30]. qPCR and similar methods have become popular for early detection of parasites in vaccine trials and experimental human infections [31,32]. Loop-mediated isothermal amplification (LAMP) is a newer alternative to PCR and qPCR. This method does not require DNA purification, utilizes simple instrumentation (water bath or a heat block), and can be completed in less than 1 h [33,34]. Several studies that compared the LAMP method to microscopy, PCR and RDT, reported high sensitivity and specificity compared with microscopy or PCR in nonpregnant populations [34–37]. The performance of the LAMP method for PM diagnosis has not yet been reported.

Whether applied to peripheral blood or placental blood samples, PCR methods yield positivity rates 20% or more above those of BS microscopy (Tables 3 & 4) [10,14–16,38–46]. Infections identified by PCR, qPCR or RDT when microscopy fails to detect parasites on BS are defined as submicroscopic or subpatent infections (although distinguishing

subpatent infection from persisting nucleic acid or antigen after parasites are cleared is not always possible). Both PCR and qPCR methods have been applied to detect submicroscopic PM, and have been used as research tools in the context of clinical trials and epidemiological studies. Although these tools are more sensitive for parasite detection compared with microscopy, the test format and the time to obtain results are not suitable for use in a primary care setting.

In our studies in Muheza, Tanzania, PM (defined by microscopy of placental BS) is significantly more frequent among primigravid compared with multigravid women, similar to earlier reports. However, sub microscopic infections showed the opposite pattern, and were more frequent in multigravid than primigravid women (Figure 1). An earlier study also reported higher rates of submicroscopic infection with increasing parity [47], whereas other studies have reported similar rates of sub microscopic infections among women of different parities [16,38,40]. An increased rate of submicroscopic infection among multigravid women suggests that acquired immunity to PM controls parasite density but does not prevent and does not completely clear infection.

Hemozoin detection

Hemozoin (or ‘pigment’) is polymerized heme produced by parasites during hemoglobin digestion. Hemozoin can be detected by polarized light [48], by the fluorescent properties of hemozoin which allows quantification in tissue [49], and by laser desorption mass spectrometry (LDMS) that identifies distinct spectral features [50]. In placental samples, polarized microscopy has aided in detecting low placental parasitemia, and in the absence of parasitemia indicates past infection [51]. However, artifacts such as formalin pigment and dust particles can mimic hemozoin [51] and can result in misdiagnosis (see below). LDMS has been evaluated as a tool for PM diagnosis [52]. LDMS detects parasites in the range of 100–1000/μl blood in samples collected from pregnant women, similar to microscopy. However, LDMS does not distinguish malaria species, and macrophages containing hemozoin can yield positive results, which complicates the differentiation of current versus past infection [52].

PM diagnosis & clinical outcomes

PM has been associated with poor outcomes such as reduced birthweight, LBW and maternal anemia in numerous studies (reviewed in [2,7]). More recently, malaria diagnoses made using RDT and PCR methods have been examined for their associations with poor pregnancy outcomes. Several studies compared PM outcomes diagnosed by microscopy to those diagnosed by PCR and RDT in either peripheral blood, placental blood or both. Clinical end points included maternal anemia, birthweight, LBW and preterm delivery. Parasites detected by microscopy, HRP2-RDT or PCR in maternal peripheral blood or placental blood were associated with mild maternal anemia (hemoglobin <11 g/dl) [16,53]. However, the association of maternal anemia to submicroscopic infection (defined by PCR, qPCR or HRP2 methods) is inconsistent, with some studies finding a relationship [16,47,53] while other studies did not [38,40,47].

In one study, reduced birthweight was associated with parasites detected by microscopy, RDT-HRP2 or PCR in placental blood, but not parasites detected in peripheral blood [15]. In another study, birthweight was associated with positive RDT-pLDH assays but not positive PCR assays performed on placental blood or peripheral blood [10]. HRP2 or pLDH detected in placental blood has been associated with increased rates of LBW in some studies [10,15,53] but not in Mozambique [16]. Positive placental blood PCR was associated with an increased rate of LBW in one study [15] but not several others [10,16,53]. Submicroscopic placental infection was associated with reduced birthweight in several

studies [15,44,45]. We and others ([42] and Figure 2) did not find an association between sub microscopic parasitemia and reduced birthweight, unlike parasitemia detected by either peripheral or placental BS, which is associated with a significant reduction in birthweight.

