MAJOR ARTICLE

A Novel Histological Grading Scheme for Placental Malaria Applied in Areas of High and Low Malaria Transmission

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Background. *Plasmodium falciparum*–infected erythrocytes sequester in the placenta and elicit an inflammatory response that is harmful to both fetus and mother. Histologic measurements during placental malaria might provide surrogate end points for interventional trials, but existing histologic schemes capture limited complexity and are not consistently used among study sites.

Methods. Using frozen-section histologic evaluation in Tanzania (high-transmission area), we established a novel grading scheme to separately quantify inflammation and pigment deposition during placental malaria ($n = 102$). To generalize this method, formalin-fixed, paraffin-embedded placental samples from Karen women in Thailand (low-transmission area) were selected from among women with documented antenatal parasitemia who were near term ($n = 18$).

Results. In the Tanzanian cohort, the inflammation and pigment-deposition scores were independently associated with birth weight, and the inflammation score was associated with chemokine levels. In the smaller cohort from Thailand, both inflammation and pigment scores were associated with birth weight, and the pigment score had an inverse trend with the number of antenatal clinic visits.

Conclusions. This semiquantitative pathological grading scheme is simple to implement and captures information that is associated with outcomes in Asia and Africa; therefore, it should facilitate the comparison and standardization of results among clinical trials across areas of differing endemicity.

Malaria during pregnancy is associated with morbidity and mortality among pregnant women and their newborns in tropical areas. During placental malaria (PM), *Plasmodium falciparum*–infected erythrocytes adhere to chondroitin sulfate A present on the trophoblast surface [1]. Parasite sequestration results in a maternal inflammatory response that can be harmful to both the mother and the fetus. Histologic features of PM correlate with poor clinical outcomes and therefore might be useful as efficacy end points for interventional trials of prophylactic or treatment drugs.

Women living in different geographical areas experience different levels of malaria exposure, and their level of immunity before pregnancy influences disease course during infection. In areas of high stable transmission, such as Tanzania, women have significant clinical immunity before pregnancy, and PM is often asymptomatic but associated with severe maternal anemia and fetal growth retardation. In these areas, PM is most frequent and severe in first-time mothers, because women develop specific immunity against placental forms of in-

Received 17 April 2010; accepted 3 June 2010; electronically published 7 October 2010.

Potential conflicts of interest: none reported.

Presented in part: American Society of Tropical Medicine and Hygiene meeting, 19 November 2009 (abstract 136); United States and Canadian Academy of Pathology meeting, 22 March 2010 (abstract 1492).

Financial support: This work was supported by a University of Washington House Staff Association grant, the Benjamin H. Kean Fellowship from the American Society of Tropical Medicine and Hygiene (to A.M.), and PREMA-EU (contract no. ICA4-CT-2001–10012). The Shoklo Malaria Research Unit is part of the Wellcome-Mahidol University–Oxford Tropical Medicine Research Program funded by the Wellcome Trust of Great Britain. The Mother Offspring Malaria Studies Project was supported by grants from the Bill and Melinda Gates Foundation (29202) and the National Institutes of Health (R01AI52059 to P.E.D.).

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The Journal of Infectious Diseases 2010; 202(10):1608–1616

0022-1899/2010/20210-0021$15.00
DOI: 10.1086/656723

fected erythrocytes over successive pregnancies. In areas of low unstable transmission, such as the Thai-Burma border, malaria is symptomatic in women of all parities and is associated with high rates of fetal loss and maternal death [2]. The approach to antenatal care and the sensitivity of the parasite to different antimalarials also differ and could contribute to differences in outcomes. In Tanzania, insecticide-treated bed nets and intermittent preventive treatment during pregnancy are routinely used as preventive interventions, but case detection is passive [3]. In contrast, preventive measures are not routine at the Thai-Burma border, where case detection is active and prompts early treatment.

Three key histologic features of an untreated PM episode—parasites, inflammation, and pigment deposition, which correspond to 3 distinct biologic timelines—were described by Garnham in 1938 [4] (Figure 1). Infected erythrocytes accumulate in the intervillous space, and parasitemia changes rapidly over the course of the parasite life cycle and in response to treatment. Maternal inflammatory cells (predominately monocyte-macrophages) also accumulate in the intervillous space, are estimated to accumulate over the course of days to weeks in susceptible women, and can persist for a brief time after treatment [4]. Malaria pigment, likely originating from degenerating pigment-laden macrophages, accumulates in intervillous fibrin and can persist for months after heavy infection during gestation or may become undetectable with adequate treatment and no further reinfection [5, 6].

