IS MALARIAL PLACENTAL INFECTION RELATED TO PERIPHERAL INFECTION AT ANY TIME OF PREGNANCY?

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Abstract. Placental infection measured by placental smear at delivery is a standard indicator, widely used to characterize malaria infection in pregnant women. However, a single measure can hardly reflect the entire history of infection during pregnancy. To investigate the relation between this indicator and peripheral infection during pregnancy, we used data collected in a randomized trial of malaria prophylaxis in 928 pregnant women in Burkina Faso, 1987–1988, during which repeated measures of peripheral infection were taken. We analyzed placental infection using a logistic model, with two methods for handling missing data. Peripheral infection during two periods of pregnancy was significantly related to placental infection at delivery, before the fifth month: OR = 2.9 [1.3; 6.3]; after 7 months: OR = 4.9 [2.7; 8.8]). Therefore, an early peripheral infection may persist throughout gestation, and placental infection is a good indicator of the women’s parasitological status during pregnancy.

INTRODUCTION

Malaria during pregnancy has serious consequences for both pregnant women and newborns. In particular, anemia is frequent in infected pregnant women and more pronounced in primigravidae than in multigravidae.1–3 Studies of placental infection have been carried out in different areas of stable endemicity and have reported rates varying between 10% and 34% of all pregnant women.4 Placental infection and low birth weight among newborns have also been shown to be correlated.5–7 Children born to malaria-infected mothers are affected by an increase in morbidity and mortality.8,9 This serious public health problem led the World Health Organization (WHO) to recommend in 1984 administration of chloroquine chemoprophylaxis during pregnancy. But due to poor compliance and increasing parasite resistance to chloroquine, the administration of curative doses of antimalarials at antenatal consultations was tested in 1996 in Malawi. Subsequently, these recommendations were changed in Eastern and Central Africa (and are progressively changing in Western Africa) to an intermittent treatment with sulfadoxine-pyrimethamine given at antenatal visits.

These studies use placental infection as a standard indicator, widely used to characterize malaria infection in epidemiologic investigations. There are other reasons why this indicator is so frequently used in epidemiologic studies. Measuring malaria infection in the placenta is fairly nontechnical, and a single measure of peripheral infection during pregnancy as any single measure, may be inappropriate to describe the true parasitological status of the mother all along pregnancy.

MATERIALS AND METHODS

Study site and design. The study took place in Banfora, a city of 35,000 inhabitants located in southwestern Burkina Faso. In this savannah area, malaria is hyperendemic, with seasonal transmission strongly influenced by rainfall (usually from June to November). At the time of the study, there was a Maternal and Child Health (MCH) center in Banfora that was associated with the Provincial Hospital. Approximately 80% of the pregnant women living in the city attended the prenatal center at least once before delivery.

The details of the study have been described elsewhere.10 In summary, between February 1987 and February 1988, all the pregnant women attending the MCH center for the first time were included in the study and randomly divided into two groups. One of these groups received a weekly controlled prophylaxis of 300 mg of chloroquine (under the supervision of an investigator), and the control group received no prophylaxis. Prior to the study, we checked to see that there was

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only very little spontaneous intake of prophylaxis in the area.
The gestational age of the women was evaluated at first visit from the date of last menses. All the women were visited at home once a week by investigators who asked about febrile episodes or intake of drugs since the last visit and took finger pricks every 2 weeks to measure parasite densities. Follow-up was continued until the end of pregnancy, and the outcome of delivery was recorded (in particular, infection of the placenta, clinical status, and birth weight of the baby).

The field study had received ethical clearance from the French Comité Consultatif National d’Ethique (CCNE) and from the ministry of health (Burkina Faso).

