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# Serum Lipids and Lipoproteins during Uncomplicated Malaria: A Cohort Study in Lambaréné, Gabon

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*Abstract.* The serum lipid profile in malaria patients has been found to differ from that of healthy controls. We investigated serum lipid profile changes in malaria patients over time compared with patients with other febrile diseases. In total, 217 patients were included in the study (111 malaria patients and 106 symptomatic controls, defined as malaria-negative febrile patients). Serum lipid levels (mmol/L) were significantly lower in malaria patients compared with those with other febrile diseases (total cholesterol [TC] = 3.26 [standard deviation = 0.94] versus 3.97 [1.22; $P < 0.001$]; high-density lipoprotein cholesterol [HDL-C] = 0.43 [0.47] versus 1.05 [0.67; $P < 0.001$], low-density lipoprotein cholesterol [LDL-C] = 2.05 [0.76] versus 2.42 [0.90; $P < 0.001$]. Triglycerides (TGs) levels were higher in malaria patients (1.81 [1.02] versus 1.11 [0.82; $P < 0.001$]). No significant differences were found for apolipoprotein A1, apolipoprotein B, and lipoprotein(a). Cholesterol levels increased toward reference values on day 28 (TC = 3.26–3.98, $P < 0.001$; HDL-C = 0.43–0.96, $P < 0.001$; LDL-C = 2.05–2.60, $P < 0.001$). TG levels decreased from 1.81 on admission to 1.76 (day 3) and 0.88 (day 28; $P = 0.130$). Lipid profile changes were not correlated with parasitemia or *Plasmodium falciparum* histidine-rich protein 2 levels. This study confirms characteristic temporary lipid profile changes in malaria. Lipid profile changes demonstrated a good accuracy to discriminate between malaria and other febrile diseases (area under the curve = 0.80 (95% confidence interval = 0.742–0.863, $P < 0.001$). Several plausible hypotheses exist regarding the pathophysiology of lipid profile changes in malaria. Further studies to elucidate the precise pathways may lead to improved understanding of the underlying pathophysiology.

## INTRODUCTION

Serum lipid profile changes have been observed during malaria infection.[1–6] A systematic review and meta-analysis (2013) showed that total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were lower in malaria patients compared with healthy controls.[7] It has been suggested that these lipid changes were lower compared with other febrile illnesses and thus characteristic of malaria.[7] However, this was concluded from a meta-analysis that included only few studies: three for TC,[8–10] two for HDL-C,[8,10] one for LDL-C,[10] and two for triglycerides (TGs).[10,11] Although TG levels seem to increase in malaria patients compared with healthy individuals, a statistical difference with other febrile diseases could not be demonstrated. A cohort study (2016) showed that serum lipids are not only altered in *Plasmodium falciparum* malaria, but similar changes also occur in *Plasmodium vivax* infections.[12] Little is known regarding apolipoprotein levels during malaria infection. Five studies investigated apolipoprotein A1 (apoA1) and B (apoB), with conflicting results.[6,13–16] A study found altered levels of serum lipids in patients with complicated malaria with a reduction in apoA1.[17] The authors suggested these findings could have malaria diagnostic potential.[17] Lipoprotein(a) [Lp(a)], an LDL-like particle with an apolipoprotein(a) covalently bound to apoB,[18] has not been studied during malaria infection to date. Similar to LDL-C levels, Lp(a) has been identified as an independent risk factor for atherosclerotic cardiovascular events, such as myocardial infarction.[19] Lp(a) serum levels are primarily genetically determined, but other factors including inflammatory state,[20] comorbidity (e.g., kidney disease and diabetes),[21] and the use of some therapeutic agents (e.g., aspirin, niacin, proprotein convertase subtilisin/kexin type 9 inhibitors, angiotensin-converting enzyme inhibitors, tamoxifen, and thyroxin replacement therapy) may also affect plasma Lp(a) levels.[22]

Although the mechanisms causing those lipid profile changes during malaria infection are not fully understood, several hypotheses have been suggested from clinical and animal studies. It is known that during acute infections, lipid profile changes can occur during the acute-phase response. In patients with severe sepsis, lipoprotein concentrations rapidly change, and can be reduced to 50% of recovery concentrations.[23] Serum TG levels may rise due to increased very-low-density lipoprotein cholesterol secretion as a consequence of fat tissue lipolysis, enhanced de novo hepatic fatty acid synthesis, and reduced fatty acid oxidation.[24] Apart from host-related acute-phase reaction theories, evidence accumulates for directly parasite-related lipid profile changes, such as the implication of host lipids in the formation of hemozoin (Hz), also known as malaria pigment.[7,25]

The current available evidence suggests that certain lipid profile changes are more pronounced during malaria

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infection compared with other febrile diseases. If an association between serum lipid profile changes and malaria could be demonstrated, further studies to elucidate the precise pathways could be conducted. Moreover, novel treatment approaches could be explored (first in vitro, and then by experiments subjecting *P. falciparum* to lipid profile or metabolism-regulating drugs). Mouse models to investigate lipid profile changes in malaria are not recommended because of important differences in lipid metabolism compared with humans. The underlying mechanisms for these differences are not yet understood.[24]

The primary objective of this cohort study was to describe the lipid profile changes during malaria compared with other febrile diseases. Secondary objectives were to evaluate the lipid profile changes over time and to correlate them with laboratory hallmarks of malaria such as hemoglobin and platelet level changes and parasitemia.

