Impact of HIV-1 Infection on the Hematological Recovery After Clinical Malaria

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Background: Anemia is the most frequent cytopenia in HIV-infected individuals and is often associated with malaria.

Objective: To assess the impact of HIV-1 on the hematological recovery after a clinical malaria episode.

Methods: In Ndola, Zambia, a region with high malaria and HIV prevalence, hemoglobin (Hb) was measured in 634 malaria patients 14 and 45 days after antimalarial treatment. Risk factors for hematological recovery were analyzed in a multivariate linear regression model.

Results: At enrollment, HIV-1–infected malaria patients had lower Hb compared with HIV-1 uninfected (122.7 vs 136.0 g/L; $P < 0.001$). In both groups, mean Hb was significantly lower at day 14 posttreatment than day 0 ($P < 0.0001$) and significantly higher at day 45 than at day 14 (HIV-1 negative: $P = 0.0001$; HIV-1 infected: $P = 0.005$). HIV-1 was a risk factor for a larger Hb decrease until day 14 ($P < 0.001$) and slower recovery until day 45 ($P = 0.048$). When considering the whole 45-day follow-up period, mean Hb increased in the HIV-1–negative group (+3.54 g/L; 95% confidence interval: 1.37 to 5.70; $P = 0.001$) but not in the HIV-1–infected group (−0.72 g/L; 95% confidence interval: −3.85 to +2.40; $P = 0.64$). HIV-1 infection as such ($P < 0.0001$), not CD4 cell count ($P = 0.46$), was an independent risk factor for a slower hematological recovery.

Conclusions: HIV-1–infected malaria patients had a slower hematological recovery after successful parasite clearance. Malaria preventive measures should be targeted to this high-risk group.

Key Words: Africa, anemia, HIV-1, malaria, treatment

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BACKGROUND

Due to the epidemiological overlap, particularly in eastern and southern Africa, coinfection with HIV-1 and malaria is a common phenomenon. Both HIV and malaria contribute to anemia in individuals and populations. In malaria-infected patients, although parasitized red blood cells (RBC) are destroyed and cleared by the reticuloendothelial system, the major contributor to anemia is the accelerated destruction of uninfected RBC, probably through immune mechanisms.[1] Additionally, impaired erythropoiesis decreases the RBC production and iron mobilization.[2] These mechanisms remain active for several weeks after a clinical malaria episode and partially explain why anemia may worsen, even after the malaria infection has been cleared.[1] Anemia is also the most frequent cytopenia during HIV infection due to bone marrow suppression by medications, hemolysis, gastrointestinal bleeding, nutritional deficiencies, various opportunistic infections (eg, mycobacterial infections), and diminished erythropoietin response.[3] Furthermore, anemia, regardless of the cause, is thought to be a strong independent predictor of mortality in HIV-infected persons, including those who have access to antiretroviral therapy.[4,5]

Few studies have addressed the role of HIV–malaria coinfection as a risk factor for anemia. Both infections have specific and different hematological effects that seem to have at least an additive effect. It has been shown that a clinical malaria episode in HIV-1–infected individuals, infants,[6] and adults[7,8] alike results in a lower mean hemoglobin (Hb) than in HIV-1–uninfected patients, with infants having a higher risk of severe anemia.[9] We have assessed in HIV-1–infected and HIV-1–uninfected adults the impact of antimalarial treatment on Hb and the results are reported below.

METHODS

This is a specific analysis of a randomized clinical trial assessing the impact of HIV immune suppression on malaria treatment efficacy.[8] The trial was performed between March

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Trial registry: http://www.clinicaltrials.gov/; reference number NCT00304980.

