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Burden of asymptomatic malaria, anemia and relationship with cotrimoxazole use and CD4 cell count among HIV1-infected adults living in Gabon, Central Africa

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ABSTRACT

Objective : This study determined the prevalence of asymptomatic Plasmodium (P.) falciparum infection and anemia in adults living with HIV/AIDS (PLHIV) and compared malaria prevalence between 858 HIV-infected (PLHIV) and 272 uninfected individuals in Gabon where such information are lacking. Factors influencing malaria and anemia were also investigated. **Patients and Methods:** Participants were screened for malaria. Available hemoglobin level, socio-demographic and use of prevention or treatment data were compared between both groups. **Results:** The prevalence of asymptomatic parasitemia was 13.5%, lower in PLHIV (7.1%) than uninfected individuals (33.8%) (p<0.01). Among the PLHIV, females (p<0.01), those aged below 25 years old (p=0.03), those with primary education (p=0.03) and those with a CD4 cell count below 200/mm3 (p=0.03) had a higher median parasitemia. Cotrimoxazole use was associated with a lower prevalence of malaria (p<0.01). Age below 25 years was independently associated with malaria in PLHIV (p<0.01). Anemia prevalence was 42.1% among the PLHIV, higher in the youngest and those with low CD4 cell count (p<0.01). P.falciparum-infected PLHIV aged below 25 years old, not under ART, with low CD4 cell count and under cotrimoxazole had the lowest median hemoglobin level. **Conclusion:** The prevalence of asymptomatic malaria is low among the PLHIV while the burden of anemia is considerable. Age below 25 years and CD4 cell count are associated factors. The cotrimoxazole use reduces the frequency of malaria.

KEYWORDS
Malaria; HIV; anemia; adults; gabon

Introduction

HIV and *Plasmodium(P.) falciparum* malaria are two important global health problems in developing countries. In 2015, 114 million malaria cases were reported in Subsaharan Africa where 26 out of the 37 million HIV-infected individuals lived [1,2]. Co-infections are considered as an emerging public health problem in tropical areas and the extensive overlap of the geographical distribution of these pathogens would make this co-infection more common in endemic areas [3]. Although malaria mortality remains confined in young children from sub-Saharan Africa, recent reports highlight an increasing frequency of *P. falciparum* infection in older children and adults in areas with changing epidemiology [4,5]. Co-morbidity influences the outcome of both diseases [3]. HIV infection can increase the risk and the severity of malaria, this effect is pronounced with increasing immunosuppression, especially when the CD4 cells count is below 200/mL [6–8]. In contrast, malaria episodes induce a non-specific lymphopenia and a decrease of CD4 cell count in HIV-1-infected adults and children; it is also associated with increasing levels of HIV RNA replication [9,10].

Over the past five years, a rebound and increasing burden of malaria, either as symptomatic cases or asymptomatic carriage, has been observed in Gabon [11,12]. Older children and adults from urban cities bear now the highest burden of *P. falciparum* infection compared to younger children [4,12]. HIV prevalence range from 2 to 13% with young adults being the most frequently infected [13]. Thus, HIV infected individuals are continuously exposed to malaria and its consequences. Both diseases are known risk factors for anemia whose prevalence is more than 40% in adults consulting in health centres of the country [4]. On the other hand, the use of Cotrimoxazole (CTX) daily prophylaxis against opportunistic infection is also associated with the reduction of malaria incidence in HIV-infected individuals [14–16]. Although it is established