Two studies reported that PM detected by either BS microscopy or by PCR only (submicroscopic infection) was associated with increased LBW deliveries [44,45]. However, other studies did not observe an association between submicroscopic infection and increased LBW [15,42]. In analyses from our cohort, both submicroscopic and microscopic placental infection were associated with increased LBW deliveries among primigravid women (submicroscopic: odds ratio: 3.025, $p = 0.03$ and microscopic: odds ratio: 3.98, $p = 0.007$), whereas among multigravid women only microscopically detected PM was associated with increased risk of LBW. Because a single infection during pregnancy contributes to poor pregnancy outcomes [2], submicroscopic infections among primigravid women may indicate an earlier patent infection causing LBW, which would also be reflected by increased pigment deposition (calculated as the percentage of fields containing pigment in intervillous fibrin) [54].

Histology to detect PM

Overview

Histology does not have a role in clinical diagnosis of PM, but serves as a valuable tool in epidemiological studies and clinical trials in which PM is an end point. Placental histology has been referred to as the ‘gold standard’ for its ability to detect sequestered parasites when none are detected in the peripheral circulation [55]. However, interpretation of placental histology carries several caveats: women with malaria detected by weekly ante natal screenings and effective treatment often have no histological changes at delivery [56,57]. Erythrocytes can be altered by histology processing methods in different ways, which can hinder recognition of true parasites or introduce artifacts that falsely appear as parasites (Figure 3).

An advantage of histology over other methods is that it identifies features of PM relevant to clinical outcomes, such as inflammatory infiltrates and hemozoin deposition. Inflammatory infiltrates occur in a subset of women with active malaria infection, particularly first-time mothers, and is strongly linked to LBW. Hemozoin can persist for months in women following treatment of a documented infection [58], and the extent of hemozoin deposition is thought to correlate with cumulative exposure [54] such that levels have been associated with gravidity, the degree of parasitemia at the time of treatment and reinfection before delivery [57]. For basic and applied research, the collection and analysis of placental tissue provides a unique opportunity to study sequestered *P. falciparum* parasite stages and their interactions with the living human host across the spectrum of presentations, from asymptomatic to severe. Challenges with placental histology studies of PM include limited standardization between laboratories in processing and scoring tissue, and limited infrastructure to properly collect, process and analyze placental samples in some tropical areas.

Methodologies

Two methodologies are most relevant for placental histology: fresh frozen versus formalin fixation with paraffin embedding (FFPE). During standard handling, both fresh frozen and FFPE processing can alter tissue in various ways that affect interpretation. Fresh frozen samples require the least amount of processing: red blood cells (RBCs) lyse (parasites remain intact) and host cell morphology can change with loss of fine cellular and nuclear detail. With FFPE tissues, some erythrocytes often diffuse out during storage and processing, and are thus not sampled for histology. During dehydration, tissue contracts and

causes an artifactual separation between trophoblast surface and RBCs, which can make adhesion of infected RBCs to trophoblasts difficult to appreciate, although this interaction has been well demonstrated by electron microscopy [59].

Formalin pigment (acid hematin) is the most damaging artifact because it is optically indistinguishable from malarial hemozoin. Buffered formalin needs to be used during fixation and processing, and tissue needs to be cut thin for fixation and processing into paraffin. Other methods to prevent formalin pigment artifact include use of non-formalin fixatives, or prompt transfer of specimens from formalin to 70% ethanol for long-term storage (which also preserves morphology). Finally, staining can introduce debris in both FFPE and frozen sections that may mimic hemozoin within erythrocytes or in fibrin.

Interpretation

Histological classification of PM is useful for epidemiological studies, and has been utilized in interventional trials. PM categories were first developed by Garnham in 1938 [60], who described a massive accumulation of inflammatory phagocytic cells in some women whereas other women experienced little or no inflammation. The most widely used classification scheme was developed by Bulmer in 1993 [55] with acute, chronic and past infection categories, based on the presence of parasitized erythrocytes and/or hemozoin in fibrin. Subsequent schemes have generally modified or refined the Bulmer classifications [61–64]. In anticipation of future interventional trials, we developed a grading scheme that used either frozen or FFPE tissues and incorporated semiquantitative scoring of inflammation and hemozoin deposition, and found both measures are independently associated with clinical outcomes [54]. The specifics of these individual grading schemes are discussed elsewhere [54], and here we will focus on common pitfalls and limitations.