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2003 and June 2005 at 4 periurban health centers in Ndola, Zambia, an area of mesoendemic malaria. HIV-1 prevalence is estimated at 25.2% among women attending antenatal clinics.[10] All individuals aged 15–50 years with fever (body temperature ≥37.5°C) and/or history of fever in the previous 48 hours, and without any other disease symptom, were screened for malaria infection (thick and thin blood film in duplicate for parasite density and species identification) and pregnancy (if applicable). Patients with a *Plasmodium falciparum* monoinfection with a density of at least 25 parasites per 200 white blood cells (WBC) (assumed to be 1000 parasites/μL) or more were included. Internal quality control was organized as recommended by the World Health Organization (WHO).[11] A written informed consent (in English and in the local language), including permission to undergo double-blinded testing for HIV-1 infection (at the central laboratory), was obtained from each patient before recruitment. Patients were randomized to either artemether–lumefantrine (AL) or sulfadoxine–pyrimethamine (SP) treatment. None of the patients took antiretroviral drugs or co-trimoxazole prophylaxis at the moment this study was conducted. Exclusion criteria included the following: pregnancy; documented intake of any sulfa drug or any antimalarial treatment, including SP or AL, within the 2 weeks before recruitment; having taken any other antimalarial drug during study follow-up; history of allergy to study drugs or known allergy to other sulfa drugs such as co-trimoxazole; nonresident of the study area; severe *P. falciparum* malaria as defined according to the WHO criteria;[11] consent withdrawal; presence of other coinfections; or clinical or laboratory findings indicating underlying chronic diseases (cardiac, renal, hepatic, malnutrition). For the latter, as HIV prevalence in adults is known to be high, standard clinical examinations and questionnaires in accordance to the WHO Integrated Management of Adult and Adolescent Illness[12] excluded with reasonable confidence persistent lymphadenopathy and HIV-related clinical events indicating HIV stages 2–4. Patients with an Hb below 70 g/L were also excluded as this is part of the definition of severe malaria.

For this analysis, we excluded patients who presented recurrent parasitemia or recurrent fever and patients who took hematinics such as ferrous sulfate, folic acid, or other medications that might influence Hb. Moderate anemia was defined as an Hb between 70 and 120 g/L in women and 70 and 130 g/L in men.[13] The study was approved by the ethical and scientific committees of the Institute of Tropical Medicine, Antwerp, Belgium, and the Tropical Diseases Research Center, Ndola.

Clinical history, symptoms, and signs were recorded, and a blood sample for parasitemia (blood slide) and genotyping (on Schleicher & Schuell filter paper) were collected at days 0, 3, 7, 14, 21, 28, 45, and at any unscheduled visit. At day 0 (before treatment), 3 mL of venous blood was collected for Hb, HIV testing, and CD4 cell count. Hb was measured by HaemoCue (HemoCue, Angelholm, Sweden) at days 0, 14, and 45 with a precision of 0.1 g/dL. The HaemoCue was calibrated before every session using the provided standard. Blood samples were sent to the central laboratory within 2 hours where HIV-1 status and CD4 count were assessed anonymously. Blood samples were tested for HIV using the Abbott Determine (Abbott Laboratories, Tokyo, Japan) test. If negative, the patient was considered HIV negative. If the sample was positive, a second test, Genie II (BioRad; Sanofi Diagnostics Pasteur, Marne La Moquette, France), was performed. If the result was discordant with the previous test, that is, negative, a final test, Capillus½ (Biognost; Cambridge Diagnostics, Galway, Ireland), was done and considered as the final result. CD4 cell count was performed on all HIV-infected individuals with direct volumetric absolute counting[14] (Cyflow Counter, Partec, Germany). A FACSCount machine (Becton Dickinson, Franklin Lakes, NJ) was used to validate the Cyflow data and served as a back up. WBC count was done using automated hematological analyzer (micro Cobas; Hoffman La Roche, Basel, Switzerland). Neither the study staff nor the patient had access to the HIV-1 test results. Patients wanting to know their HIV-1 status were offered voluntary counseling and testing adjacent to the study. Patients were encouraged to attend the health facility outside scheduled visits if they felt ill.