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that HIV and malaria worsen each other, PLHIV are not targeted by preventive strategies; and, as a common feature in adults from highly malaria endemic settings, they would also be frequently asymptomatic. Studies estimating the burden and the transmission of malaria usually do not target this population, although they could contribute to parasite reservoir. Indeed malaria transmission is also maintain by asymptomatic or submicroscopic parasite carriers [17]. HIV-infected individuals which are not always screened for malaria, could either be frequently infected by *P. falciparum*, as they are not targeted by the control strategies such as ITNs or chronically infected. Therefore, asymptomatic PLHIV would represent an important and unknown parasite reservoir. Anemia, a consequence of the silent parasite multiplication, would also be frequent among them. Together with sulfadoxine-pyrimethamine which is used for malaria prevention during pregnancy, CTX use can also select drug resistant parasites whose circulation would also contribute to the persistence of malaria transmission [18,19]. Little is known about CTX prophylaxis coverage among HIV infected individuals, as well as malaria-HIV co-infection prevalence in Gabon. Providing estimate of malaria and anemia burden in this vulnerable population will help for the design of appropriate intervention and control strategies at the time of the observed rebound of malaria morbidity in Gabon. The present study aimed at determining the frequency of asymptomatic malaria and related or unrelated anemia among PLHIV living in Gabon. The study also looked at the influence of demographic variables, bednet, CTX, antiretroviral treatment (ART) use and CD4 cell count on malaria and anemia. A subgroup of HIV-uninfected individuals served as control group for this evaluation. These data will serve as baseline and could help for the design of malaria and anemia control strategies which would incorporate the needs of this vulnerable group.

Patients and methods

Study sites

The study was carried out between February and June 2016 in the care and treatment centers of three main cities of Gabon (Libreville, Oyem, Koulamoutou). Malaria prevalence ranges from 24% in Libreville to 53% at Oyem (Data from DPM). Libreville is the capital city of Gabon where almost 50% of the 1.8 million inhabitants of the country live, Oyem and Koulamoutou are rural cities located in Northern and South Eastern parts of the country, respectively. The climate is equatorial with two rainy and two dry seasons, temperatures ranges of 23–33 °C and 80% humidity on average. Malaria transmission is perennial in these areas and *P. falciparum* is the main species (96%).

Study design and population

A cross sectional study was conducted from February to June 2016. Patients living with HIV (PLHIV) attending their routine follow-up visits in three HIV care and treatment centers (CTC) were invited to participate in the study. For the comparison of malaria prevalence, data from volunteer adults participating in a survey on asymptomatic malaria and/or intestinal parasite prevalence during the same period were also collected. Only individuals who accepted to be tested for HIV and malaria and who gave their written informed consent were invited. Inclusion criteria for the present study were the same in both groups: age above 17 years, agreement to be tested for HIV for those not followed at the CTC, an absence of fever (tympanic temperature ≥37.5 °C) or history of fever the day of the screening and during the week preceding the consultation, other clinical symptoms suggestive of malaria, absence of antimalarial drug uptake the last two weeks, absence of any other severe medical condition and sickle cell disease, permanent residence in the study area, agreement to fill the questionnaire and written informed consent. Children, pregnant women, febrile patients, any HIV infected individual who has been on CTX and ART treatment for less than one month as well as all those who did not consent to participate were not included.

Sample size estimation

The sample size calculation was based on 5% precision with 95% level of confidence and an expected prevalence of asymptomatic malaria of 13.9% [11]. This expected prevalence was based on the assumption that the prevalence of positive blood smear will be at least comparable to the last reported prevalence (13.9%) observed in Dienga Gabon. The literature review highlighted frequencies of asymptomatic malaria in PLHIV varying from 2.2 to 12% [6,18,19]. Given that, when these prevalence were taken into account, the sample size calculation gave a lower minimum size than that obtained with the prevalence of asymptomatic malaria found in Gabon, the latter was used for the calculation. Using the following formula for sample size calculation for prevalence study [20]: $N = (Z^2 \times p(1 - p))/d^2$ with $Z = 1.96$; p = previous prevalence of microscopic asymptomatic malaria = 13.9%; d = precision, a minimum of 184 screened individuals in each group (PLHIV, HIV-uninfected volunteers) was necessary. Consenting participants were prospectively included in the study sites during the defined study period which was estimated sufficient to include the required number of participants. All eligible individuals who presented at the study centers during this predefined study period (February to June 2016) were included, even after the required sample size was reached.