**Biological methods.** Samples from maternal blood and placenta were collected for serological analysis and were examined for malaria parasites. Samples were identified with a number, stained, refrigerated as needed, and sent twice a week to the ORSTOM laboratory in Bobo-Dioulasso, where slide examination and serological assays were carried out. Thick and thin blood films and placenta smears were stained with Giemsa. At least 200 fields were examined for malaria parasites before a slide was considered negative. More than 95% of the parasites we identified were *Plasmodium falciparum.*

**Selection of subjects.** The original cohort included 1,522 pregnant women. Women whose placenta was not analyzed were excluded (*N* = 550). Most of them did not deliver at hospital. We also excluded women with a declared duration of pregnancy over 10 months and those for whom we were unable to determine the exact date of last menses (*N* = 44). Finally, 928 women were analyzed (see Table 1 for characteristics of the sample).

**Statistical methods.** We compared the distribution of women’s characteristics between analyzed and excluded women using χ² and Wilcoxon tests for qualitative and quantitative variables, respectively.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cohort of analysis</th>
<th>Excluded women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Gestational rank} )</td>
<td>( N )</td>
<td>( % )</td>
<td>( N )</td>
</tr>
<tr>
<td>1-2</td>
<td>291 (31.4%)</td>
<td>204 (34.4%)</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>241 (26.0%)</td>
<td>167 (28.2%)</td>
<td>0.10</td>
</tr>
<tr>
<td>5-6</td>
<td>193 (20.8%)</td>
<td>122 (20.6%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 6</td>
<td>203 (21.8%)</td>
<td>100 (16.8%)</td>
<td></td>
</tr>
<tr>
<td>( \text{Age} )</td>
<td>( N )</td>
<td>( % )</td>
<td>( N )</td>
</tr>
<tr>
<td>( \leq 21 \text{ years} )</td>
<td>207 (22.3%)</td>
<td>143 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>22-25</td>
<td>215 (23.2%)</td>
<td>146 (25.6%)</td>
<td>0.61</td>
</tr>
<tr>
<td>26-30</td>
<td>253 (27.3%)</td>
<td>146 (24.6%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 30</td>
<td>253 (27.2%)</td>
<td>159 (26.7%)</td>
<td></td>
</tr>
<tr>
<td>( \text{Ethnic group} )</td>
<td>( N )</td>
<td>( % )</td>
<td>( N )</td>
</tr>
<tr>
<td>Peuls</td>
<td>45 (5.0%)</td>
<td>50 (8.8%)</td>
<td></td>
</tr>
<tr>
<td>Mossi</td>
<td>165 (18.3%)</td>
<td>84 (14.9%)</td>
<td></td>
</tr>
<tr>
<td>Bobo</td>
<td>61 (6.8%)</td>
<td>40 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>Dioula</td>
<td>95 (10.5%)</td>
<td>71 (12.6%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Gourounsi-Dagari</td>
<td>40 (4.4%)</td>
<td>23 (4.1%)</td>
<td></td>
</tr>
<tr>
<td>Lobi-Senoufo</td>
<td>399 (44.2%)</td>
<td>243 (43.0%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>97 (10.8%)</td>
<td>54 (9.5%)</td>
<td></td>
</tr>
<tr>
<td>( \text{Body mass index} )</td>
<td>( % )</td>
<td>( N )</td>
<td>( % )</td>
</tr>
<tr>
<td>( \approx 20 )</td>
<td>198 (24.4%)</td>
<td>66 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>20-22</td>
<td>212 (26.1%)</td>
<td>69 (25.2%)</td>
<td>0.98</td>
</tr>
<tr>
<td>22-24</td>
<td>201 (24.7%)</td>
<td>68 (24.8%)</td>
<td></td>
</tr>
<tr>
<td>( \approx 24 )</td>
<td>202 (24.8%)</td>
<td>71 (25.9%)</td>
<td></td>
</tr>
<tr>
<td>Trial prophylaxis</td>
<td>502 (54.1%)</td>
<td>289 (48.7%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Placental infection was studied through a logistic regression model as a function of peripheral blood infection after adjustment on several cofactors. Placental infection was coded 1 if present, 0 otherwise. For peripheral blood infection, 3 binary variables were associated with infection according to gestational age at blood collection, less than 4 months, 5 or 6 months, 7 months or more. “Positive” was defined as the presence of parasites in at least one blood sample and “negative” as the absence of parasites in all samples during each gestational age period.