Rare infected erythrocytes are difficult to detect by histology and there are concerns regarding both sensitivity and specificity. Extended searching of individual sections for rare parasites increases the risk of false positive, since it becomes more likely that artifact will be encountered. Extensive processing of RBCs in histological sections (described above) affects their morphology and can introduce pigment artifact (Figure 3). While BS have well-established criteria for defining positives (WHO), there are no published or standardized criteria that define a positive tissue section. Furthermore, the literature can be confusing as when the description ‘pigment within RBC’ does not explicitly state whether parasite cytoplasm can be visualized [55]. In the current era of decreasing transmission and increasing interventions, rates of placental parasitemia are expected to decrease. With low rates of true positives, the proportion of false-positive malaria diagnoses (using any tool) will increase.

Several methods can be more sensitive than histology to detect parasites, such as RDT and PCR described above. Hypothetically, thick and thin smear of mechanically extracted placental blood will allow for increased sensitivity (more RBC and better preserved RBCs) and increased specificity with the use of strict diagnostic criteria. Past infection can be determined much more reliably in histology studies, and is based on the presence of hemozoin deposition in fibrin. However, as noted above, formalin pigment can mimic hemozoin, and unfortunately most studies do not comment on the specimen quality, nor whether samples were excluded due to background artifact or read in the presence of formalin pigment.

Histology, quality control & quality assurance

Because PM histology is retrospective, there has been little scrutiny of it as a ‘gold standard’, unlike other histologic diagnoses where a false positive can lead to a disastrous

intervention. In order for histology to be a reliable metric in future studies, it can be argued that specificity should be favored over sensitivity. False negatives are expected in the presence of more sensitive tests including PCR, RDT or placental BS, whereas a high false-positive rate would result in incorrect assessment of the accuracy of other diagnostic tests, such as RDT, that could have widespread clinical utility to screen pregnant women. Without robust quality control/quality assurance, studies that report a high rate of histology positives in the setting of negative results by other methods (PCR, RDT and placental BS) should be interpreted with caution.

Histopathological processing and interpretation takes considerable training. This is often conducted as one-on-one training sessions and targeted workshops. A placental histology slide set has recently been made available through the Malaria Research and Reference Reagent Resource Center. An analysis of intraobserver variability has not been performed, which would provide objective confidence intervals for interpreting the literature. Standardized approaches for proficiency testing and training of staff would also lead to increased consistency between study sites.

Multiple reading of histological sections can improve sensitivity and specificity. However, the majority of studies have used single reads. Several studies have incorporated multiple reads [16,55,62,63,65,66] including reads by microscopists at different institutions [55,66]. BS, PCR and RDT results can be used to confirm histology reads, and have potential to enhance training for future studies. Negative results with other diagnostic tools can be used to identify false positives, and positive results with other tools should prompt a second histologic examination to exclude a false negative, keeping in mind that histology may be less sensitive.

Placental histology versus placental BS

Similar to placental BS, placental histology is more sensitive than peripheral BS for detecting PM (33 vs 21%, respectively, and 52.5 vs 17.4%, respectively) [67,68]. Placental histology was also found to be more sensitive than placental BS in several studies, with diagnoses by histology occurring at a rate 1.2–1.7-fold higher than placental BS [16,69–71]. In the Mother–Offspring Malaria Studies Project experience, only one among 207 parturient women without detectable parasites in placental BS had parasites detected by histology, and in that unusual case parasites appeared as a single nidus in one intervillous space [72]. PM detected by histology has uniformly been associated with poor pregnancy outcomes such as reduced birthweight, LBW and maternal anemia.