## MATERIALS AND METHODS

**Study design, setting, and patients.** Strengthening the Reporting of Observational Studies in Epidemiology guidelines were followed in reporting this study.[26] This cohort study was conducted in the Hospital Albert Schweitzer (HAS) in Lambaréné, a town of 25,000 inhabitants situated within the Central African rainforest area of the Moyen-Ogooué Province, Gabon. Patients admitted between April 2014 and April 2015 were screened for eligibility. Adult patients (aged $\geq$ 16 years) admitted to the hospital with fever (defined as tympanic temperature of $\geq$ 38.0°C) or a history of fever and suspected malaria were included. For all patients, written informed consent was obtained, and a thick smear was performed. Patients testing positive for malaria with a thick smear were included in the "malaria group." Febrile patients testing negative (thick smear) for malaria were included in the "symptomatic control group." Blood was drawn before start of the antibiotic/antimalarial/antiviral treatment. Uncomplicated malaria was treated with a fixed-dose artesunate–amodiaquine combination following Gabonese guidelines. Antipyretics and other drugs were used as prescribed by the attending physician. Malaria patients were followed up on day 3 (D3) and D28 following diagnosis and treatment initiation (D0). To increase the follow-up rate, patients were sent a reminder by SMS and were called up to three times. Control patients were not followed up any further. Refund of travel expenses was provided. Clinical information, malaria test results, and blood samples were collected following standard procedures. The study was approved by the Scientific and the Ethics Review Committees of the Center des Recherches Médicales de Lambaréné (CERMEL) and conducted according to good laboratory practice and good clinical practice/International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines. In addition, the study was endorsed by the Ethics Committee of the Academic Medical Center of the University of Amsterdam, the Netherlands.

**Case definitions.** Malaria cases were defined as individuals with fever (measured, or history provided of fever in the past 24 hours) and any level of parasitemia due to *Plasmodium* species. Uncomplicated malaria was defined as a patient who presents with symptoms and a positive parasitological test (microscopy) but with no features of severe malaria. There were no patients with severe malaria according to World Health Organization (WHO) definition.[27] Symptomatic controls (not further diagnosed) were defined as patients presenting with symptoms and (a history of) fever, but testing negative for malaria (microscopy). Malaria thick and thin smears were prepared following standard procedures. Thick smears were assessed by well-trained laboratory staff and independently by a clinical researcher according to the Lambaréné method.[28]

**Clinical laboratory methods.** Blood samples from malaria patients were collected on D0, D3, and D28; and for symptomatic controls on D0. Hematological analysis was performed in the laboratory of the CERMEL using an ABX Pentra 60® (HORIBA Ltd, Kyota, Japan) analyzer. All samples were processed according to standard protocols and stored at –80°C within 3 hours after collection until analysis. The serum lipids were analyzed following standard procedures. For measurement of Lp(a) levels, an apo(a)-independent assay was used (Architect; Abbott, Chicago, IL).[29] *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP-2) (Malaria Antigen CELISA; Cellabs, Sydney, Australia) was measured on D0 and D28 in malaria patients with antigen-detection enzyme-linked immunosorbent assay (ELISA) at 450 nm in a SUNRISE ELISA reader (Tecan, Männedorf, Switzerland).

**Outcomes and variables.** The outcomes were levels of TC, HDL-C, LDL-C, TGs, apoA1, apoB, and Lp(a). Parasitemia was calculated using microscopy. *Pf*HRP-2 is directly correlated with total viable parasite mass. The main outcomes were mean differences of these variables between malaria patients and symptomatic controls. Predefined factors considered to correlate with serum lipid levels were age, sex, body mass index, and waist circumference (cm). The number of hours since the last consumed meal was noted (as reported by the patient) because this can correlate with serum TG levels.

**Sample size calculation.** To determine the sample size, differences between two means were calculated. To define the expected differences to be found, reference values as documented in the laboratory were used. European reference values were applied since no reference values were available for Gabon or the Central African region. Normal HDL-C measures ranged from 0.78 to 1.74 mmol/L, LDL-C from 2.51 to 5.23 mmol/L, apoA1 from 1.0 to 2.1 mmol/L, TC from 3.7 to 6.5 mmol/L, and TGs from 0.5 to 2.0 mmol/L. These reference values differ for age and sex. The parameter with the smallest range was used to calculate our group size, which was HDL-C. To detect a change (difference in group means) in HDL-C of 0.1 mmol/L blood in the patient population compared with the symptomatic controls, with a confidence interval (CI) of 95%, a power of 80% and an standard deviation of 0.24 in both groups, the number of patients required in each group (sample size) was calculated as 91.

**Statistical methods.** For statistical analyses, the IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY) was used. Categorical variables are described as absolute numbers or percentages, and continuous variables are presented as medians and interquartile ranges (IQRs). A Kolmogorov–Smirnov test was used to determine the distribution of continuous variables. Fisher's exact test was used for comparisons of categorical variables. For data with

a normal distribution, an unpaired *t* test (Student's *t* test) was used for determining continuous variables. The Mann–Whitney *U* test was used to determine differences between nonnormally distributed continuous variables. The repeated measures general linear model (repeated measures analysis of variance [ANOVA]) was used to analyze variables with repeated measures. Correlation coefficients were determined according to Spearman's rank correlation test. For a prediction model, a binary logistic regression method was used in combination with receiver operating characteristic (ROC) curves. A $P$ value of $< 0.05$ was used as level of significance in all analyses. Data were assessed for completeness by the first author (Benjamin J. Visser). If more than 10% of data for a certain variable was missing, a missing data analysis was conducted. Finally, a meta-analysis[7] of lipids in malaria studies, conducted by the authors in 2013, was updated including the findings of the present study. Methods of the meta-analysis were described previously.[7]