We considered the following adjustment factors: woman’s age, gestation rank, ethnic group, district of habitation, occupation, intake of malaria treatment (other than prophylaxis), sex of newborn, and woman’s body mass index (weight/height²), which reflects the overall nutritional status. Continuous variables were transformed into categorical variables (four categories corresponding approximately to each quartile).

From the prophylaxis data collected during follow-up, we generated two binary variables, corresponding to a dose of chloroquine assumed to be effective (three 300 mg tablets) for each follow-up period, before and after 6 months of pregnancy.

To account for the duration and position of the pregnancy with respect to the malaria transmission period, we created a four level categorical variable termed “transmission period” variable (Figure 1). We considered that transmission occurred from mid-June to mid-December, as shown through earlier entomological studies in a neighboring area. For instance, code 2 corresponds to the last 3 to 6 months of gestation during the transmission period, and code 4 corresponds to the first 3 to 6 months of gestation during the transmission period.

We first carried out a univariate logistic regression analysis to select the adjustment cofactors. For polytomous variables, when odds ratios had similar values, categories were pooled. Variables for which *P* value was less or equal to 0.3 were further included in a subsequent multivariate logistic regression model. The variables were then introduced together in a multivariate logistic regression and were eliminated step by step using the backward method and likelihood ratio test. At this level, only variables for which the *P* value was less than 0.05 were kept. Finally, we performed a logistic regression with all the significant adjustment cofactors and peripheral blood infection at each period of pregnancy.

Peripheral parasitemia was unavailable in two main cases: either the woman had not entered the study at a given moment of pregnancy, or (much less often) she was not home when the investigator visited her. For these two reasons, much data were unavailable or missing (65.2% in the first period of pregnancy, 25.4% in the second period of pregnancy, and 6.2% in the third period). To account for this, two different methods were used: the “missing data indicator,” a method that consists in adding a category to peripheral infection variable for missing value (i.e., peripheral parasitemia can take three distinct values, negative, positive or unknown), and a multiple imputation technique for missing data, the MICE method (multiple imputation by chained equations), based on the Gibbs algorithm, recently implemented with S+ language and described by Van Buuren and others. We used the following 13 variables when carrying out the multiple imputation: woman’s age, parity, district of residence, sex of newborn, newborn’s birth weight, duration of pregnancy, the
two prophylaxis variables, onset of delivery by transmission period, placental infection, and the three variables indicating parasitemia by period of pregnancy. We simulated 20 data sets of imputed data and we used 10 iterations.

To measure the prediction accuracy of the model using MICE method, we estimated for women who had peripheral infection evaluation during the three periods the number of infected placentas predicted by the model, taking into account peripheral infections and significant adjustment cofactors.

All analyses were done using S + 2000 (Statistical Sciences Inc., Seattle, WA) and SAS 8.2 (SAS Institute Inc., Cary, NC).

RESULTS

Characteristics of study sample. We compared the 594 excluded women and the 928 analyzed women for ethnic group, age, gestational rank, and prophylaxis, as shown in Table 1. There was no difference between these two groups, except for the proportion of women included in the prophylaxis group, slightly higher in the analyzed group (54.1% versus 48.7%, \(P = 0.04\)). The groups did not differ as to the gestational age at inclusion (median, 21.7 and 21.0 for analyzed and excluded women, respectively, \(P = 0.09\)).

The median duration of pregnancy was 8.4 months (extreme values, 4 and 10 months). Median of the follow-up duration was 3.4 months (interquartile interval [2.3; 4.2]). Median of the women’s gestational age when entering the study was 5 months (distribution shown on Figure 2).

Results of the univariate analysis are shown in Table 2.