Expert commentary & five-year view

Current WHO guidelines recommend that RDT assays must achieve 90% specificity at 95% sensitivity to be implemented for malaria diagnosis [102]. In a recent evaluation of RDT performance, many kits achieved this threshold in nonpregnant populations [101]. However, among PM studies, RDT met this standard in only one study [13]. Although RDT-HRP2 has higher sensitivity compared with BS or RDT-pLDH on peripheral blood samples, it fails to detect many PM cases detected by placental BS. This is especially common in areas with high malaria transmission levels where women with substantial systemic immunity often have negative peripheral BS and/or remain asymptomatic during infection. In three of four studies (Table 1), using peripheral BS as a reference increases the sensitivity and specificity of many RDTs, but the sensitivity and specificity of RDT were lower when placental blood microscopy was used as a reference, raising a critical question of whether peripheral BS is an adequate reference. During PM, parasites sequester in the placenta, and most of the parasites collected from the peripheral blood of pregnant women during the antenatal period or at delivery bind to the placenta receptor chondroitin sulfate A. That indicates that the

sequestered parasite biomass in pregnant women is often limited to placental intervillous spaces, which represents a small proportion of the total body vasculature. In addition, normal pregnancy-related hemodilution increases plasma volume to a greater degree than RBCs volume (2.5–3.75 l and 1.2–1.5 l, respectively) [73]. Thus, 1 ml of blood from a pregnant women will contain fewer parasites (and parasite antigens) compared with 1 ml of blood collected from a child or a nonpregnant adult. These two factors, biomass concentration in the placenta and hemodilution, could be the main reasons for the lower performance of RDTs to detect PM. For these reasons, diagnostic performance assessed in nonpregnant populations, who are often presenting with symptoms, may not be extrapolated to PM. On the other hand, PM provides an opportunity to relate parasite biomass (placental parasitemia at delivery) with RDT sensitivity. Future testing of RDTs should focus on relating test performance with placental parasitemia.

Owing to the spread of drug resistance, the accuracy of RDTs to detect PM is critical when considering alternatives such as intermittent screening and treatment instead of intermittent preventive treatment with sulphadoxine-pyrimethamine [74]. In areas of low malaria transmission, a single infection during pregnancy negatively affected fetal development [2], emphasizing the importance of further evaluating diagnostic tools that can be implemented at the point of care. Several ongoing studies are relating RDTs performance during pregnancy with placental infection at term that may contribute to a better selection of RDT for PM diagnosis.

Although RDT-HRP2 has higher sensitivity compared with BS or RDT-pLDH on peripheral blood samples, it fails to detect many PM cases detected by placental BS. This is especially true in areas with high malaria transmission levels where women with substantial systemic immunity often have negative BS and/or remain asymptomatic during infection. One weakness of HRP2-based tests is the persistence of antigen for several weeks after effective treatment, which reduces the test specificity. PM is associated with inflammatory immune responses [75–79], with increased levels of soluble mediators like TNF-α, TNF receptor I, TNF receptor II and IL-10 in peripheral blood [80,81]. TNF receptor II may be increased in women with submicroscopic infection as well [81]. Other proposed biomarkers to detect PM include a combination of three markers (sFlt1, leptin and C-reactive protein) [82] and soluble endoglin, which increases during PM and, like sFlt1, is a marker of preeclampsia [83]. These biomarkers may not be specific for PM, and a combination of an inflammatory marker together with a parasite marker has the potential to increase the sensitivity and specificity to detect infection and to improve care during pregnancy.

Evaluation of PM at the time of delivery by placental examination plays an important role in epidemiological studies as well as in clinical trials in which PM is an end point. Placental histology has been referred to as a gold standard. The reported higher sensitivity of placental histology compared with placental BS could have various explanations, including differences in placenta sampling methods, and false positives by histology. Additional studies that compare histology to placental BS will be valuable, as the latter requires minimal laboratory infrastructure. Standardization of quality assurance/quality control for placental histology is also needed to ensure reliability and comparability between clinical studies.

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Key issues

- Timely treatment and effective management of pregnancy malaria (PM) is hindered because current tools to detect infection in peripheral blood do not have sufficient sensitivity and specificity.
- The sensitivity and specificity of diagnostic tools like rapid diagnostic tests should be evaluated in relation to placental parasite biomass.
- Standardization of placental histology, the ‘gold standard’ for PM detection, is needed to allow comparison between studies.
- Inflammatory markers associated with PM have the potential to be included in future diagnostic tests together with parasite-specific markers for timely and accurate detection of PM.