## RESULTS

**Participants.** In total, 525 patients were screened for eligibility. A total of 217 patients were included in the study (Figure 1). The mean age and sex did not differ significantly

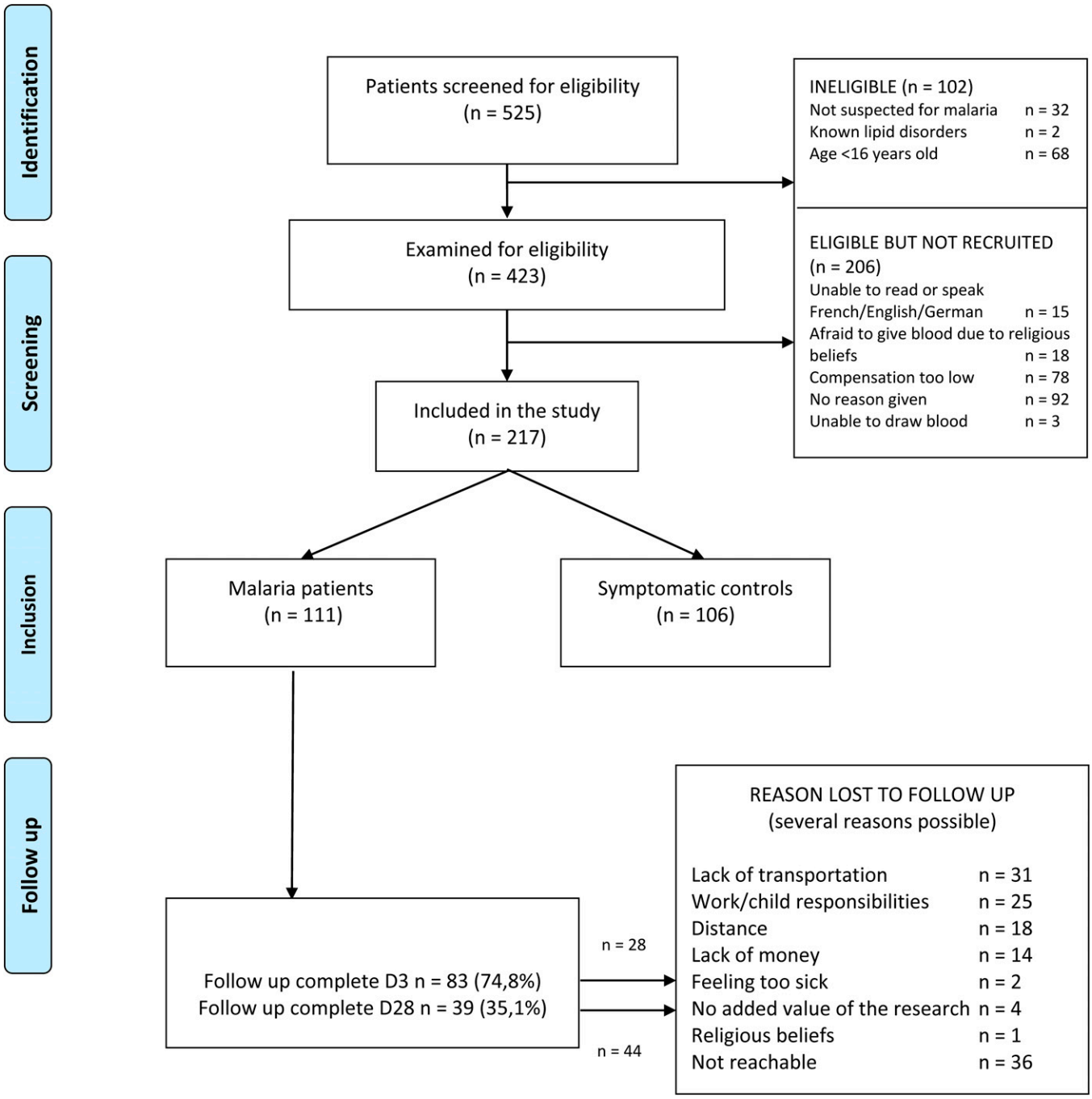


FIGURE 1. Strengthening the Reporting of Observational Studies in Epidemiology flow diagram.

between the patients screened for eligibility and the patients included in this study.

For the follow-up visits, 83 (74.8%) malaria patients presented on D3, and 39 (35.1%) on D28. The main reasons for lost to follow-up were unreachable (patient could not be reached by mobile phone), lack of transportation, work/child responsibilities, travel distance, and lack of money. Baseline characteristics of lost to follow-up participants did not significantly differ from participants who were present at follow-up visits (data not shown).

Table 1 describes the baseline characteristics of patients enrolled in this study, stratified according to the two predefined groups "malaria patients" and "symptomatic controls." Baseline characteristics did not differ significantly except for the symptoms of fever, cough, headache, and vomiting. There were no patients with severe malaria according to WHO definition.[27] The body temperature was significantly higher in the malaria group compared with the controls at the time of inclusion in the study (37.7°C [36.5–39.0] versus 37.3°C [36.5–38.0] $P = 0.024$). At the time of screening for eligibility, all participants had fever (tympanic temperature $\geq$ 38.0°C). Our population was relatively young (mean age = 33 years, IQR = 20–43), with a well-balanced male/female ratio (49:51). Most patients came from neighborhoods in and around Lambaréné. The parasitemia geometric median was 3,900/μL. Data on medical care before admission were available for 215 (99%) of the included patients. The majority ($N$ = 188, 87.4%) had used some