In areas of endemicity, pathologic features of PM correspond to clinical outcomes. Maternal inflammation is mostly seen in first-time mothers and is linked to decreased birth weight [7–9] and anemia [9]. Pigment present in immune cells or fibrin has been associated with reduced birth weight [8, 10]. The presence of pigment in women without active parasitemia (past infection) has been associated with decreased birth weight in some studies [9, 11] but not in others [8, 12, 13]. Differences in outcomes associated with past infections may be related to the sensitivity of different methods for detecting parasites, especially during very low-level parasitemia. Along the Thai-Burma border, an area of low transmission, pathologic changes of PM were rare in women without evidence of recent infection [6].

A pathologic classification scheme for PM was developed by Bulmer in 1993 [14] to reflect the chronology of the infection. This scheme infers active or acute infection when parasites are present in the intervillous spaces with or without pigment in intervillous monocytes, chronic infection when parasites are present in the intervillous spaces along with pigment as deposits or in macrophages within fibrin, and past-chronic infection when pigment is present in the absence of parasites (Figure 1*A*). Subsequent modifications and additional grading schemes were developed by Ismail et al [15] and Rogerson et al [9], whose schemes were similar to the Bulmer scheme, and by Davison et al [16] to incorporate other features of placental injury, including intervillous inflammatory cells.

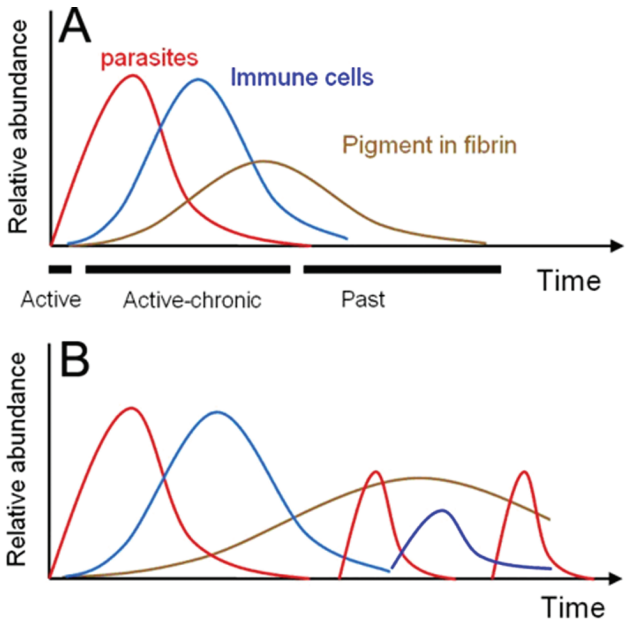

Figure 1. Schematics of a hypothetical single placental malaria (PM) episode in a susceptible woman (*A*) and recrudescences and reinfections in a single woman that can lead to varying degrees of parasitemia, inflammation, and pigment (*B*), especially when the drugs for intermittent preventive treatment or treatment are not effective.

The application of the existing PM grading schemes is limited, because chronic PM is a broad category encompassing varying degrees of pathology, scoring criteria vary by study site, and in general the degree of inflammation is not independently considered. In contrast, a scheme using 2 parameters (inflammation and fibrosis) is widely used in monitoring progression and treatment of chronic viral hepatitis [17]. We have developed a 2-parameter semiquantitative grading scheme that scores the degrees of inflammation and pigment deposition during PM. The proposed scheme is simple to perform, corresponds to pregnancy outcomes on 2 continents, and may improve the comparison and standardization of results among clinical trials across areas of differing endemicity.

METHODS

Study sites. Placental samples from study populations living in 2 areas of different malaria endemicity were analyzed. Informed consent was obtained at both sites. Women living in the high-transmission area around Muheza, Tanzania, were enrolled in the Mother Offspring Malaria Studies (MOMS) project during their deliveries at the Muheza Designated District Hospital. These women received antenatal care according to Tanzanian national guidelines, including mostly passive malaria

case detection and 1–2 presumptive antimalarial treatments. Women from refugee and migrant populations living in an area of sporadic transmission along the Thai-Burma border received weekly antenatal care with active malaria screening at 1 of 5 clinics operated by the Shoklo Malaria Research Unit. Antenatal care was provided free of charge, and a subset of women were enrolled in clinical trials. The demographic characteristics of the cohorts are presented in Table 1.

Malaria diagnosis and sample selection. In the Tanzanian cohort, PM was detected using microscopic examination of Giemsa-stained thick and thin smears of blood extracted from placental tissue by mechanical grinding. Placental parasite density was quantified as the percentage of infected erythrocytes. The primary analysis was done among PM-positive women to identify features that are specifically linked to poor outcomes in this group. In the cohort from the Thai-Burma border, malaria episodes were detected using peripheral blood smear at weekly antenatal clinic visits and at delivery, with additional examination of mother peripheral blood and placental blood samples collected by incision at delivery. Samples were selected from among women with peripheral blood parasitemia at least 2 weeks before delivery. Only women with recent infection were included to avoid examining negative histopathology samples [6] and to facilitate comparison with the Tanzanian cohort.