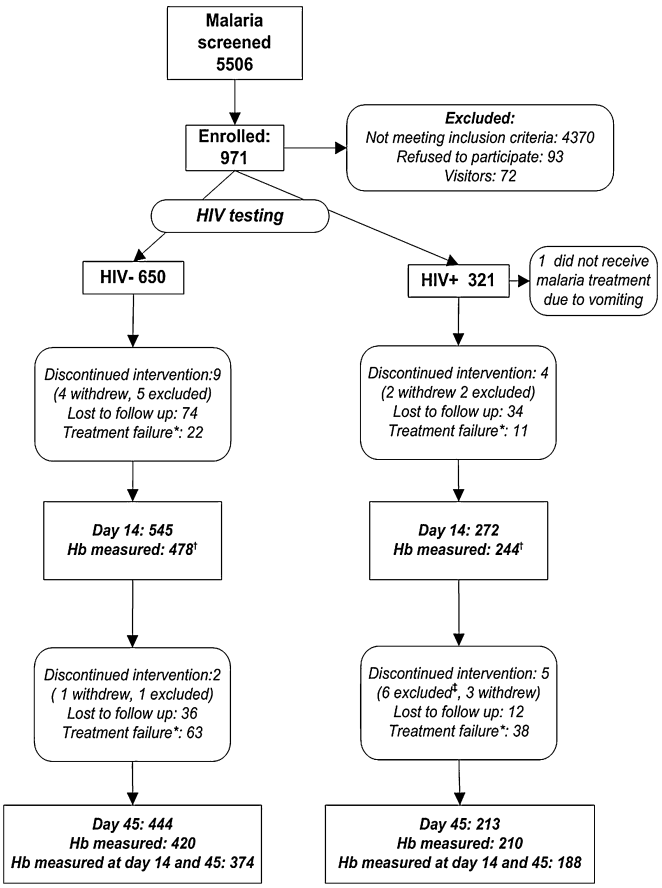
Statistical Analysis

This analysis was performed to assess the importance of HIV-1 infection as a risk factor for anemia in patients with clinical malaria and the impact of malaria treatment on the hematological recovery. The primary endpoint was the Hb increase 45 days after successful antimalarial treatment. Adequate clinical and parasitological response for this analysis was defined as no malaria infection and no fever at day 45. Data were double entered and cleaned in Epi-info (version 6.04b; Centers for Disease Control and Prevention). Proportions were compared using the χ^2 or Fisher exact test (when required); Student t test was used for continuous variables. A paired t test was used for within-patient comparisons. Nonnormally distributed variables were transformed or nonparametric tests (Wilcoxon or Kruskal–Wallis) used. Univariate linear regression analyses were performed. All possible interactions up to order 2 were tested and all modifying risk factors or reporting P value <0.10 were maintained in a multivariate linear regression model. All reported P values are 2 sided. All analyses were performed using STATA statistical analysis software package version 10 (Stata Corp, College Station, TX).

RESULTS

Of the 971 patients enrolled, 818 were still followed up at day 14 (range 11–18 days) (Fig. 1). Hb was measured in 88.3% of them. At day 45 (range 42–58 days), 661 patients enrolled fulfilled the selection criteria and Hb was measured in 634 of them (92.9%). Among them, 217 (32.8%) were HIV-1 infected. Hb was not available at day 14 for 11.5% (51/444) of HIV-1–negative patients and 10.1% (22/217) of HIV-1–infected patients. Hb was not available at day 45 for 5.4% (24/444) of HIV-1–negative patients and 1.4% (3/217) of HIV-1–infected patients. Patients not included in the analysis at any follow-up visit had similar demographic and clinical characteristics as the patients included.

At enrollment, mean Hb [122.7 g/L, 95% confidence interval (CI): 119.7 to 125.8, vs 136.0 g/L, 95% CI: 134.1 to 137.9; $P < 0.001$] and WBC count 5.0×10^6/μL vs 5.3×10^6/μL;



* Treatment failure were at different time points and only treatment failures on day 45 were included in a seperate analysis.
† Patients exluded between day 14 and 45 showed same results at day 14 an at enrolmentas those shown in the final analysis.
‡ Four patient with fever without parasitaemia were excluded from the analysis.

FIGURE 1. Study flowchart.