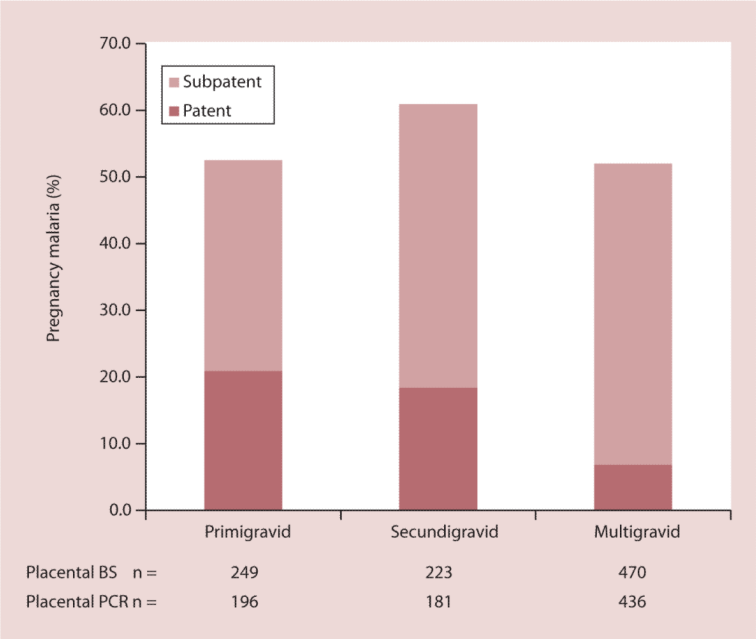

Figure 1. Patent and subpatent pregnancy malaria

Patent parasitemia was defined by blood smear microscopy. Subpatent parasitemia was defined by nested PCR as previously described by Snounou *et al* [28].

BS: Blood smear.

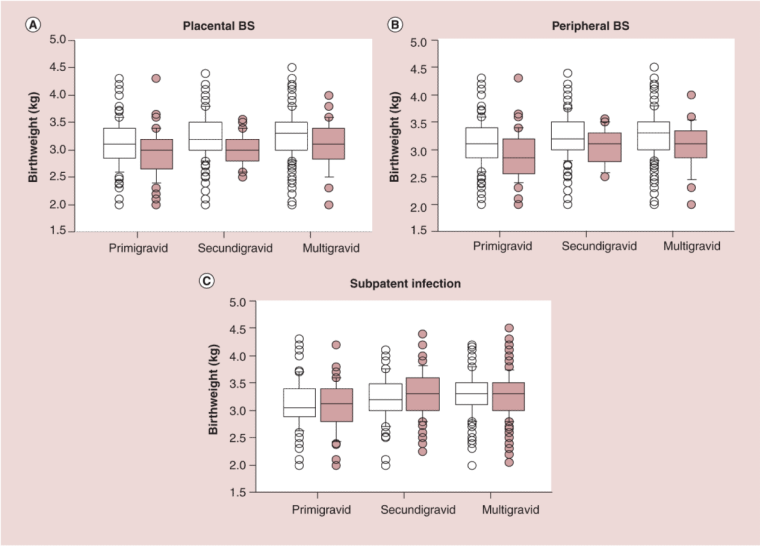

Figure 2. Patent, subpatent pregnancy malaria and birthweight

Birthweight was compared between offspring born to uninfected mother (open boxes) and mothers with PM (filled boxes) diagnosed by **(A)** placental BS, **(B)** peripheral BS at delivery and **(C)** subpatent infection defined as placental blood PCR+/placenta BS-. The numbers that follow indicate the number of offspring from uninfected and infected women, respectively, in each gravid group. **(A)** 191, 49; 168, 36; 396, 31. **(B)** 200, 40; 181, 23; 407, 20. **(C)** 130, 60; 94, 73; 213, 181. Differences in birthweight between offspring born to uninfected mothers and those with PM defined by placental or peripheral BS were significant ($p < 0.05$) in all the gravid groups.

BS: Blood Smear; PM: Pregnancy malaria.