After selection through univariate and multivariate analysis, the following cofactors were significantly related to a high risk of placental infection: onset of delivery during the malaria transmission period (either first half or second half of transmission period), primigravidae or secundigravidae, absence of effective prophylaxis after 6 months of pregnancy. These cofactors were further included in the multivariate model with the three peripheral parasitemia variables. Results of the combined analysis of the 20 logistic regressions carried out respectively from the 20 imputed data sets using the MICE method concerning the variables peripheral infection during pregnancy and all the significant adjustment cofactors are shown in Table 3. Similar results obtained through missing data indicator method are presented in Table 4.

With both methods, the occurrence of peripheral parasitemia at the beginning and at the end of pregnancy was significantly related to placental infection, whereas peripheral parasitemia in the middle of pregnancy wasn’t. Moreover, there was a strong relation between peripheral infection in the middle and at the end of pregnancy (\(OR = 7.0, 95\% \) confidence interval [3.9 to 12.5]). All the formerly related adjustment factors remained significant.

Two hundred seventy-seven women were evaluated for peripheral infection during the three predefined periods of pregnancy. Table 5 shows the observed number of placental infections according to the sequence of the peripheral infection during the three periods of pregnancy, in comparison to the number of infected placentas predicted by the model, taking into account adjustment factors.

DISCUSSION

The aim of this work was to study the correlation between placental infection and peripheral infection history during pregnancy, two indicators of malaria infection for pregnant women, while adjusting for the other factors known to influence placental infection. We found a strong correlation between placental infection and peripheral infection at the end of the pregnancy, but also, more surprisingly, at the beginning of the pregnancy.
Five hundred ninety-four women had to be excluded from
the 1,522 in the initial cohort because of unavailable depen-
dent variable or outlying values in the duration of pregnancy.
When the women we excluded were compared with those
included in our study, no difference between the two groups
was apparent (except for the intake of prophylaxis, with only
5% less women under prophylaxis among the women we ex-
cluded compared with those selected). Thus, we believe there
is good evidence that excluding these 594 women did not lead
to selection bias, and that the cohort we analyzed was repre-
sentative of the initial cohort.

The only variable we could use for estimating the beginning
of pregnancy was the date of last menses declared by the
women. This variable produced very few unlikely values (27)
and the median duration of pregnancy (36 weeks) was quite
plausible. Other studies performed in developing countries
have shown this variable to be fairly reliable in spite of pos-
sible recall biases.\textsuperscript{18}

It has been generally observed that pregnant women are
more susceptible to malaria infection, especially primi-
gravidae (and to a lesser extent, secundigravidae). A common
explanation is that pregnancy is associated with a decrease in
immunity, which is more pronounced in primigravidae than in
multigravidae. More recently, several studies have shown that
this increase in susceptibility could be related to the property
of parasitized erythrocytes to adhere to chondroitin sulphate
A (CSA) expressed by the syncytiotrophoblast. The decreas-
ing susceptibility to pregnancy-associated malaria with in-
creasing parity is reflected in the acquisition of antibodies
specific to parasites variant antigens expressed on the surface