Questionnaire

After consent had been obtained, a pre-tested questionnaire was administered for completion to each participant by a trained team member. Briefly, demographic (age, sex, marital status and education level), self-medication with antimalarial, self-reported possession and use of bed net, anti-retroviral treatment (ART) type and duration of use, cotrimoxazole (CTX) use and duration of prophylaxis, were recorded. According to the national guidelines, the drugs administered as first line ART were Zidovudine or Tenofovir plus Lamivudine plus Efavirenz or Nevirapine, for the second line treatment, Abacavir or Zidovudine plus Didanosine plus Lopinavir/r were recorded. Among patients under ART and CTX, only those with at least one month treatment duration were included. Results of laboratory analysis (Hb level, blood film result with parasite density, parasite species and density, CD4 cell count) were also recorded.

Laboratory procedures

Malaria diagnosis

A rapid diagnostic test (SD BIOLINE, SD Standard Diagnostics Inc., South Korea) was performed directly on site according to the manufacturer's recommendations, and the results were communicated to the physicians for appropriate management. Thick films were used to confirm the diagnosis, as previously described [20]. Briefly, the slides were dried and stained with 20% Giemsa for 20 min. We then read 100 oil-immersion fields were read, and the parasitemia was determined as the number of parasites per microliter of blood. Smears were considered as negative if no parasite was detected on 100 oil-immersion fields. Thin blood smears were used for species identification.

Hematology

Recent available Hb measurements were collected from the print outs of the PLHIV files. Hemoglobin level is measured using the automated ABX micros ES 60 (Horiba®) system according to the manufacturer instructions. This system is the same in the public health structures when available. Daily quality control and monthly blood control analysis are carried out to validate each test run. Anemia was defined as an Hb concentration below 11 g/dL. Hb measurements were not part of the study on asymptomatic malaria and/or intestinal parasite study.

HIV infection diagnosis

HIV test was conducted using the immuno-chromatographic rapid Alere Determine™ HIV 1/2 Ag/Ab Combo (Alere ® California, USA). Positive samples were subsequently confirmed according to the national guidelines at the CTC where they could benefit for free case management and counselling in case of positivity. *Determination of CD4 + T-lymphocyte count*

The CD4 cell count was performed at each CTC by BD FACS Count® Machine (BD Biosciences). CD4 T-cell counts were categorized according to WHO as low (<200/µL) moderate (200–499/µL) and high (≥500/µL) [21].

Data management and statistical analysis

Data generated were double entered into excel file, reviewed cleaned, then imported and analyzed using STATA 13.0 (Statacorp, College Station, USA). Patient characteristics were summarized using medians and interquartile ranges for continuous variables and proportions for categorical variables. Differences between groups were assessed using chi-squared or Fisher's exact tests if there were less than five expected values for proportions. Student's *t*-test and analysis of variance (ANOVA) or the Kruskal-Wallis test were used, as appropriate, for continuous variables. Crude odds ratio (cOR) and 95% confidence intervals (CI) were used to assess the association of risk factors and malaria among HIV-infected individuals. The presence of risk factors was also compared between PLHIV and HIV uninfected individuals. For all these tests, the difference was considered significant if $p < 0.05$. All the reported p values are for two-tailed tests. Logistic regression was performed to estimate the adjusted odds ratio between malaria status according to the presence or absence of HIV infection or within HIV infected individuals and all variables with a p value <0.20 in bivariate analysis. Because many cells in the table had low counts, multivariate mixed logistic regression model using likelihood ratio estimation for discrete choice modeling of small datasets was performed. Adjusted OR with their 95% CI were then calculated. Associations were found significant if p values were <0.05, a trend was indicated if p values were between 0.05 and 0.10.

Ethical considerations

Approval of the Ministry of Public Health and of the National Ethics Committee were obtained in February 2015. All participants received information about the study from team members assisted by community health workers, and written consent was obtained before their enrollment. The importance of the study for both personal and public health, its objectives, the procedures used were explained to all participants. They were also informed that their personal information would be kept strictly confidential, and they could withdraw their participation at any time without giving explanations.

The survey included free medical consultations for the participants and the administration of appropriate treatment according to national recommendations, with referral to an identified specialist when required. Biological testing was offered to participants, and the results were supplied to their physicians, for case management.