Histology. Paraffin-embedded blocks were previously generated from tissue samples collected at the Thai-Burma border [6]; Giemsa-stained slides were made from these samples (Figure 2*A* and 2*B*). In Tanzania, placental tissue was placed in polyvinyl alcohol, snap-frozen in liquid nitrogen, and stored at −80°C. Sections were made on a cryostat and were air-dried, methanol-fixed, and Giemsa-stained for 12 min (Figure 2*C*–2*E*). The proposed grading scheme is detailed in Results. PM episodes were also classified as acute or chronic [14], with chronic PM defined by the presence of pigment in fibrin (>1 in 50 high-power fields).

Biomarker analysis. Quantitative polymerase chain reaction (PCR) was performed as described elsewhere [20]. Briefly, total RNA was extracted from frozen placental cryosections by means of RNeasy Mini kits (Qiagen), and real-time PCR was performed using SYBR Green Master mix, an ABI Prism 7500 system (Applied Biosystems), and intron-spanning primers for CXCL13 and for KRT7 (a gene expressed by the trophoblast).

Statistical analysis. Analyses were performed using Statview and SAS software (SAS Institute). Continuous and categorical variables were analyzed using the Student *t* test and the Fisher exact test. Bar graphs are presented as means ± standard errors. The percentages of infected erythrocytes were log transformed before analysis. Logistic regression and analysis of variance were used for multivariate analyses.

RESULTS

The inflammation score. The inflammation categories are qualitative and are intended to reflect distinct biologic entities (Figure 3*A*). Minimal inflammation (I) describes cases with no appreciable intervillous inflammation. Pigmented monocytes are rare, and the intervillous space white cell density is not increased above the level of peripheral white blood cells expected in transit. Inflammation present (II) describes cases with mononuclear cells sequestering in the intervillous space, particularly pigment-laden macrophages. This is a broad category describing the intermediate stage between no inflammation and massive intervillositis. Massive intervillositis (III) is a distinct entity in which the intervillous space contains sheets of densely packed mononuclear cells [21–23]. Massive intervillositis was reported in 6.3% of PM cases in southern Tanzania [8], 7 (6.9%) of 102 PM cases in MOMS project in northeastern Tanzania, and 3 (1.7%) of 175 women who had malaria during pregnancy at the Thai-Burma border [6]. Massive intervillositis is a rare finding in the absence of malaria, in which it is associated with recurrent miscarriage [21].

The pigment-deposition score. The pigment-deposition score is semiquantitative and can be rapidly assessed by quantifying the percentage of high-power fields that are positive for pigment in fibrin in intervillous spaces (Figure 3*B*). The scoring method excludes pigment in erythrocytes or monocytes. Malaria pigment is golden brown after Giemsa staining and can be identified on either paraffin or frozen sections. The use of a 60× objective is recommended, because pigment is present in fine granules and at least 60 fields in the intervillous space are counted. The total field count excludes stromal tissue in the decidua, basal plate, and stem villi. Although the total amount of fibrin may be variable, the total field count includes fields in intervillous spaces that do not have fibrin.

The categories are determined as follows: I (<10% of fields positive), II (10%–40%), and III (>40%). The cutoff values of 10% and 40% per high-power field corresponded to the 50th and 90th percentiles, respectively, for both multiparous Tanzanian PM-positive women and for the selected cohort from Thailand (all parities). Among Tanzanian first-time mothers with PM, these values corresponded to the 20th and 75th percentiles, respectively.

A null category could be considered for women if pigment is absent, particularly if the purpose of the study is to detect previous infections during drug trials. In the MOMS project, pigment was absent in 10% of PM-positive women, and these women had a trend toward increased birth weight and multiparity (data not shown). We elected to include these cases in category I for the present study, because the criteria for pigment exclusion would require a greater amount of tissue examined and would be problematic in formalin-fixed tissue samples with

Table 1. Malaria Epidemiology, Therapy, and Antenatal Care during Pregnancy in the Tanzania and Thailand Cohorts