TABLE 1
Baseline characteristics

| | Malaria patients ($N$ = 111) | Symptomatic controls ($N$ = 106) | $P$ value |
|---|---|---|---|
| Age, years | Mean: 33.0 | Mean: 34.8 | Mann–Whitney $U$ test (0.659) |
| Kolmogorov–Smirnov ($P \leq 0.001$) | Median: 27.0 | Median: 30.0 | |
| | IQR: 42.3–21.0 | IQR: 43.8–20.3 | |
| *Plasmodium* spp. | | | |
| *P. falciparum* mono infection | 111 | NA | |
| Mixed infection (*P. falciparum* with *P. ovale*, *P. malariae*, or *P. vivax*) | 0 | NA | |
| Sex | | | $\chi^2$ test (0.344) |
| Male | 51 (45.9%) | 55 (51.9%) | Fisher's two-sided test (0.414) |
| Female | 60 (54.1%) | 51 (48.1%) | |
| Health insurance | | | $\chi^2$ test (0.270) |
| Yes | 61 (55.0%) | 62 (58.5%) | |
| No | 46 (41.4%) | 34 (32.1%) | Fisher's two-sided test (0.315) |
| Missing | 4 (3.6%) | 10 (9.4%) | |
| Last meal in hours, mean (IQR) | 18.9 (5.5–24.5) | 13.5 (1–17) | 0.05 |
| Clinical symptoms | | | Fisher's two-sided test |
| Fever (> 38.0°C) | 102 (91.9%) | 78 (73.6%) | **< 0.001** |
| Cough | 22 (19.8%) | 36 (34.0%) | **0.021** |
| Shortness of breath | 21 (18.9%) | 23 (21.7%) | 0.617 |
| Headache | 93 (83.8%) | 76 (71.7%) | **0.032** |
| Altered mental status | 1 (0.9%) | 2 (1.9%) | 0.615 |
| Diarrhea | 33 (29.7%) | 36 (34.0%) | 0.560 |
| Vomiting | 66 (59.5%) | 38 (35.8%) | **0.001** |
| Abdominal pain | 46 (41.4%) | 41 (38.7%) | 0.781 |
| Weight loss | 22 (19.8%) | 34 (32.1%) | **0.044** |
| Night sweats | 45 (40.5%) | 30 (28.3%) | 0.064 |
| Urinary tract symptoms | 10 (9.0%) | 11 (10.4%) | 0.820 |
| Wounds | 2 (1.8%) | 2 (1.9%) | 1.000 |
| SIRS criteria | 6 (5.4%) | 3 (2.8%) | 0.500 |
| Other clinical symptoms | | | |
| Weight, kg | 61.1 (50–67) | 62.2 (51–70) | 0.583 |
| Height, cm | 164.3 (160–170) | 168.1 (161–175) | **0.010** |
| BMI (nonnormal), kg/m$^2$ | 22.8 (19.4–24.5) | 22.4 (19.1–24.2) | 0.624 |
| Temperature (axillary), °C | 37.7 (36.5–39.0) | 37.3 (36.5–38.0) | **0.024** |
| Heart rate, beats/minute | 87.7 (78–100) | 86.1 (72–99) | 0.501 |
| Blood pressure systolic (nonnormal), mmHg | 117.8 (110–130) | 117.9 (110–130) | 0.647 |
| Blood pressure diastolic (nonnormal), mmHg | 64.9 (60–70) | 69.4 (60–80) | **0.003** |
| Respiratory rate (nonnormal), breaths/minute | 17.0 | 19.1 (15–24) | 0.421 |
| EMV score (nonnormal) | 15.0 | 14.9 | 0.526 |
| Abdominal circumference, cm | 78.4 (70–84) | 80.5 (72–90) | 0.316 |
| HIV status | | | |
| HIV-positive | 7 | 10 | 0.41 |
| HIV negative | 22 | 31 | |
| Unknown | 82 | 65 | |
| Malaria parasites | | | |
| Minimum–maximum | 48–366,000 | NA | |
| Median | 3,900.0 | | |
| Mean | 32,261.21 | | |
| IQR | 15,150.0–1,294.3.0 | | |

BMI = body mass index; EMV = Glasgow Coma Scale; E = eye response; M = motor response; V = verbal response; HIV = human immunodeficiency virus; IQR = interquartile range; SIRS = Systemic Inflammatory Response Syndrome. For categorical variables, the absolute number is given with the percentage, and for continuous variable medians are given with their IQRs. $\chi^2$ tests were used for comparisons of categorical variables, Mann–Whitney $U$ tests to assess differences for nonnormally distributed continuous variables, and unpaired $t$ tests for normally distributed variables. Bold values represent values $P$ value < 0.05.

TABLE 2
Lipid levels (mmol/L) in the malaria group versus symptomatic controls

| | Malaria patients (on admission) | Symptomatic controls (on admission) | |
|---|---|---|---|
| Serum lipid parameters | Mean (SE, SD) | Mean (SE, SD) | *P* value |
| Total cholesterol (nonnormal) | 3.26 (0.09, 0.94) | 3.97 (0.12, 1.22) | **< 0.001** |
| HDL-C (nonnormal) | 0.43 (0.05, 0.47) | 1.05 (0.07, 0.67) | **< 0.001** |
| LDL-C (nonnormal) | 2.05 (0.07, 0.76) | 2.42 (0.09, 0.90) | **< 0.001** |
| Triglycerides (nonnormal) | 1.81 (0.09, 1.02) | 1.11 (0.08, 0.82) | **< 0.001** |
| Apo A1 (nonnormal) | 1.01 (0.04, 0.39) | 1.09 (0.04, 0.42) | 0.161 |
| Apo B | 0.80 (0.02, 0.23) | 0.80 (0.02, 0.23) | 0.934 |
| Lipoprotein(a) (mg/L) (nonnormal) | 306.83 (22.85, 238.61) | 301.37 (21.58, 221.11) | 0.954 |

Apo A1 = apolipoprotein-A1; Apo B = apolipoprotein B; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SD = standard deviation; SE = standard error. Mann–Whitney *U* test was used to assess differences for nonnormally distributed continuous variables, and unpaired *t* test for normally distributed variables. Bold values represent values *P* value < 0.05.

kind of medicine for their current illness before presenting to the HAS. Data on health-care-seeking behavior is provided in Supplemental Table 1. Hematological parameters of malaria versus symptomatic control patients are depicted in Supplemental Table 2.