\begin{table}
\centering
\caption{Factors related to placental infection, univariate analysis (Banfora, Burkina Faso, 1987–1988)}
\begin{tabular}{llll}
\hline
Variable & \textit{O/N}* & OR & \textit{P} \\
\hline
Parasitemia before 4 months of pregnancy
Negative & 19/274 & 1† & 0.001 \\
Positive & 12/49 & 4.35 & \\
Unknown & 73/605 & 1.84 & \\
Parasitemia at 5–6 months of pregnancy
Negative & 47/599 & 1† & < 0.001 \\
Positive & 21/94 & 3.4 & \\
Unknown & 36/235 & 2.1 & \\
Parasitemia after 7 months of pregnancy
Negative & 54/776 & 1† & < 0.001 \\
Positive & 41/94 & 10.3 & \\
Unknown & 9/58 & 2.5 & \\
Position of delivery according to the malaria transmission period‡
Beginning of no transmission & 9/200 & 1† & < 0.001 \\
End of no transmission & 23/284 & 1.9 & \\
End of transmission & 41/221 & 4.8 & \\
Beginning of transmission & 31/223 & 3.4 & \\
No transmission & 32/484 & 1† & < 0.001 \\
Beginning of transmission & 31/223 & 3.2 & \\
End of transmission & 41/221 & 2.3 & \\
Gestational rank‡
\begin{itemize}
\item > 6 & 14/203 & 1† & < 0.001 \\
\item 5–6 & 18/193 & 1.4 & \\
\item 3–4 & 18/241 & 1.1 & \\
\item 2 & 27/158 & 2.8 & \\
\item 1 & 27/133 & 3.4 & \\
\end{itemize}
\begin{itemize}
\item > 2 & 50/637 & 1† & < 0.001 \\
\item 1–2 & 54/291 & 2.7 & \\
\end{itemize}
Effective prophylaxis§ after 6 months of pregnancy
Yes & 12/368 & 1† & < 0.001 \\
No & 92/560 & 5.9 & \\
Effective prophylaxis§ before 6 months of pregnancy
Yes & 9/290 & 1† & < 0.001 \\
No & 95/638 & 5.5 & \\
Age
\begin{itemize}
\item \leq 21 years & 45/266 & 2.4 & 0.007 \\
\item 22–25 years & 21/215 & 1.3 & \\
\item 26–30 years & 18/230 & 1† & \\
\item > 30 years & 19/210 & 1.2 & \\
\end{itemize}
Ethnic group
\begin{itemize}
\item 0.72 & \\
\item 0.38 & \\
\end{itemize}
\begin{itemize}
\item Intake of treatment (no prophylaxis) & 0.86 & \\
\item Sex of newborn & 0.88 & \\
\item Body mass index & 0.54 & \\
\end{itemize}
\hline
\end{tabular}
\textsuperscript{OR, odds ratio.}
\textsuperscript{* Number of infected placentas/total number of placentas.}
\textsuperscript{† Baseline category.}
\textsuperscript{‡ Transmission period is defined from mid-June to mid-December.}
\textsuperscript{§ At least three 300 mg intakes.}
\end{table}
of infected erythrocytes. In our analysis, we pooled primigravidae and secundigravidae in the first class, as no clear difference in placental infection showed up. Moreover this allowed us to balance the numbers within each class. Recent studies suggest that secundigravidae are as likely to be infected by malaria than primigravidae.

We had to deal with a lot of unavailable information relating to peripheral parasitemia at the beginning of pregnancy (for instance, it is impossible to determine the parasitological status during the first term of pregnancy of a woman included in the study at the fifth month of gestation) and with some missing data (absence at an investigator's visit). This is not surprising, as most studies performed in sub-Saharan Africa have shown that women only go to their first antenatal visit only in the second half of pregnancy. In such settings, it is thus vital to deal with large amounts of missing data. However, few epidemiologic studies have stressed the importance of early pregnancy in terms of malaria infection. Brabin observed that women were more likely to be infected during the first term of pregnancy, and Taha and others show that the risk of low birth weight was highest when multiple malaria episodes occurred earlier in pregnancy. This indicates an early scientific interest for the beginning of pregnancy. Lastly, we would like to stress that in spite of much unavailable data, peripheral infection status during the first period of pregnancy was identified for 323 women—the most extensive information collected on malaria infection in early pregnancy.

Biostatistical studies have shown that limiting analysis to observations with complete data drastically reduces the sample size (and thus the power of tests) and could also lead to selection bias. More accurate techniques have been developed and implemented and have been gaining wider attention among epidemiologists over the past few years. Nowadays, it is known that one of the best ways to handle missing data is to use the multiple imputation method.

We nevertheless decided to use two different methods to deal with our unknown data: a simple, commonly used method (missing data indicator) on the one hand, and because of the quantity of unknown data (particularly for peripheral infection in the first part of pregnancy), a multiple imputation method (MICE), a recent technique based on the Gibbs algorithm, on the other hand. The former method increases power, but may not correct bias. The latter gives better results than standard methods for handling missing data (such as missing data indicator or simple imputation).