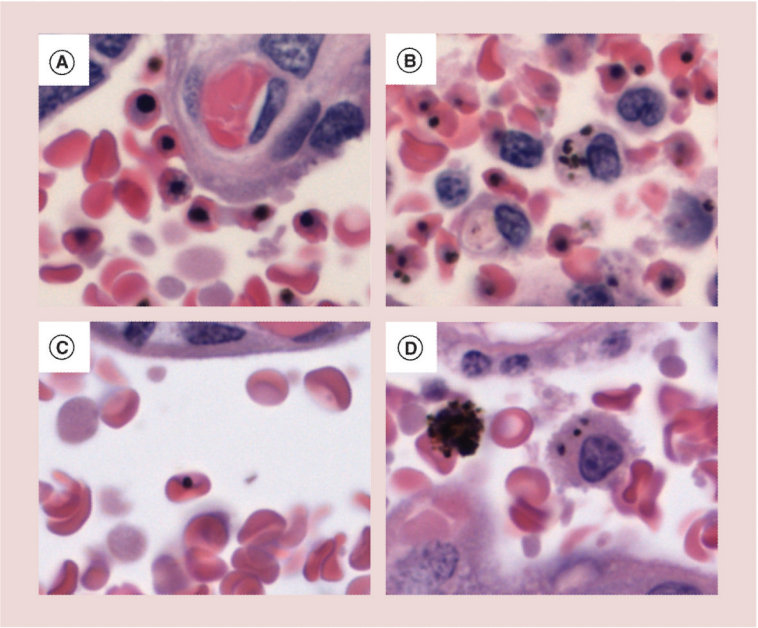

Figure 3. Risk of false-positive histology due to processing artifact

In well-preserved and well-processed cases, **(A)** parasitized red blood cells are readily identifiable and **(B)** hemozoin-containing leukocytes can be seen. In cases compromised by formalin pigment, **(C)** red blood cells artifactually contain formalin pigment and **(D)** cells contain formalin pigment mimicking packed red blood cells and hemozoin-laden macrophages, respectively. Formalin pigment in stroma or fibrin often resembles hemozoin (data not shown). ×600 magnification of hematoxylin- and eosin-stained formalin fixation with paraffin embedding sections obtained from women delivering in Mbarara, Uganda [53].

Table 1

Performance of rapid diagnostic tests of peripheral blood for pregnancy malaria diagnosis.

Study (year)	Test (peripheral blood)	Kit (source)	Reference	Sensitivity and specificity (%)	Ref.
Leke *et al.* (1999)	HRP2	ICT Malaria Pf (Amrad ICT, Sydney, NSW, Australia)	Peripheral BS	94.4, 90.6	[13]
			Placental BS	89, 94.9	
Mankhambo *et al.* (2002)	BS		Placental BS	52.1, 92.7	[10]
	pLDH	OptiMAL (Flow, Inc. Portland, OR, USA)	Peripheral BS	70.7, 93.8	
			Placental BS	38.4, 90.8	
Mockenhaupt *et al.* (2002)	BS		Placental BS	42, 97	[14]
	HRP2	ICT Malaria Pf/Pv (BD, Heidelberg, Germany)	Placental BS	80, 90	
	BS		Placenta PCR	27, 100	
	HRP2	ICT Malaria Pf/Pv (BD, Heidelberg, Germany)	Placenta PCR	56, 97	
Singer *et al.* (2004)	BS		Placental BS	82, 86	[15]
	HRP2	MAKROmed Pty, Ltd., (Johannesburg, South Africa)	Peripheral BS	96, 67	
			Placental BS	95, 61	
			Placenta PCR	92, 59	
Vanderjagt *et al.* (2005)	pLDH	OptiMAL (Flow, Inc., OR, USA)	Peripheral BS	15, 98	[11]
Mockenhaupt *et al.* (2006)	BS		Placental BS	50, 98	[53]
	HRP2	ICT Malaria Pf/Pv (BD, Heidelberg, Germany)	Placental BS	78, 89	
Tagbor *et al.* (2008)	pLDH	OptiMAL (DiaMed AG, Cressier, Swizerland)	Peripheral BS	96.6, 85.4	[12]
Kyabayinze *et al.* (2011)	HRP2	Diagnosticks, Malaria Pf cassette (SSA Diagnostics and Biotech Systems, Goa, India)	Peripheral BS	96.8, 73.5	[18]
			Placental histology	80.9, 87.5	
Dhorda *et al.* (2012)	BS		Peripheral PCR	36.4, 99.6	[17]
	HRP2	Paracheck Pf (Orchid, Goa, India)	Peripheral PCR	31.8, 100	
Mayor *et al.* (2012)	BS		Placental histology	65.2, 97.8	[16]
	HRP2	SD Bioline (Standard Diagnostics)	Placental histology	78.3, 93.4	

BS: Blood smear; ICT: Immunochromatographic test; RDT: Rapid diagnostic test.