**Lipid profile changes.** The mean values of serum lipids (mmol/L, except Lp(a) mg/L) for the two groups are depicted in Table 2. TC, HDL-C, and LDL-C were significantly lower in malaria patients compared with controls on the day of admission. TG concentrations were significantly higher compared with controls. No differences were observed in apoA1, apoB, and Lp(a) on the day of admission between both groups. There was no significant difference in the number of hours since the last consumed meal between the malaria and symptomatic control groups (18.9 hours versus 13.5 hours, $P = 0.05$). No clinical relevant correlations between lipid levels and time of the last meal could be demonstrated (TC: $R = -0.095$, $P = 0.359$; HDL-C: $R = -0.123$, $P = 0.232$; LDL-C: $R = -0.091$, $P = 0.379$; TGs: $R = 0.079$, $P = 0.442$; apoA1: $R = 0.074$, $P = 0.471$; apoB: $R = -0.094$, $P = 0.358$, Lp(a): $R = -0.021$, $P = 0.039$). Table 3 shows lipid levels in malaria patients on D3 and D28. Assuming that D28 is reflecting "back-to-normal" serum lipid levels, following successful malaria treatment, HDL-C demonstrates a significant increase (55% lower levels on admission [D0] compared with D28) compared with other lipids (TC = 18.1% lower, LDL-C = 21.0% lower, TGs = 105.3% higher, apoA1 = 14.7% lower, apo B = 6.9% lower, and Lp(a) = 41.8% lower when compared with D28). HDL-C levels continued to decrease on D3 and recovered to reference values on D28. TC levels reached reference values within 28 days. The raised TG levels on D0 recovered and decreased to reference values on D3 and D28. Lp(a) levels declined on D3 but increased significantly on D28. No strong correlations could be found between lipid levels and parasitemia in malaria patients except for a weak correlation between HDL-C and parasitemia ($R = -0.259$, $P = 0.010$). As well, no strong correlation could be demonstrated between lipid levels and *Pf*HRP-2 D0 ($R = 0.151$, $P = 0.159$). Full results of correlations of parasite counts and lipids are shown in Supplemental Figures 1–2, Supplemental Tables 3 and 4. Subgroup analysis showed no significant differences in high concentrations versus low concentrations of *Pf*HRP-2 and the extent of the lipid profile changes. These calculations were corrected for pretreatment with antimalarials, which may affect *Pf*HPR-2 production. The mean *Pf*HRP-2 concentration in malaria patients on admission (D0) was 2.37 (95% CI = 2.15–2.58) versus 0.74 (CI = 0.38–1.10) on D28 ($P = 0.001$, Wilcoxon signed-rank test).

**Update of meta-analysis.** A meta-analysis of serum lipid profile changes conducted by the authors before was updated using the present study data.[7] The forest plots are shown in Supplemental Figures 3–6 . Forest plot 1 shows the mean difference for TC in malaria patients versus symptomatic controls: 0.78 mmol/L (95% CI = 0.32–1.23, $I^2 = 86\%$, $Z = 3.33$, $P = 0.0009$). Forest plot 2 shows the mean difference for HDL-C in malaria patients versus symptomatic controls: 0.45 mmol/L (95% CI = 0.22–0.68, $I^2 = 77\%$, $Z = 3.81$, $P = 0.0001$). Forest plot 3 shows the mean difference for LDL-C in malaria patients versus symptomatic controls: 1.02 mmol/L (95% CI = –0.25 to 2.29, $I^2 = 98\%$, $Z = 1.57$, $P = 0.12$). Forest plot 4 shows the mean difference for TGs in malaria versus symptomatic controls: 0.52 mmol/L (95% CI = 0.02–1.02, $I^2 = 91\%$, $Z = 2.02$, $P = 0.04$). Only random effect models were used. This meta-analysis update further strengthens the findings in the present cohort study.

TABLE 3
Lipid levels (mmol/L) on day 0 (D0), D3, and D28 in malaria patients

| Lipid parameters on admission and follow-up (only malaria patients) | D0 (*N* = 111, 100%) | D3 (*N* = 83, 74.8%) | D28 (*N* = 39, 35.1%) | *P* value |
|---|---|---|---|---|
| Total cholesterol | 3.2589 (–18.1%) | 3.4051 | 3.9787 | **< 0.001** |
| High-density lipoprotein cholesterol | 0.4260 (–55.7%) | 0.4048 | 0.9621 | **< 0.001** |
| Low-density lipoprotein cholesterol | 2.0492 (–21.0%) | 2.2064 | 2.5944 | **< 0.001** |
| Triglycerides | 1.812 (+105.3%) | 1.7578 | 0.8828 | 0.130 |
| Albumin | 40.16 | 38.94 | 40.05 | 0.128 |
| Apolipoprotein A1 | 1.0128 (–14.7%) | 0.7705 | 1.1877 | **0.001** |
| Apolipoprotein B | 0.8012 (–6.9%) | 0.9135 | 0.8603 | 0.920 |
| Lipoprotein(a) (mg/L) | 306.83 (–41.8%) | 285.76 | 527.62 | **0.003** |

Bold values represent values *P* value < 0.05.