Results

A total of 1130 asymptomatic individuals were enrolled into the study, 858 (75.9%) were PLHIV and 753 (65.0%) were females; 751 (66.6%) were enrolled in Libreville and 379 (32.1%) in the rural cities. Age was known for 1106 participants; it ranged from 17 to 96 years. The median age was significantly higher among PLHIV (43[35–52] years old) compared to the HIV uninfected individuals (27 [18–42] years old) ($p < 0.01$). Indeed, almost half of the patients of this group were less than 25 years old while this age group represented only 4.8% of the PLHIV (Table 1). The main characteristics of the two groups are presented in Table 1. The two groups differed according to the education level, the bednet use, and the history of previous antimalarial treatment. Among the PLHIV with available data, the median CD4 cell count was 337[180–524] cell/mm^3, the median Hb level 11.5[9.5–12.9] g/dL, 76.2% ($n = 576$) were under first line ART, 8.5% ($n = 64$) under second line.

The global malaria prevalence was 13.5% ($n = 153$), significantly lower among the PLHIV (7.1%, $n = 61$) compared to the HIV-uninfected participants (33.8%, $n = 92$) ($p < 0.01$). When the *P. falciparum*-infected individuals were compared, the PLHIV had a lower median parasitaemia and a higher median age; HIV-positive and HIV negative groups differed according to the frequency of bednet users (52.6% vs. 34.0% respectively) (Table 2). In both groups, the majority of infected individuals was female, not married and had a primary or secondary educational level (Table 2).

Among the PLHIV, the frequency of positive blood smears (PBS) was higher in the youngest (not significantly), in individuals who used a bednet and those who were not under CTX prophylaxis (Table 3). The median parasite density was also significantly higher among the youngest, the female, the individuals having less than 200 CD4/mm^3 and those with no education or no history of previous antimalarial intake (Table 3). In the bivariate analysis, age below 25 years and anemia were risk factors associated with malaria parasite infection while in the multivariate analysis, young age (<25 years) remained an independent risk factors significantly associated with *P. falciparum* infection, as shown in Table 4. The median CD4 cell count was comparable between the patients with a PBS (364[181–518] CD4 cell/mm^3) and those free of malaria (319[167–581] CD4 cell/mm^3) ($p = 0.69$). In another hand, the frequency of PLHIV under CTX was significantly higher (39.4%; $n = 307/779$) among those with a negative blood smear than those infected by *P. falciparum* (23.2%; $n = 13/56$) ($p = 0.02$). These groups did not differ according to the ART use. The overall prevalence of anemia in the PLHIV participants with measured Hb was 42.1% ($n = 211/501$). Although there was no statistically significant difference, the prevalence of malaria was 5.1% in anemic individuals and 2.5% in the non-anemic ones ($p = 0.11$). The median Hb level

Table 1. Patient characteristics.

	HIV negative ($N = 272$)		HIV positive ($N = 858$)	
	n	%	*n*	%
Age in years (N = 1106)				
<25	118	45.4	41	4.8
25–54	104	40.0	652	77.1
≥25	38	14.6	153	18.1
Sex (N = 1130)				
Male	89	32.7	288	33.6
Female	183	67.3	570	66.4
Matrimonial status (N = 839)				
Married	50	56,8	344	45,8
Not married	38	43,2	407	54,2
Education level (N = 809)				
No or primary	42	48.3	141	19.5
Secondery	41	47.1	394	54.6
High	4	4.6	187	25.9
Bednet use (N = 781)				
No	66	48.2	437	67.9
Yes	71	51.8	207	32.1
Previous antimalarial treatment (N = 863)				
No	38	77.6	779	95.7
Yes	11	22.4	35	4.3
Anemia (N = 484)				
No			277	57.2
Yes			207	42.8
Exposed to ART (N = 756)				
No ART			116	15.3
ART			640	84.7
Exposed to cotrimoxazole (N = 835)				
No CTX			515	61.7
CTX			320	38.3
CD4 cell count in mm^3/µL (N = 521)				
<200			153	29.4
200–499			219	42.0
≥500			149	28.6

Table 2. Comparison of studied factors between P. falciparum-HIV positive and P. falciparum-HIV negative individuals.