Variable	Tanzania, Africa (2002–2005)	Thailand, Asia (1995–2002)
Malaria incidence during pregnancy	34% of women reported treatment for acute malaria	~5%–30% with microscopy-confirmed episodes during pregnancy (varied by site); <1 *Plasmodium falciparum* infection per woman per year; <1.5 *Plasmodium vivax* infections per woman per year
Cases of malaria due to non–*P. falciparum* species during pregnancy	<1%	~60% (*P. vivax*)
Case detection method during pregnancy	Passive (screening available at ANC)	Active (weekly visits) and passive (24-h malaria screening available)
Placental malaria incidence at delivery	12.6% of all women, 19.4% of first-time mothers	<1%
Women receiving ANC	36% with documented ANC attendance, 83% with reported IPTp exposure	>90% attend ANC in refugee camp
No. of ANC visits, mean (range)	4 (1–12), where documented by ANC card	Weekly ANC >50% during first trimester; 15–20 visits
Preventive treatment	SP-IPTp	None because of high levels of MDR *P. falciparum* strains; SP not used in Thailand for >30 years
Women reporting IPTp use	83%	NA
No. of IPTp doses, mean (range)	1 (0–3), where documented by ANC card	NA
Drugs used to treat malaria during pregnancy	ACT, quinine	Quinine, mefloquine, artesunate monotherapy, ACT
Efficacy of available drugs		
Limited	High-level resistance to first-line therapy (SP), 2002–2008	Quinine and mefloquine monotherapy, 1995–2002
Currently efficacious	ACT (artemether-lumefantrine), quinine; for severe malaria, intravenous quinine	During first trimester, quinine and clindamycin for 7 days; during second and third trimesters, ACT; for severe malaria, intravenous artesunate
Bed net use	62% for any net, 19% for treated net	~90% for treated net
Screening for anemia: Hb or HCT	At most ANC visits	Every 2 weeks
Nutritional supplements	Ferrous and folic acid at most ANC visits	At each visit, prophylactic ferrous (200 mg daily) and folic acid (5 mg/week); for treatment, ferrous (400 mg twice daily) and folic acid (5 mg daily)
Antihelminthic policy	All women: stool and urine testing	Anemic women: stool testing
Prevalence of HIV infection among pregnant women	5%–7% (from Somi et al [18])	<0.5% (from Plewes et al [19])

NOTE. ACT, artemisinin-based combination therapy (as of 2009, artesunate plus clindamycin for 7 days); ANC, antenatal care; HIV, human immunodeficiency virus; IPTp, intermittent preventive treatment in pregnancy; MDR, multidrug resistant; NA, not available; SP, sulfadoxine-pyrimethamine.