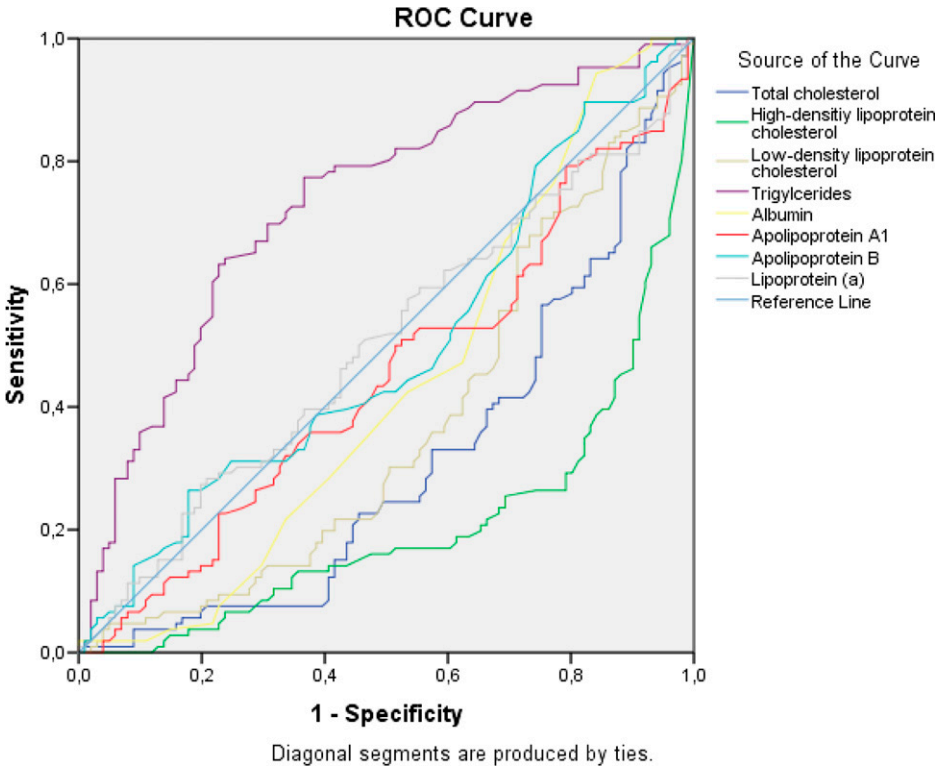


FIGURE 2. Receiver operating characteristic (ROC) curve of lipids differentiating malaria from other febrile diseases.

**Accuracy of serum lipids for differentiating malaria from other febrile diseases.** The area under the ROC (AUC) indicates the accuracy of different lipids to differentiate between malaria and other febrile diseases (Figure 2 and Table 4). The ROC curves demonstrate a decreased HDL-C (AUC = 0.207, $P < 0.02$) and raised TGs (AUC = 0.732, $P < 0.042$) to be moderate predictors of malaria. The predictive values of other lipids were low and not significant. However, the ROC curves of a prediction model, combining serum lipids and lipoproteins, showed a high accuracy to differentiate malaria from other febrile diseases (AUC = 0.80 [95% CI = 0.742–0.863; $P < 0.001$ for TC, HDL-C, LDL-C, TG, apoA1, apo B, and Lp(a)]) (Figure 3 and Table 5).

## DISCUSSION

This prospective cohort study of 217 patients demonstrated that malaria has an impact on serum lipids in patients presenting with malaria in Lambaréné comparable to previous reports.[30,31]

TABLE 4
AUC for serum lipids for differentiating uncomplicated from other febrile diseases

| Lipid | AUC |
|---|---|
| Total cholesterol | 0.311 |
| High-density lipoprotein cholesterol | 0.207 |
| Low-density lipoprotein cholesterol | 0.371 |
| Triglycerides | 0.732 |
| Albumin | 0.435 |
| Apolipoprotein A1 | 0.448 |
| Apolipoprotein B | 0.495 |
| Lipoprotein(a) | 0.501 |

AUC = area under the curve.

In malaria patients, TC, HDL-C, and LDL-C levels were lower compared with patients with other febrile diseases. In particular, HDL-C levels were extremely low in malaria patients. TG levels were raised during acute malaria infection. In accordance, previous studies and a recent meta-analysis showed congruent lipid profile changes during malaria infection.[2–5,7,8,10,11,13,14,24,32–47] The meta-analysis (2013) was updated with the data of the present study, further strengthening the findings of that study.[7]

The extent of these serum lipid profile changes was not related to parasitemia, in contrast to three other studies.[33,34,48] Two other studies also did not find an association with parasitemia.[14,35] This may be explained by the fact that differences in our malaria population (all cases were uncomplicated malaria) were too marginal to yield significant differences.