	PLHIV	HIV-Negative participants	*P*
Median age [IQR] years	42.5 [37.8–50.0]	26.3 [20.0–36.8]	<0.01
Age groups, n (%)			
<25 years	9 (14.8)	44 (50.0)	<0.01
25–54 years	43 (70.5)	35 (39.8)	<0.01
≥55 years	12 (19.7)	9 (10.2)	0.10
Median parasitemia [IQR]p/µL	117 [14.0–1069]	1221 [63.0–7662]	<0.01
Sex, n (%)			
Female	43 (70.5)	54 (58.7)	0.40
Male	18 (28.5)	38 (41.3)	0.09
Marital status, n (%)			
Single	26 (61.9)	8 (61.5)	0.76
Married	5 (38.5)	16 (38.1)	0.99
Education level, n (%)			
None or primary	10 (24.4)	3 (23.1)	0.78
Secondary	20 (48.8)	10 (76.9)	0.07
High	11 (26.8)	0 (0.0)	–
Bednet use, n (%)			
Yes	20 (52.6)	16 (34.0)	0.08
No	18 (47.4)	31 (66.0)	0.08
Previous antimalarial treatment			
Yes	3 (6.4)	6 (17.6)	0.21
No	44 (93.6)	28 (83.4)	0.22

Table 3. Malaria prevalence and parasite density among the PLHIV.

	INFECTED, % (n/N)	*P*	PARASITEMIA [IQR]	*P*
Age		0.15		0.03
<25 years	14.6 (6/41)		2810 [30–2577]	
25–54 years	6.6 (43/652)		173 [44–1264]	
≥55 years	7.8 (12/153)		14 [7–36]	
Sex		0.50		<0.01
Female	7.5 (43/570)		183 [21–1400]	
Male	6.3 (18/286)		53 [8–234]	
Education level		0.67		0.03
None or primary	7.1 (10/141)		5600 [5600–7700]	
Secondary	5.1 (20/393)		53 [7–77]	
High	5.9 (11/185)		7 [5–77]	
Bednet use		<0.01		0.08
Yes	9.7 (20/207)		147 [12–1195]	
No	4.1 (18/437)		77 [12–2345]	
Previous antimalarial treatment		0.72		0.04
Yes	8.6 (3/35)		8 [4–23]	
No	5.6 (44/779)		77 [14–1400]	
ART use		0.25		–
1st line	6.9 (40/576)		105 [14–764]	
2nd line	1.6 (1/64)		–	
No	6.9 (8/116)		112 [14–1508]	
Cotrimoxazole use		0.02		0.19
Yes	4.1 (13/320)		70 [18–1332]	
No	8.3 (43/515)		65 [14–764]	
Anemia		0.26		0.46
Yes	5.3 (11/207)		147 [18–2345]	
No	3.2 (9/277)		28 [18–92]	
CD4 cell (number/µL)		0.73		0.03
<200	5.9 (9/153)		773 [14–5180]	
200–499	4.6 (10/219)		219 [101–745]	
≥500	4.0 (6/149)		77 [45–705]	

*Calculated among patients with available data (see Table 1).

tended to be lower in PLHIV with a PBS (9.2[6.3–12.8] g/dL) compared to patients free of malaria (11.1[9.2–12.7] g/dL) ($p = 0.13$). Anemia was also not found to be associated with ART and CTX use although patients without ART and those who were under CTX prophylaxis had a lower median Hb level (Table 5). The proportion of anemic patients significantly decreased and the median Hb significantly increased with increasing age ($p < 0.01$) and with increasing CD4 cell count ($p < 0.01$) (Table 5). Indeed, there was a positive correlation between the Hb level and age (Rho = 0.13; $p = 0.02$); and Hb level and CD4 cell number (Rho = 0.23; $p < 0.01$).