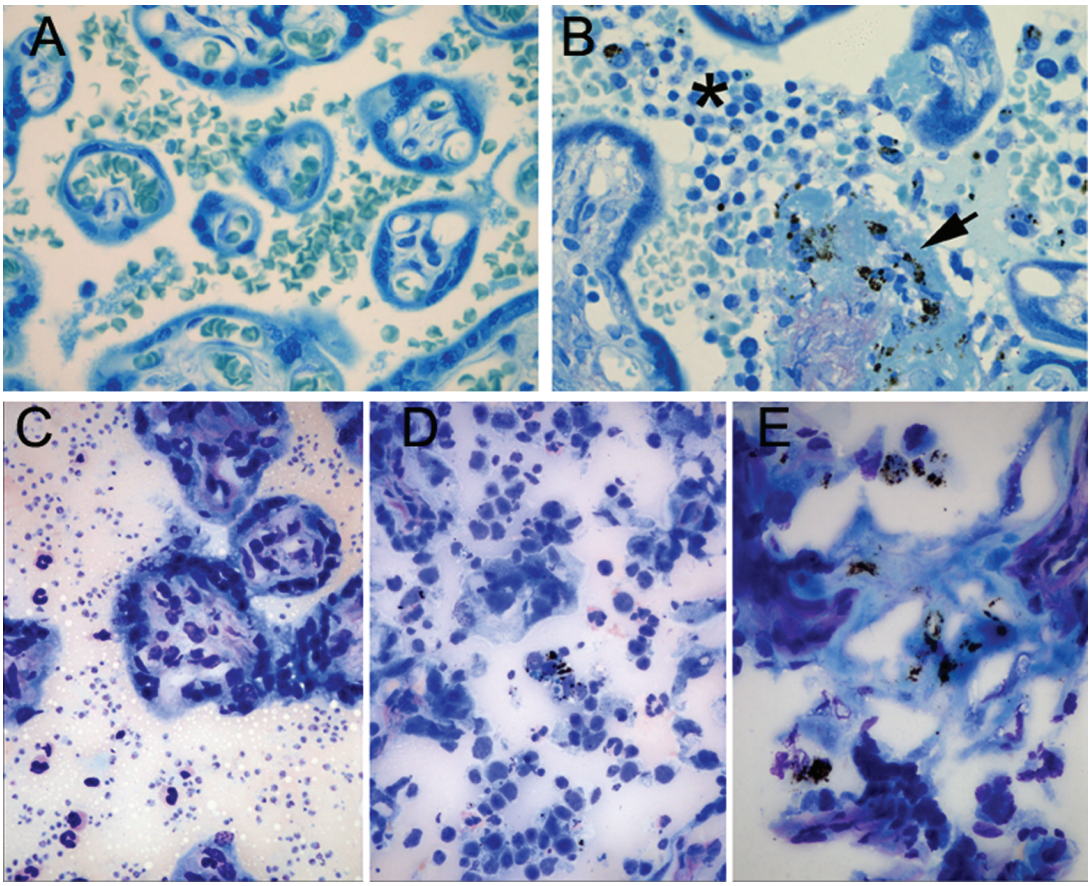

Figure 2. *A* and *B*, Formalin-fixed, paraffin-embedded placental tissue section from women with placental malaria (PM) at the Thai-Burma border. *A*, Remote infection, with no histologic alteration. *B, Plasmodium falciparum* infection at delivery, with inflammatory infiltrate (*asterisk*) and pigment deposition in fibrin (*arrow*). *C–E*, Fresh-frozen, Giemsa-stained sections from women with PM in Tanzania. The features of placental malaria are readily identified: parasites (*C*), inflammation (*D*), and pigment (*E*) deposition in fibrin. Original magnification, ×400.

even a scant amount of formalin pigment deposition. Among PM-negative first-time mothers in Tanzania, histologic evidence of past infection was not associated with decreased birth weight (58/104; $P = .462$).

The Tanzanian cohort. Of 984 women with singleton deliveries in Muheza, Tanzania, 124 (12%) had PM, as determined by placental blood smear. Tissue samples were available for frozen-section histologic examination for 102 (82%) of the 124 women. On the basis of the traditional histologic grading scheme, chronic PM was associated with a decrease in mean birth weight, compared with acute PM; the difference approached statistical significance (0.21 kg; $P = .057$). When the cohort was stratified by inflammation and pigment-deposition scores on the basis of the new scheme (Figure 4), both scores were associated with birth weight. Pigment and inflammation scores were strongly related ($P = .005$) but nevertheless were independently associated with birth weight by analysis of variance ($n = 101$; $P = .016$ and $P = .005$, respectively). Inflammation and pigment scores of III were independently associated with low birth weight by logistic regression analysis ($n = 101$; $P = .003$ and $P = .017$, respectively).

Primiparous women were analyzed as a subgroup because first-time mothers are at risk for poor outcomes (Figure 5). On the basis of the traditional grading scheme, chronic PM was not associated with a significant decrease in mean birth weight, compared with acute PM (0.22 kg; $P = .46$). Pigment and inflammation scores were related ($n = 47$; $P = .042$), and birth weight was inversely associated both with inflammation and with pigment scores; however, by multivariate analysis neither pigment nor inflammation was independently associated with birth weight. Placental parasitemia was inversely associated with pigment deposition. Placental messenger RNA levels of CXCL13, a PM biomarker, were 200-fold higher in placental tissue samples with an inflammation score of III than in samples with an inflammation score of I and were 16-fold higher than in samples with a pigment score of III than in samples with a pigment score of I.

The Thai-Burma cohort. Samples collected during a his-

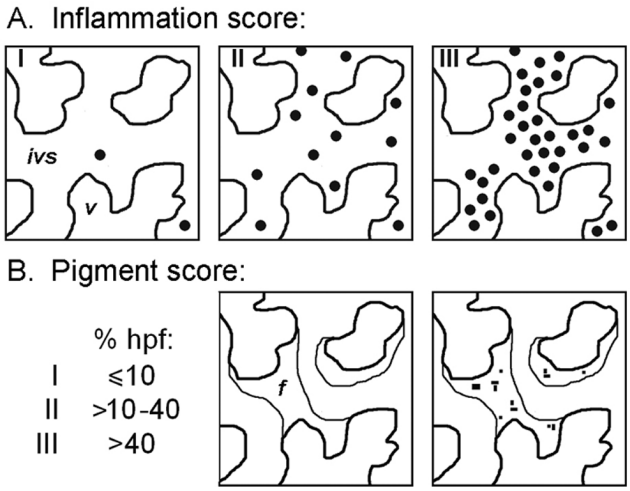

Figure 3. Schematic demonstrating placental villi (v) for grading the histologic features of placental malaria (PM). *A,* Categories of maternal inflammation in the intervillous spaces (ivs): I, minimal; II, present; and III, massive. *B,* Cutoff values (percentage of 60× high-power fields [hpf]) for categorizing malarial pigment deposition in intervillous fibrin (f).

topathologic study at the Thai-Burma border from 1995 to 1997 [6] were reviewed. Of the 175 women with malaria episodes from whom placental samples were obtained, 18 had *P. falciparum* infection within the 2 weeks before delivery (median, 2 days; range, 0–10 days) and were included in the analysis. Inflammation and pigment scores of I–III were observed. A single sample had both pigment and inflammation scores of III; no inflammation was identified in 10 samples, and no pigment was identified in 5 of 18 samples. Inflammation and pigment were not significantly related in this small sample size ($P = .28$).