$P = 0.02$) were significantly lower in HIV-1–infected individuals (Table 1, Fig. 2A). Prevalence of anemia was also significantly higher in HIV-1–infected patients (47.5%, 95% CI: 40.7 to 54.3) as compared with HIV-1–negative ones (25.7%, 95% CI: 21.7 to 30.0) ($P < 0.001$) (Fig. 2B). Among HIV-1–infected patients, low Hb was associated with low CD4 cell count ($P < 0.001$), female gender ($P < 0.0001$), and low bodyweight ($P < 0.03$).

By day 14, posttreatment, mean Hb compared with day 0 had decreased both in HIV-1–uninfected (−4.2 g/L; 95% CI: −5.8 to −2.5; paired *t* test: $P < 0.0001$) and HIV-1–infected patients (−5.0 g/L; 95% CI: −7.2 to −2.8; paired *t* test: $P < 0.0001$), though the Hb decrease between the 2 groups of patients was not statistically significant (HIV-1 positive vs HIV-1 negative: $P = 0.23$). However, mean Hb at day 14 was significantly lower in HIV-1–infected than HIV-1–uninfected patients (116.9 g/L; 95% CI: 114.2 to 119.7 vs 132.4 g/L; 95% CI: 130.6 to 134.2; $P < 0.0001$) (Fig. 2A). Similarly, the prevalence of anemia between days 0 and 14 increased both in HIV-1–negative (31.6%, 95% CI: 27.0 to 36.4; $P = 0.06$) and HIV-1–infected patients (64.1%, 95% CI: 56.9 to 70.8; $P < 0.0001$) (Fig. 2B). A larger Hb decrease at day 14 was associated with HIV-1 infection, higher parasite load, higher Hb values at enrollment, and SP treatment (all $P < 0.001$) (Table 2). In HIV-1–infected patients, CD4 cell count at enrollment was not associated with Hb changes at day 14 ($P = 0.78$).

Between days 14 and 45, Hb increased in both HIV-1–uninfected (+7.8 g/L; 95% CI: 5.7 to 10.0; paired *t* test: $P = 0.0001$) and HIV-1–infected patients (+4.2 g/L; 95% CI: 1.2 to 7.0; paired *t* test: $P = 0.005$) (Fig. 2A). Such increment was lower in HIV-1–infected than in HIV-1–uninfected patients ($P = 0.04$). Anemia prevalence decreased to 16.2% (95% CI: 12.8 to 20.1) in HIV-1–uninfected patients ($P < 0.001$) and to 45.7% (95% CI: 38.8 to 52.7) in HIV-1–infected ones ($P < 0.0001$) (Fig. 2B). A lower Hb increase between days 14 and 45 was not only associated with HIV-1 infection but also with a lower parasite load ($P < 0.001$), higher Hb values at enrollment ($P = 0.001$), female gender ($P = 0.002$), and older age ($P = 0.03$) (Table 2). The magnitude of hematological recovery was not influenced by impaired cellular immunity as measured by CD4 count ($P = 0.46$).

When considering the whole 45-day follow-up period, Hb significantly increased, compared with day 0, in HIV-1–uninfected patients (+3.54 g/L; 95% CI: 1.37 to 5.70; paired *t* test: $P = 0.001$) but not in HIV-1–infected patients (−0.72 g/L; 95% CI: −3.85 to 2.40; paired *t* test, $P = 0.64$). The difference in mean Hb between HIV-1–infected (122.5 g/L; 95% CI: 119.3 to 125.7) and HIV-1–uninfected patients (139.2 g/L; 95% CI: 137.0 to 141.4) became more pronounced by day 45 ($P < 0.0001$) (Fig. 2A). Compared with enrollment, prevalence of anemia at day 45 was significantly lower in HIV-1–uninfected patients ($P = 0.0006$) but not in HIV-1–infected ones ($P = 0.88$) (Fig. 2B). Older age ($P < 0.002$), female gender ($P < 0.001$), higher Hb values at enrollment ($P < 0.001$), and HIV-1 infection ($P < 0.001$) were all risk factors for a smaller Hb increment at day 45 (all $P < 0.001$; Table 2, last column).