MICE is a more flexible method than other algorithms equally based on Markov chain Monte Carlo, such as those described by Schafer, because MICE does not hypothesize a known joint distribution of the observations. The MICE method and other multiple imputation procedures were compared with real data, and the MICE method produced slightly better results when predicting new observations. Finally, the imputation of categorical variables is easy with MICE. Variables with missing data can be used in the imputation

### Table 3

Factors related to placental infection through logistic regression: The MICE method was used to deal with missing data (Banfora, Burkina Faso, 1987–1988)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Baseline category</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral infection as a function of gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4 months</td>
<td>Negative</td>
<td>2.9</td>
<td>1.3–6.3</td>
<td>0.01</td>
</tr>
<tr>
<td>5–6 months</td>
<td>Negative</td>
<td>1.3</td>
<td>0.7–2.6</td>
<td>0.45</td>
</tr>
<tr>
<td>≥ 7 months</td>
<td>Negative</td>
<td>4.9</td>
<td>2.7–8.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational rank ≤ 2</td>
<td>≥ 2</td>
<td>2.1</td>
<td>1.3–3.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Delivery at the beginning of transmission period</td>
<td>During the nontransmission period</td>
<td>3.2</td>
<td>1.8–5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delivery at the end of transmission period</td>
<td>During the nontransmission period</td>
<td>3.3</td>
<td>1.8–5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No effective prophylaxis after 6 months of pregnancy</td>
<td>Yes</td>
<td>4.5</td>
<td>2.4–9.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, confidence interval.

### Table 4

Factors related to placental infection through logistic regression: The missing data indicator method was used to deal with missing data (Banfora, Burkina Faso, 1987–1988)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Baseline category</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral infection as a function of gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4 months</td>
<td>Negative</td>
<td>3.3</td>
<td>1.3–8.4</td>
<td>0.02</td>
</tr>
<tr>
<td>5–6 months</td>
<td>Negative</td>
<td>1.8</td>
<td>1.0–3.2</td>
<td>0.06</td>
</tr>
<tr>
<td>≥ 7 months</td>
<td>Negative</td>
<td>1.2</td>
<td>0.6–2.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Gestational rank ≤ 2</td>
<td>≥ 2</td>
<td>1.3</td>
<td>0.8–2.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Delivery at the beginning of transmission period</td>
<td>During the nontransmission period</td>
<td>5.5</td>
<td>3.2–9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delivery at the end of transmission period</td>
<td>During the nontransmission period</td>
<td>1.9</td>
<td>0.8–4.2</td>
<td>0.1</td>
</tr>
<tr>
<td>No effective prophylaxis after 6 months of pregnancy</td>
<td>Yes</td>
<td>2.4</td>
<td>1.5–3.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, confidence interval.

*At least three 300 mg intakes.
model, and the S+ MICE library is freely available on the Web.

Nevertheless, some potential bias may still exist, because of the high number of women with unknown early peripheral parasitemia. However, MICE is known to give accurate results when used with the proportion of missing data is high.\textsuperscript{28} Moreover, to improve algorithm efficiency, we performed 20 imputations instead of the recommended 5 imputations, and we repeated 10 iterations instead of the recommended five to ensure good convergence. This was confirmed through similar results obtained after performing 10 new iterations of the algorithm.

Finally, we feel quite confident with our results for two main reasons. First, both methods (missing indicator data and MICE) led to very similar results, with the same significant factors, and particularly a significant correlation between the infection at the beginning of pregnancy and placental infection. Second, Table 5 shows that the predicted numbers of infected placentas are very close to the actual numbers. This shows that the model resulting from the combined analysis of the 20 imputed data sets gives a good prediction of the observed data. Consequently, we believe the method we used was appropriate to answer our questions with rigour.