We conducted preliminary analyses of this small sample size to examine trends in relationships between scores and clinical outcomes. Pigment deposition together with inflammation by histologic examination was associated with decreased birth weight (Figure 6). Estimated gestational age did not significantly differ by inflammation or pigment category, although it decreased with increasing pigment. The number of antenatal clinic visits (where women were screened and promptly treated for malaria) was inversely related with pigment, but the relationship was not significant. CXCL13 was not examined in these samples because of formalin fixation.

Comparison across study sites. A formal analysis across study sites was limited by differences in study design, malaria diagnosis, and histologic technique. Samples from the Thai-Burma border were compared with those from Tanzanian first-time mothers, the group that has the least immunity and is most susceptible to PM. Pigment deposition was significantly lower in the Thai-Burma cohort ($P = .002$), but the presence or degree of inflammation did not significantly differ (18 Karen vs 47 Tanzanian; $P = .267$). The parasite densities in the placenta samples were not examined across sites because of the differing modalities of tissue processing and malaria diagnosis.

DISCUSSION

The clinical and epidemiologic features of PM vary widely with malaria transmission levels, as do control measures. We have developed a 2-parameter grading scheme measuring inflammation and pigment deposition that is simple to use and captures more information than earlier schemes. We found that the criteria for histologic classification are applicable in an African setting with high malaria transmission and an Asian setting with low transmission. Furthermore, the scores were significantly related to pregnancy outcomes in a relatively large Tanzanian cohort, and similar trends were found in a small study of Karen women in Thailand. On the basis of these preliminary findings, we propose that this scheme be further evaluated in clinical trials, in which it may facilitate comparison and standardization of results between sites.

As a result of differences in exposure, immunity, and treatment, malaria episodes are complex. Chronic PM can result from several factors, including low host immunity and lack of effective antenatal control measures. Women can be inoculated multiple times during pregnancy, be treated or not treated adequately, and experience reinfections and recrudescences (Figure 1*B*) [24, 25]. For example, in a recently published treatment study at the Thai-Burma border 253 women had a median of 2 episodes of malaria (range, 1–11 episodes; *P. falciparum* and *Plasmodium vivax*) detected and treated during pregnancy [26].

In the present study, pigment deposition and inflammation were associated with decreased birth weight at 2 distant sites. In Tanzania, both pigment deposition and inflammation were independently associated with low birth weight in the overall cohort but not the primiparous subgroup. Inflammation determined by histologic examination was strongly associated with CXCL13, a proposed biomarker of inflammatory PM [23],

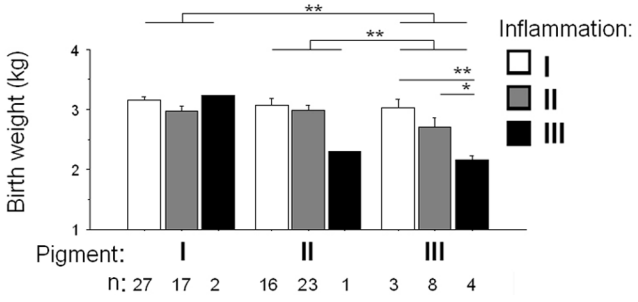

Figure 4. Inflammation and pigment scores in relation to birth weight in Tanzanian women of all parities. *$P < .05$; **$P < .01$.