This is the first study evaluating the association between *Pf*HRP-2 and lipid profile changes. This analysis was conducted to find a potentially more reliable correlation between parasite biomass and lipid profile changes. *Pf*HRP-2 was not measured on D3 because of the long clearance time. It generally takes 2–4 weeks (sometimes longer) for *Pf*HRP-2 levels to become negative after adequate antimalarial treatment.[49]

*Pf*HRP-2 response on treatment, because of its long half-life, is limping behind parasite clearance.[49] In the present study, *Pf*HRP-2 concentrations had decreased significantly on D28 indicating adequate antimalarial treatment response. However, the degree of serum lipid profile

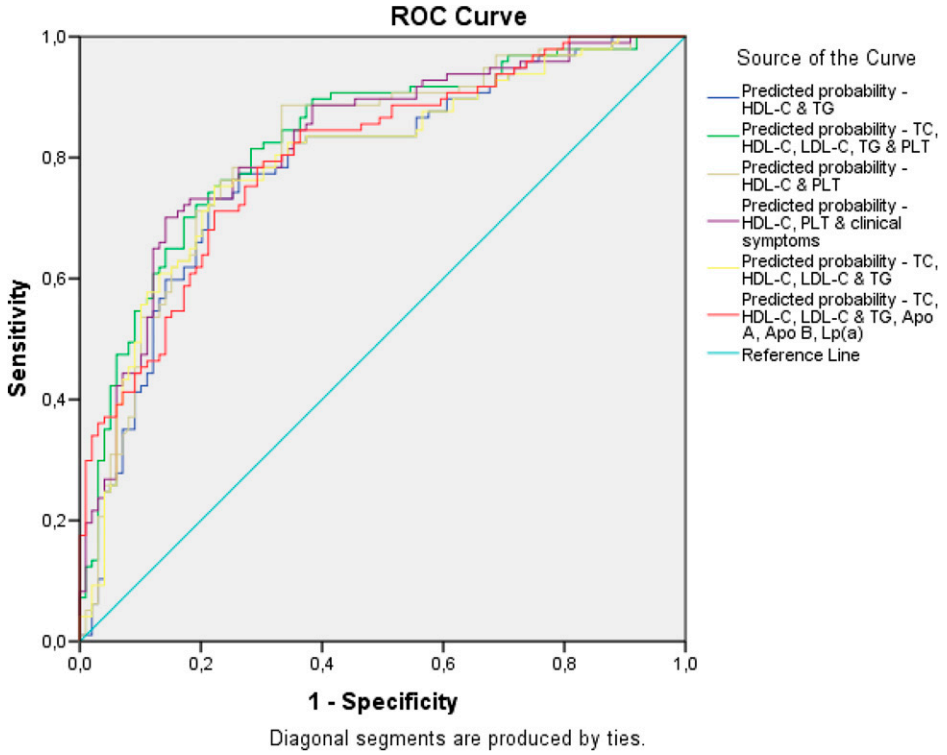


FIGURE 3. Receiver operating characteristic (ROC) curve of combination of lipids in differentiating malaria from other febrile diseases.

changes did not correlate with *Pf*HRP-2. Initial peripheral blood parasitemia is not always correlated with severity of disease, because in falciparum malaria, microvascular sequestration of parasitized erythrocytes occurs in the organs. The concentration of *Pf*HRP-2 in the peripheral blood is directly related to the total parasite biomass and can be associated with disease severity and outcome in univariate analysis.[50] The weak association between the extent of lipid level changes and *Pf*HRP-2 in this study can indicate that the extend is not correlated to disease severity of malaria. However, the most important reason in the present study is that the malaria population is quite homogeneous, all malaria cases were uncomplicated, and no one developed severe malaria, therefore, vast differences were not expected. Other, less probable explanations include variations across populations; this can be caused by variations in the structure of *Pf*HRP-2 proteins linked with different clones of the malaria parasite. Polymorphisms in the sequencing of *Pf*HRP-2 can cause changes in the number of antibodies bound to the protein.[51] Other reasons such as deletions of the *Pf*HRP-2 genes in some populations, biological variations of *Pf*HRP-2, and variation across sample types may also play a role.

This study also reviewed the usefulness of lipid profile changes for diagnosing malaria. It has been suggested in the literature that a raised TG level[11] and a lowered HDL-C level[14] are useful indicators to differentiate between uncomplicated and severe malaria or between malaria and other febrile diseases.[37] An animal study demonstrated the potential of differentiating and identifying animals with cerebral malaria at an early asymptomatic stage.[52] However, the malaria mouse model does not often replicate the pathophysiology of malaria infections in humans,[53] and therefore we do not recommend to study lipid metabolism (which differs significantly in humans[24]) in a mouse model.

The present study demonstrated TGs and HDL-C as separate markers to be of moderate accuracy in differentiating malaria from other febrile diseases, whereas the

TABLE 5
AUC for combinations of serum lipids and lipoproteins, platelets, and clinical symptoms

| Lipid | AUC | 95% Confidence interval (all $P < 0.001$) |
|---|---|---|
| HDL-C and TG | 0.786 | 0.721–0.851 |
| TC, HDL-C, LDL-C, TG, and PLT | 0.830 | 0.772–0.888 |
| TC, HDL-C, LDL-C, and TG | 0.796 | 0.732–0.859 |
| HDL-C and PLT | 0.813 | 0.752–0.874 |
| HDL-C, PLT, and clinical symptoms | 0.824 | 0.765–0.882 |
| TC, HDL-C, LDL-C, and TG, Apo A, Apo B, Lp(a) | 0.802 | 0.742–0.863 |

Apo A1 = apolipoprotein A1; Apo B = apolipoprotein B; AUC = area under the curve; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Lp(a) = Lipoprotein (a); PLT = platelets; TG = triglyceride; TC = total cholesterol. Clinical symptoms: headache, vomiting, and fever.

combination of lipid profile changes shows a good accuracy in differentiating malaria from other diseases. However, the widespread availability of highly specific malaria rapid diagnostic tests in endemic areas, indicate very little, if any, clinical value of these markers to aid establishing a malaria diagnosis.