Discussion

The results of this study, which aimed at estimating the burden of malaria and anemia in asymptomatic PLHIV living in Gabon, suggest that HIV-positive patients attending routine care in CTC are less likely to have asymptomatic malaria than HIV-negative patients. The overall malaria prevalence (13.5%) was similar to that reported in 2011(13%) in villages of the country [22]. So, when considering the population of HIV-uninfected included in this study, the rate of positive blood smears is almost three fold higher (33.8%) than in 2013, but comparable to that recently found in the inhabitants of Dienga (25–30%), a rural area located in the Southeast part of Gabon where HIV prevalence is low (2%) [11]. This is in agreement with the continuous increase of malaria prevalence observed since the last six years in the country [4,12]. The frequency of malaria among PLHIV (7.1%) is relatively consistent with that found in studies in Cameroon (7.3%), but lower than malaria-HIV co-infection rates noticed in Equatorial Guinea (13.8%), in Ghana (11.75%) and in Nigeria (18.5%) [23–26]. Then, PLHIV who live in Gabon are also at lower risk of being infected by *P. falciparum*, considering the reports from countries cited above. This low prevalence of co-infection in this population could be attributed to the health seeking attitude of HIV patients, the scaling up of care and treatment centers in endemic countries and thus the better accessibility to health care and systematic biological tests in case of clinical events. Malaria have been shown to be more severe in this population. Thus, they probably seek promptly for health care and malaria infected patients will quickly go down with the disease and seek medical attention faster [27]. Other factors that could be responsible for the low prevalence of malaria in this population are the use of preventive strategies such as the bednets and the CTX-based chemoprophylaxis which is recommended for the protection against opportunistic infection in PLHIV in the country. Indeed, the regular use of ITNs is associated with a lower prevalence of malaria in some studies where the majority of patients reported to have slept under ITNs [23,27–29]. The impact of bednets was not observed in the present study. In fact, most bednets are not ITNs or are not re-impregnated ITNs. Likewise, adults who are not targeted by the free ITN distribution, buy their bednet, impregnated

Table 4. Factors associated with the presence of P. falciparum parasites in HIV individuals (Uni and Multivariate analysis).

Variables		CrudeOR	Crude Lower 95%	Crude Upper 95%	Crude P	Adj. OR	Adj. Lower 95%	Adj. Upper 95%	Adj. P
Age (years)	25–54 vs. <25	9.01	3.44	23.59	<0.01	21.53	3.43	199.74	<0.01
	≥55 vs. <25	9.78	2.9	32.93	<0.01	5.42	0.79	50.09	0.08
Sex	Male vs. Female	0.65	0.32	1.28	0.21	0.42	0.07	2.1	0.30
Matrimonial status	Married vs. Not married	0.98	0.28	3.75	0.98	0.83	0.17	4.0	0.81
Bednet use	Bednet used vs. No Bednet used	2.15	0.9	5.25	0.08	0.48	0.08	2.2	0.35
Previousantimalarial treatment	Yes vs. No	0.32	0.06	1.31	0.11				
Anemia	Yes vs. No	7.33	1	152.32	0.05				

Table 5. Factors associated with anemia in HIV infected participants.

	N	ANEMIC (%)	*p*	Median Hb [IQR]	*p*
AGE (N = 479)			<0.01		0.04
<25 years	18	61.1		10.5 [8.8–11.1]	
25–54 years	392	45.1		11.3 [9.4–12.8]	
≥55 years	69	27.5		12.1 [10.4–13.4]	
ART (N = 443)			0.10		0.05
1st line	332	40.1		11.5 [9.8–13.0]	
2nd line	43	48.8		11.4 [9.6–12.7]	
None	68	54.4		10.2 [8.2–12.3]	
COTRIMOXAZOLE PROPHYLAXIS (N = 482)			0.20		0.03
Yes	197	46.2		10.8 [8.9–11.9]	
No	285	40.3		11.5 [9.9–12.8]	
CD4 CELL COUNT (N = 403)			0.01		<0.01
<200	111	54.0		10.7 [9.0–12.1]	
200–500	177	33.3		11.5 [9.5–12.8]	
>500	115	36.5		11.7 [10.2–13.2]	
POSITIVE BLOOD SMEARS (N = 484)					
Yes	20	55.0	0.26	9.2 [6.9–12.8]	0.27
No	464	42.2		11.1 [9.2–12.7]	

or not, bearing in mind their current status. Nevertheless, other studies did not observed any association between the use of ITNs and the prevalence of malaria [30].