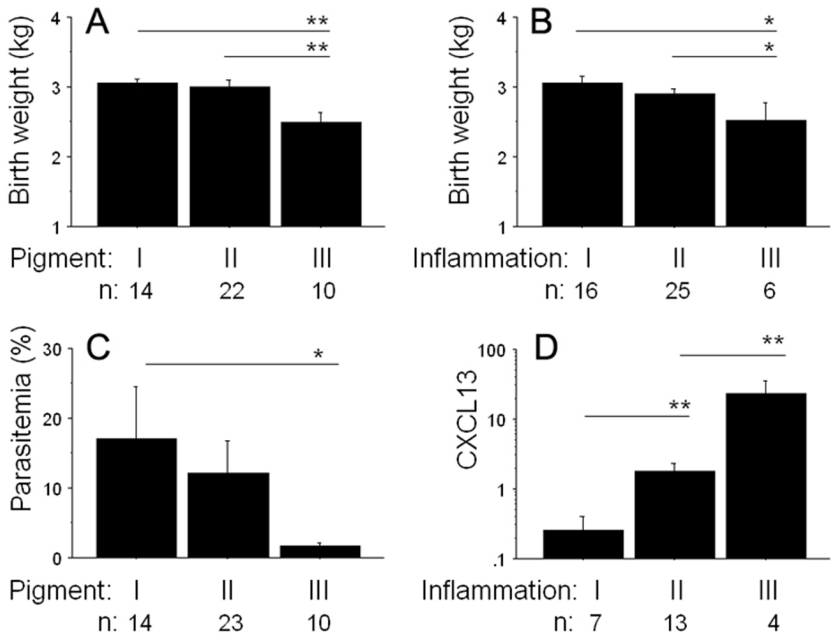

Figure 5. Inflammation and pigment scores examined in Tanzanian first-time mothers. Shown are the relationships between birth weight and pigment score (*A*), birth weight and inflammation score (*B*), placental parasitemia and pigment score (*C*), and placental CXCL13 transcript levels (fold change over cytokeratin 7 expression) and inflammation score (*D*). $*P < .05$; $**P < .01$.

and has been linked to maternal peripheral blood levels of interleukin 10 [28], suggesting the usefulness of these or other markers to monitor placental inflammation before delivery.

At the Thai-Burma border, the relationship between pathologic features of malaria and outcome differs from that in areas of high stable transmission. In one study in which all episodes of peripheral parasitemia were detected by active screening during pregnancy and treated, both antenatal *P. fal-*

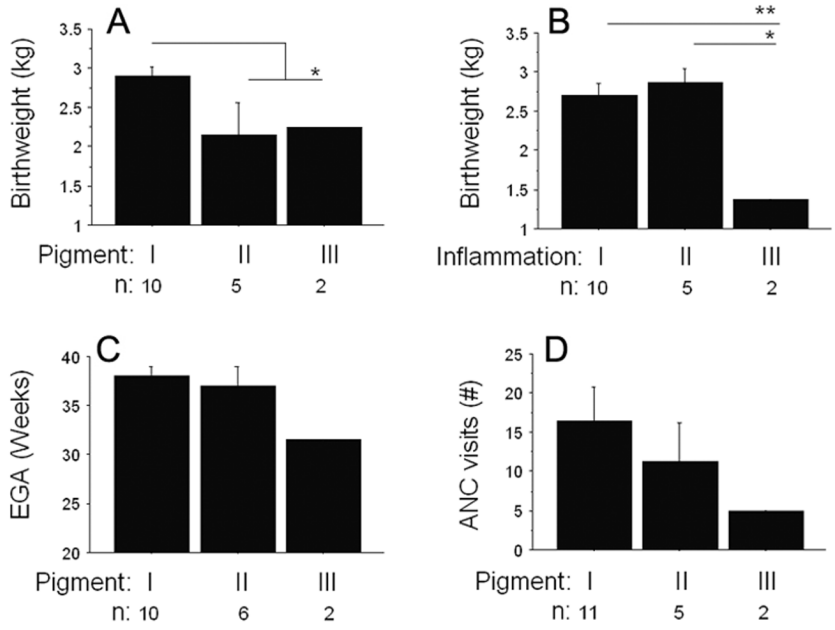

Figure 6. Inflammation and pigment scores in Karen women who had received a diagnosis of *Plasmodium falciparum* malaria within 2 weeks before delivery. Shown are the relationships between birth weight and pigment score (*A*), birth weight and inflammation score (*B*), estimated gestational age (EGA) and pigment score (*C*), and number of antenatal clinic (ANC) visits and pigment score (*D*). $*P < .05$; $**P < .01$.

ciparum and *P. vivax* infections were associated with birth weight reduction [27], although the effect was greater with *P. falciparum* than with *P. vivax*. No placental pigment was observed in 33% (16/49) of women with documented *P. falciparum* infection during pregnancy [6]. However, pathological changes were more likely to be observed when malaria was diagnosed during the month before delivery, and more pigment (in immune cells and fibrin) was observed when malaria was diagnosed during the week before delivery [6].