No significant changes could be found for apoA1, apoB, and Lp(a) levels. Lp(a) levels are mainly determined genetically, and the fact that no difference could be demonstrated between malaria patients and symptomatic controls does not exclude a role of Lp(a) in the pathogenesis of malaria. Lp(a) levels were approximately normal on admission (reference value for males and females: < 300 mg/L), slightly decreased on D3 and increased on D28 (527.62 mg/L; $P = 0.003$, ANOVA). Long-term increased Lp(a) levels are a modest risk factor for atherosclerotic diseases, especially myocardial infarction. Lp(a) levels are normally stable over time, but may be affected by patient-related factors including pro-inflammatory state, comorbidity, and use of therapeutic agents.[54] Increased Lp(a) levels have been described in infections before, potentially resulting in hypercoagulability with reduced fibrinolysis.[55] Lp(a) levels have, to the best of our knowledge, not been described before in malaria patients but may play a role in the coagulopathy seen in malaria.[56]

In the present study, apoA1 and apoB levels did not differ significantly between malaria patients and symptomatic controls, in contrast to previous studies. A study in 255 Nigerian children showed significant differences in apoA1 levels between uncomplicated and severe malaria and healthy controls, and suggested these as of potential prognostic utility in the management of malaria.[17] However, the results of other studies investigating apoA1 and apoB are conflicting.[6,13,14,16]

The possible implications and mechanisms behind the lipid level alterations remain to be elucidated. Several theories have been described in detail before.[7] In patients with acute infection[57–62] or with severe sepsis, lipid concentrations can occur rapidly (D0) and can decrease to 50% of the recovery concentrations.[23] HDL-C is found to decrease in patients with sepsis, but not as extreme as found in malaria patients in this study.[23] TG levels were higher in the malaria group. TG levels can be raised during sepsis.[63] Fever and vomiting were overrepresented in the malaria group; therefore, these symptoms may be marker of disease severity and sepsis, explaining the observed TGs profiles. Some other studies described specific lipid pattern changes during acute viral and bacterial infections.[64–66]

Because no data on lipid levels before the malaria or febrile episode were available, it was impossible to ascertain whether lipoprotein levels were low because of the malaria episode, or whether the patients were susceptible to malaria infection because of low serum lipids caused by another mechanism. In one study, decreased levels of HDL-C and TC were associated with a larger risk for nosocomial infections.[67] On the other hand, the treatment of malaria resulted in the normalizing of lipid levels, demonstrating inverse temporality and suggesting malaria is the cause of serum lipid alterations.

Apolipoproteins have been implicated in the prevention of growth of malarial parasite in the intraerythrocytic stage,[68] or to play a role in the level of parasitemia.[69] Another parasite-related theory is the Hz theory. Hz, also known as malaria pigment, is the end product of the plasmodial detoxification process of free heme.[25] The amount of Hz within ringforms/trophozoites and macrophages relates only poorly to parasitemia. Further research could elucidate the precise biological pathways. This should be done in in vitro models or in vivo studies in primates, and not in nonprimates such as a rodent model. The most important reason is that cholesterol metabolism differs between rodents and primates. In nonprimates such as rodents, rabbits, and dogs, inflammation and infection result in a raised TC levels and hepatic cholesterol synthesis, whereas in primates and humans either no change or a decrease in cholesterol and LDL levels are observed.[24,70] The underlying mechanisms for these differences are not fully understood.

There are some limitations to this study. First, due to limited availability of diagnostic services it was not possible to diagnose fully the symptomatic control patients. However, likely diagnoses in this group were bacterial infections including tuberculosis, urinary and pulmonary tract infections, and viral illnesses, such as dengue and chikungunya. While all those patients were treated according to the clinical practice, the pattern of confirmed diagnoses remained patchy. Second, all malaria patients were diagnosed by microscopy with a *P. falciparum* monoinfection. Mixed malaria infections may have been missed by the medical technician and second reader. Third, microscopy-based diagnosis could have misallocated patients. Malaria polymerase chain reaction (PCR)–based confirmation was not conducted in this study setting, most importantly because Gabon is a high-transmission country for malaria, meaning that many people are asymptomatic carrier of malaria parasites or have a low-level parasitemia because of partial immunity. Detecting a PCR-positive blood sample does not necessarily represent an indication for antimalarial treatment of the patient (and subsequently allocation to our "malaria group").[71] Literature is scarce about the role and importance of subclinical parasitaemia, for example, in the development of partial immunity.[72] Fourth, a large number of patients were lost to follow-up, possibly due to the high mobility of the Gabonese population and the unavailability of a local fieldworker. Fifth, this study was a hospital-based study, so the lipid profile changes presented in the current study may not be fully representative for malaria episodes in the community particularly in semi-immune adults. Finally, no reference values for common lipid parameters were available for Gabon or the Central Africa region. Therefore, European reference standards were used.

In conclusion, certain serum lipid profile changes are characteristic for malaria. This study confirms and strengthens previous findings regarding lipid profile changes in malaria. The mechanisms underlying these changes are most probably a combination of parasite- and host-related (i.e., related to an acute-phase reaction) mechanisms. Serum lipid profile changes have been repeatedly suggested as an accurate marker to differentiate between malaria and other febrile diseases. However, the usefulness of lipid markers to differentiate between malaria and other diseases was low and of no clinical value when rapid diagnostic tests or microscopy is available. Further research should not be focused on lipid profile changes in patients (as evidence is accumulating), but rather on the biological pathways, to increase our

knowledge of the pathogenesis of malaria, potentially opening avenues to explore novel treatment options.

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