The use of cotrimoxazole has been associated with a lower risk of malaria in children, pregnant women, or severely immunocompromised patients; its discontinuation also increases malaria prevalence and parasitemia [6,15,19,31]. In this study, the prevalence of malaria among participants who were not under CTX prophylaxis was twofold higher than those from individuals who were under otrimoxazole prophylaxis (8.3% vs. 4.1%). Furthermore, there were more patients under CTX in the PLHIV group with no malaria than in the *P. falciparum*-infected PLHIV group. Recently, this sulfa combination drug was recommended to all PLHIV living in malaria endemic countries due to its recognized activity against protozoal parasites, more precisely the *Plasmodium* species [19,20,32]. This recommendation was not established in Gabon at the time of the study period. Only pregnant women, patients with CD4 cell count below 350/mm^3 and those with opportunistic infections are under CTX, this would explain the insufficient CTX coverage in our HIV infected population.

The protective effect of CTX against the occurence of malaria was also reported to increase in patients on ART compared to the untreated ones [33]. Direct impact of ART on the frequency of malaria was not observed here, neither in terms of positive blood smear frequency rate, nor on parasite density. Opposite results are sometimes reported, with a higher prevalence found in patient under ART compared to those who were not [34]. Our results may be related to the small sample size of PLHIV with a PBS, or other factors such as low adherence to treatment regimens which was not investigated here. The median parasitemia was also comparable between patients according to the use or not of ART. Nonetheless, patients receiving the second line treatment were less frequently infected compared to those under the first line treatment. Some ART such as protease inhibitors which are included in the recommended second line treatment may inhibit *P. falciparum* growth and then reduce malaria incidence [3,34]. Unfortunately, this analysis could not be performed here.

A recent history of fever treated with an antimalarial drug was not associated with a lower frequency of positive blood smears, but with a lower parasite density. This emphasizes the need of a biological testing especially in case of fever, since in this population, the prevalence of malaria is low and other more serious causes of fever which cannot be managed by self-medication only could exist.

A significant association was observed between the prevalence of malaria and age in PLHIV ($p < 0.01$). PBS were mostly observed in the youngest participants which had also the highest parasitemia (Table 3). Malaria is known to be more common in youngest non pregnant or pregnant PLHIV [24,36]. This is in accordance with the changing epidemiological profile of malaria observed in the country after the implementation of ACT, bednets and Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine during pregnancy. Indeed, data from epidemiological surveys and MNCP highlighted an increasing prevalence of malaria with increasing age in Gabon since 2011 [4,12]. Thus, the relationship between malaria and age among the PLHIV follows that of the general population. On the other hand, the predominance of women among *P. falciparum*-infected individuals is not surprising, the prevalence of HIV is twofold higher among women (5.8%) compared to men (2.2%); they represent a large proportion of the participants involved in this study. Most of these women are screened for HIV during their pregnancy, those who are HIV-infected are more likely to continue their follow-up after the post-partum period in the CTC while HIV-infected men attend less frequently these centers. Some authors reported that malaria-HIV coinfection was more prevalent among younger women, while others highlighted a higher prevalence in man than in female [7,24,24,29].

The immunocompromised state of HIV-infected patients, defined as a low CD4+ T cell count, is associated with a higher risk of acquiring parasitic infections including malaria, according to some reports [6,29,35]. The mean CD4+ cell count was not significantly different in participants with a PBS when compared to that of their uninfected counterparts. This is in agreement with other studies in which neither tuberculosis, nor malaria prevalence and incidence have an effect or were associated with the CD4 count [23,36]. However, *P. falciparum* infection rate was more frequent in PLHIV with less than 200 CD4 cells/µL who had also the higher median parasite density, in agreement with the assumption of an association between the severity of malaria and the decline in CD4+ T lymphocytes [6,29,37].