This is the first study, to our knowledge, to document conserved histopathologic features in pregnancy malaria between Africa and Asia. Although a formal analysis across study sites is precluded by differences in study design and histologic technique, samples from Tanzanian first-time mothers were compared with selected samples representing the small number of Karen women who experienced *P. falciparum* malaria during the last 2 weeks of pregnancy. This time frame was chosen to maximize the likelihood of finding histologic changes, including the presence of pigment [6]. The histologic degree of placental inflammation did not significantly differ between these 2 groups; however, there was significantly decreased pigment deposition in the Karen women. This finding likely reflects the increased inoculation rate and limited treatment and antenatal care efficacy in Tanzania, compared with the effect of early detection and prompt treatment at the Thai-Burma border, because pigment deposition was inversely related to the frequency of antenatal care visits among Karen women. These differences might also reflect the differing biologic timelines that regulate inflammation and pigment deposition during PM: inflammation changes more dynamically in response to parasitemia, whereas pigment persists for a longer duration (Figure 1). Interpretation of the associations of inflammation and pigment with birth weight would have been improved if data on gestational age were available for all study sites and would have allowed the association to be examined in growth-restricted term infants.

The present study was not powered to analyze the result of human immunodeficiency virus (HIV) coinfection ($n = 4$; Tanzanian cohort). Because both HIV infection and malaria affect birth weight and pregnancy outcome, HIV infection could alter the reported findings. Several studies have found that there is an increased risk of placental malaria with HIV coinfection in areas of endemicity [29, 30] and that PM episodes are more likely to involve higher-level parasitemia and increased pigment deposition by histologic examination [29]. HIV-coinfected women exhibit altered humoral immune responses to placental infected erythrocytes [31], and immune cells isolated from the intervillous spaces in coinfected women exhibit decreased interleukin 12 production [32] and contain an expanded $CD16^+$ monocyte subset [33]. In areas of low transmission—such as the Thai-Burma border, where the prevalence of HIV infection is low [19]—the effect of HIV coinfection on PM episodes has not been determined.

Here, we have demonstrated that the new grading scheme is applicable on both frozen and formalin-fixed, paraffin-embedded sections. A direct comparison of histologic examination of frozen sections and of formalin-fixed, paraffin-embedded samples to detect the features of PM was not performed and would require both methods to be performed on the same sample population. In formalin-fixed, paraffin-embedded tissue, fine details of cellular morphology are preserved and erythrocytes remain intact, whereas in frozen tissue cellular architecture is disrupted and erythrocytes lyse. The formation of formalin pigment or acid hematin during prolonged storage or during processing of formalin-fixed, paraffin-embedded tissue can obscure malarial hemozoin, often precluding analysis; formalin pigment formation is reduced with smaller tissue samples, generous amounts of buffered formalin, and prompt passage through graded alcohols after fixation. Frozen tissue is handled minimally, decreasing the concern of washing out intervillous contents. Frozen-section histologic examination is not routinely available in most rural tropical areas; however, the infrastructural costs are significantly less than those for formalin-fixed, paraffin-embedded histologic examination. Frozen sections have several advantages: no formalin pigment artifact, reduced laboratory infrastructure, and preserved tissue that yields high-quality RNA [23, 24], antigens [23], protein, and DNA for use in molecular analyses.

In the Tanzanian cohort, parasitemia was diagnosed by placental blood smear, and parasitemia per se is not included in the proposed grading scheme. Placental blood smears may not be available for all studies, with diagnosis and quantification of parasitemia relying on examination of histologic sections or other ancillary tests. As reported by Ewing et al [34] and in our experience (A.M. and M.F.), the identification of low-level parasitemia (<1%) by histologic examination is challenging because of routine dehydration and sectioning of erythrocytes and can be precluded by intraerythrocyte formalin pigment formation. Parasitemia levels >5% may be readily detected and quantified in histologic sections.

In the present study, PM refers strictly to the placental sequestration of *P. falciparum*–infected erythrocytes, because it is not known whether all episodes of *P. falciparum* malaria during pregnancy have a placental phenotype and there is no evidence at this time that *P. vivax* sequesters in the placenta. The mechanisms by which malaria episodes (both *P. falciparum* and *P. vivax*) during the first or second trimester lead to poor birth outcomes are not known, particularly in the absence of malaria-specific histologic features at term. Pathologic studies of miscarriages associated with both *Plasmodium* species or longitudinal studies that incorporate ultrasound measurements and biomarkers of infection would be of great interest.

In conclusion, we provide a semiquantitative pathological grading scheme that is simple to implement, specifically documents inflammation, and is associated with outcomes. Future clinical trials may benefit from this scheme for evaluation of histologic end points and for comparison across study sites.

Acknowledgments

We thank Billie Davidson for performing initial histologic examination on the cohort of Karen women, Ronald S. Veazey for coordinating the transfer of samples, Corinne Fligner for contributing to the pathological analyses, and Wonjong Moon for contributing to demographic analyses of the Tanzanian cohort.

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