In our adjustment factors, we observed that taking prophylaxis at the end of pregnancy provided a significant fivefold protection from placental infection. This confirms prior findings.\textsuperscript{15} Other randomized trials show a protective effect of prophylaxis on placental infection or peripheral parasitemia.\textsuperscript{26–31}

Gestational rank also appeared to be an important risk factor, as in primigravidae and secundigravidae the risk of placental infection was twofold that of multigravidae. This result is not surprising because increased susceptibility has been frequently observed in primigravidae. It is consistent with other studies, placental infection rate being generally evaluated to be one and half to two times higher in primigravidae than in multigravidae.\textsuperscript{4}

We found also a threefold increase of placental infection risk for deliveries occurring during the first or the second part of the transmission period. This is a logical result because these women are more susceptible to be infected just before delivery.\textsuperscript{4,10,32}

Peripheral parasitemia at the end of pregnancy is a very strong risk factor for placental infection (a fivefold increase). This was already suggested by the first observations of placental infection.\textsuperscript{33} Later on, several studies showed that placental infection was very often associated with a peripheral infection on the day of delivery (see, for example, Ref. 34), thus confirming the importance of late maternal infections. Also, there may be intermittent recirculation of parasites to the periphery, but this does not appear to happen frequently.

Our most original result concerns the influence of parasitemia before the fifth month of pregnancy. Univariate analysis showed that the three parasitemia variables (at the beginning, in the middle, and at the end of pregnancy) were significantly correlated with placental infection at delivery. Adjustment for the three periods of pregnancy ensures that there is indeed a proper effect of this early stage of pregnancy, independently of a late infection. Probably because of the strong correlation between parasitemia in the middle of pregnancy and parasitemia at the end of pregnancy, parasitemia in the middle of pregnancy was not significant any more in the multivariate model. Peripheral parasitemia at the beginning of pregnancy seems to be a risk factor indeed for placental infection, even when adjusted for infection at the end of pregnancy, while a later peripheral infection (during the second period of pregnancy) does not appear to influence placental infection. This suggests that a peripheral parasitemia at the beginning of pregnancy may persist within the placenta throughout gestation, with possibly more severe consequences on the placenta and the newborn, than a later infection.

Few studies have analyzed maternal infection. A study in Kenya\textsuperscript{22} showed a different parasitological status at the beginning of pregnancy compared with the end of pregnancy, with notably higher parasitemias during the first term than in the rest of pregnancy. This particular parasitological status at the beginning of pregnancy is consistent with our findings and suggests a lower immune protection at the beginning of pregnancy compared with the end of pregnancy. A large scale prophylaxis trial in Malawi\textsuperscript{34} showed, first, that placental infection was more frequent in women who were infected at enrollment in the study than those who were not infected at enrollment, and second, that placental infection was more frequent in women who were infected at enrollment in the study and also later during pregnancy than in women who were not infected at enrollment in the study but infected only later during pregnancy. These results are consistent with our own results and strongly suggest that early peripheral infection during pregnancy may be a particularly important risk factor for placental infection. Two studies investigated precisely the relationship between peripheral parasitemia and placental infection.\textsuperscript{13,14} Although they showed, as we did, the importance of the late part of pregnancy, they did not conclude on the beginning of pregnancy. The reasons may be small sample sizes, the absence of adjustment for cofactors, and for the latter study\textsuperscript{14} the particular features of the study area (low transmission, associated P. vivax malaria).

The aim of this work was to compare two different ways of characterizing malaria infection in pregnant women. As far as we know, no published study has yet related placental infection and peripheral infection during successive periods of pregnancy. Although placental infection gives only a binary response (infected or not at delivery), without taking into account the history of maternal infection during pregnancy, our results showed that this indicator is more than just an expression of a recent peripheral infection just before delivery. Although we cannot completely exclude a coincidence...
between high exposure to malaria and repeated episodes of parasitemia, we think that placental infection may also reflect an early peripheral infection during pregnancy. We feel it is a good indicator of a woman’s parasitological status during pregnancy. Moreover, insofar as placental infection has been reported to be related with a newborn’s weight at birth, it would be interesting to evaluate the consequences of an early peripheral infection during pregnancy in terms of birth weight. This will be examined in a forthcoming study.

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REFERENCES