Hematological abnormalities are often described during malaria and among HIV-infected individuals. Even so, data in co-infected patients or in PLHIV living in endemic areas are scarce. Anemia is the most common hematological complication of HIV infection [38]. In the population of PLHIV, the median values of hemoglobin tended to be lower and the prevalence of anemia higher in those having asymptomatic parasitaemia when compared to those who had a negative blood smear. The median Hb level was 2 g lower and the PBS rate two fold higher in this group compared to the malaria negative participants. The absence of a statistical significance of these differences could be attributed to the small number of *P. falciparum*-infected patients, but also to the fact that asymptomatic individuals usually have a low parasite density. In previous study, the frequency of anemia was not negligible among pregnant (37%) or non-pregnant adults (51.5%) [4,39]. The prevalence of anemia in PLHIV (57.2%) is comparable to the rates reported in pregnant and non-pregnant individuals in Libreville, Northeast Nigeria (60.6%), Dar es Salam (56.0%), but lower than that of Ghana (67.5%) [39–41]. Almost two thirds (64.3%) of participants with malaria-HIV co-infection were anemic, in line to findings from elsewhere in Africa [42].

The data obtained also allow the analysis of the factors associated with anemia in PLHIV. A lower Hb level and a higher prevalence of anemia were recorded in younger PLHIV, those who were not on ART and PLHIV with a low CD4 cell count. These factors are linked to a rapid progression of the HIV disease which is marked by a general immunosuppression with a depletion of all the hematological parameters. Apart from the chronic inflammation in PLHIV, during the disease progression, the increase of the viral load induces an increase of cytokine-mediated myelo-suppression which subsequently impairs erythropoietin; some opportunistic pathogens could also infiltrate the bone marrow and disrupt erytropoiesis [43]. Thus, as indicated by others, anemia is correlated with the depression of the immune status; as it has also been shown to favor adverse consequences of HIV infection and to worsen the disease progression, its prevention should be systematic in PLHIV and this population should be carefully monitor and screened for adverse outcomes in case of anemia [43].

This study has some limitations. First, the relatively small sample size of *P. falciparum*-infected PLHIV could have resulted in a lack of statistical significance when recognized protective or risk factors were screened. Even so, it is not sure that increasing the number of participants would have increased the frequency of PLHIV with a positive blood smear. The findings of this study may need to be confirmed in a larger population of malaria positive participants. Second, two groups with different baseline characteristics were compared. Apart from age, the groups differed according to their education level, the bednet use, and the history of a previous antimalarial treatment, factors which were not found to be statistically different between *P. falciparum*-infected PLHIV and *P. falciparum*-infected HIV-negative individuals. This is probably due to the fact that generally the age of majority of HIV-infected ranges between 25 and 45 years, whereas HIV-uninfected individuals who are frequently seen in public health centres are people with low socio-economic or young adults looking for a job; individuals with higher income go more frequently to consultation in private clinics [13]. To the opposite, the majority of PLHIV is followed-up in the HIV CTCs where appropriate case management as well as ART are freely provided. Third, some factors such as the Hb levels in HIV-uninfected individuals, the AIDS stages, and

the viral load which have been shown to be associated with the presence of malaria and/or anemia, were not recorded. However, the main findings of this study, the first reported in Gabon, are in accordance with those reported by other authors [14,24,42,44].

Conclusion

In conclusion, this study shows that the prevalence of asymptomatic *P. falciparum* infection is lower in PLHIV compared to HIV- uninfected individuals living in Gabon, while anemia is more common in this population. Young age, low education level, female sex are associated with a higher parasite density whereas CTX is associated with a lower plasmodial infection rate in PLHIV. Larger studies comparing asymptomatic and symptomatic *P. falciparum*-infected and *P. falciparum*-uninfected PLHIV are needed to draw information which should help for the management and the design of preventive strategies against malaria and anemia in PLHIV.

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The authors declare that there is no conflict of interests.

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