Patients With Treatment Failure at Day 45

Fifty-two patients (30 HIV-1–negative and 22 HIV-1–infected patients) had a recurrent parasitemia at day 45. In these patients, mean Hb at enrollment (132.3 g/L in HIV-1–negative patients and 124.5 g/L in HIV-1–infected patients) was similar than in those without recurrent parasitemia. The mean difference of Hb between days 0 and 45 was negative in both HIV-1–negative (−7.8 g/L; paired *t* test $P = 0.09$) and HIV-1–positive patients (−8.8 g/L; paired *t* test $P = 0.06$) and almost reached statistical significance despite the very low statistical power.

DISCUSSION

In our study, as expected, at enrollment, Hb was lower in HIV-1–infected than in HIV-1–uninfected malaria patients, and after successful malaria treatment, both groups showed a fall and rise in Hb within the follow-up period. After multivariate analysis, HIV infection was a risk factor for a more important Hb decline and a slower hematological recovery. Therefore, 45 days after successful malaria treatment, the mean Hb in HIV-infected patients was similar to that during the malaria attack, whereas HIV-negative patients were already recovering.

TABLE 1. Baseline Characteristics of Successfully Treated Malaria Patients by HIV-1 Status and Treatment Outcome

	HIV-1 Negative (n = 444)	HIV-1 Positive (n = 217)	*P*
Mean weight (SE), kg	57.0 (0.5)	56.8 (0.8)	0.84
Women (%)	192 (43.2)	120 (55.3)	0.04
Mean age (SE), yr	25.4 (0.4)	30.7 (0.5)	<0.001
Mean body temperature (SE), °C	37.2 (0.6)	37.3 (0.9)	0.33
Mean WBC count (SE), n × 10^9/L*	5.2 (0.1)	5.0 (0.1)	0.18
Mean Hb (SE), g/L†	136.0 (0.1)	122.7 (0.2)	<0.001
Anemia (%)†	114 (25.7)	103 (47.5)	<0.001
Mean (geometric) parasite density (95% CI), parasites/μL*	8483 (7523 to 9566)	8553 (7148 to 10235)	0.94
Gametocytes prevalence, n (%)	12 (2.7)	9 (4.1)	0.32
Treatment AL (%)§	249 (56.1)	113 (52.1)	0.33
Median CD4 counts (interquartile range), cells/μL*‡	—	271 (185–431)	—
CD4 cell count <200 (%)	—	29.7	—
CD4 cell count <300 (%)	—	54.4	—

*Data on WBC were missing in 2 HIV-1–negative and 6 HIV-1–positive patients.
†Patients with Hb <7 or any underlying disease were excluded; WHO definition of anemia <120 g/L in women; <130 g/L in men and other patients treated with SP.
‡CD4 cell count missing in 35 (16%) HIV-1–positive patients.
§Other patients were treated with salfadoxine-pyrimethamine.

In this selected cohort, compared with HIV-negative malaria patients, the HIV-1–positive malaria patients were older, had a slightly lower Hb level, and women were more represented. These findings are consistent with the epidemiology of HIV-1 in Zambia and with the overall trial findings.[10,8]

Patients with an underlying disease such as those with symptomatic AIDS or severe malaria, including hyperparasitemia and severe anemia (Hb < 70 g/L), were excluded. Therefore, our study patients represent a selected group with an Hb higher than that found in the whole population of HIV-1–infected individuals with clinical malaria.