Table 2

Rapid diagnostic test performance for the diagnosis of pregnancy malaria: placental blood.

Study (year)	RDT test	Kit (source)	Reference	Sensitivity and specificity (%)	Ref.
Singer *et al.* (2004)	HRP2	MAKROmed Pty, Ltd. (Johannesburg, South Africa)	Placental BS	95, 72	[15]
			Placental PCR	89, 76	
Singh *et al.* (2005)	HRP2	Paracheck Pf (Orchid, Goa, India)	Placental BS	93.3, 84.4	[84]
	HRP2	ParaHITf (Span Diagnostics, Inc., Surat, India)	Placental BS	87.5, 97	
Sarr *et al.* (2006)	HRP2	MAKROmed Pty, Ltd., (Johannesburg, South Africa)	Placental BS	100, 68	[85]
Kyabayinze *et al.* (2011)	HRP2	Diagnosticks, Malaria Pf cassette (SSA Diagnostics and Biotech Systems, Goa, India)	Placental BS	80.9, 87.5	[18]

BS: Blood smear; RDT: Rapid diagnostic test.

Table 3

PCR and quantitative PCR performance for the diagnosis of pregnancy malaria: peripheral blood.

Study (year)	Proportion infected by PCR or qPCR	Proportion infected: BS peripheral blood	Proportion infected: BS placental blood	Submicroscopic infection	Ref.
Mockenhaupt *et al.* (2002)	336/530 (PCR)	172/530		164/358	[14]
Mankhambo *et al.* (2002)	70/135 (50/68 peripheral and/or placental BS+) (PCR)	41/509	73/509	20/67 peripheral and/or placental BS	[10]
Saute *et al.* (2002)	101/181 (PCR)	156/672		36/101	[38]
Adam *et al.* (2005)	40/125 BS- (PCR)	17/142		40/125	[39]
Walker-Abbey *et al.* (2005)	212/278 (PCR)	63/278	75/278	137/203 compared with placental BS	[40]
Perrault *et al.* (2009)	52/157 (qPCR)	25/157	27/157	25/130 compared with placental BS	[41]
Rantala *et al.* (2010)	51/475 (qPCR)	11/475		41/464	[42]
Campos *et al.* (2011)	27/84[†] (PCR)	11/84[†]	8/84[†]	19/76 compared with placental BS	[43]

[†] *Plasmodium falciparum* and *Plasmodium vivax*.

BS: Blood smear; qPCR: Quantitative PCR.

Table 4

PCR performance for the diagnosis of pregnancy malaria: placental blood.

Study (year)	Proportion infected by PCR	Proportion infected: BS peripheral blood	Proportion infected: BS placental blood	Submicroscopic infection	Ref.
Mankhambo *et al.* (2002)	70/135 (50/68 peripheral and/or placental BS+)	41/509	73/509	20/67 peripheral and/ or placental BS-	[10]
Singer *et al.* (2004)	247/484	204/690	151/693		[15]
Walker-Abbey *et al.* (2005)	147/278	63/278	75/278	72/203	[40]
Adegnika *et al.* (2006)	30/130 BS-	13/145	14/145	30/130	[44]
Newman *et al.* (2009)	57/356		30/356	27/326	[45]
Perrault *et al.* (2009)	56/157	25/157	27/157	29/130 compared with placental BS	[41]
Campos *et al.* (2011)	22/84†	11/84†	8/84†	14/76 compared with placental BS	[43]
Elbashir *et al.* (2011)	34/107		33/107‡	19/74§	[46]
Mayor *et al.* (2012)	98/272		46/272¶	57/226	[16]

† *Plasmodium falciparum* and *Plasmodium vivax*.

‡ Placental histology.

§ 18/33 Infected placenta by histology were negative by PCR.

¶ By placental histology BS: Blood smear.