At day 14, a larger Hb decline was associated with higher parasitemia, higher Hb at enrollment, and SP treatment. This confirms that the risk factors responsible for a lower Hb

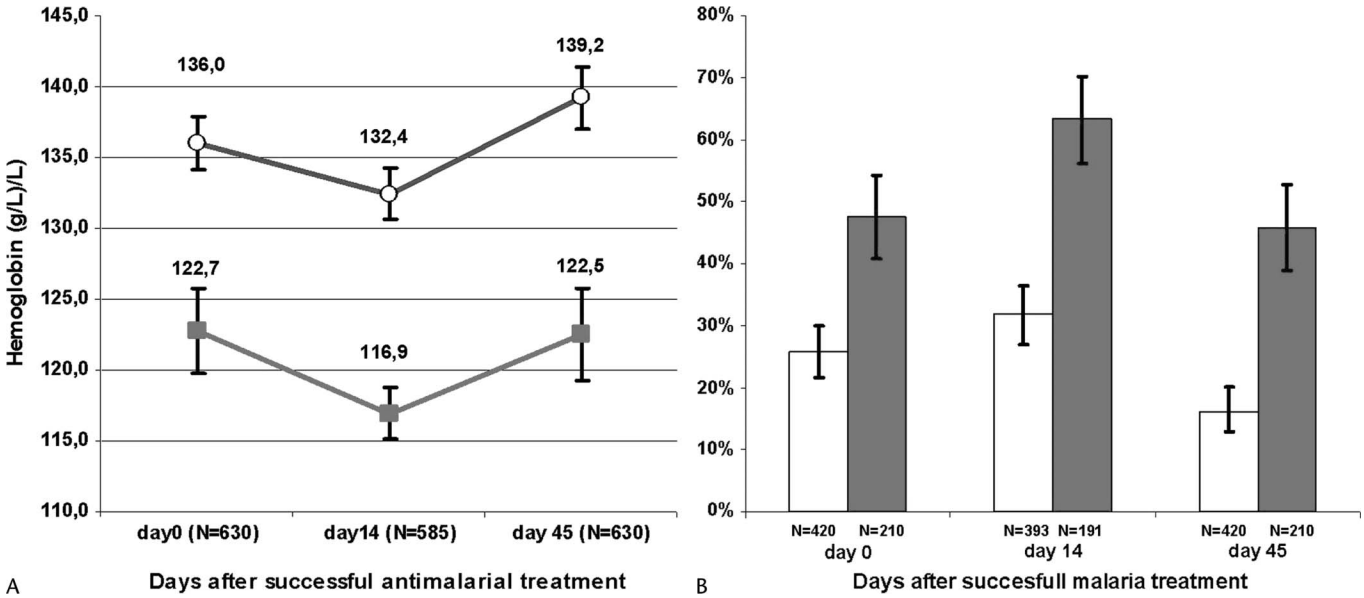


FIGURE 2. A, Mean Hb g/L of patients at enrollment, 14 and 45 days after successful malaria treatment by HIV-1 status. Values shown with 95% confidence intervals. Successful treatment signifies no recurrent parasitemia till day 45. HIV negative: day 14 vs day 0: P < 0.0001; day 45 vs day 0: P = 0.001. HIV positive: day 14 vs day 0: P < 0.0001; day 45 vs day 0: P = 0.64. B, percentage of patients being anemic at enrollment, 14 and 45 days after successful malaria treatment by HIV-1 status. 95% confidence intervals are shown. Anemia followed the WHO definition Hb <120 g/L for women and Hb <130 g/L for men. Successful treatment signifies no recurrent parasitemia till day 45. HIV negative: day 14 vs day 0: P = 0.06; day 45 vs day 0:P = 0.0006. HIV positive: day 14 vs day 0: P < 0.0001; day 45 vs day 0:P = 0.88.

TABLE 2. Association of Hb Change and Other Risk Factors in Adult Patients With Nonsevere Clinical Malaria at Enrollment and 14 and 45 Days After Successful Antimalarial Treatment

	Day 14 vs Day 0 (n = 588)				Day 45 vs Day 14 (n = 562)				Day 45 vs Day 0 (n = 630			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
Risk Factors for Hb Change	**Slope**	***P***	**Slope**	***P***	**Slope**	***P***	**Slope**	***P***	**Slope**	***P***	**Slope**	***P***
Hb at enrollment (g/L)*	−0.35	<0.001	−0.38	<0.001	−0.05	0.19	−0.16	0.001	−0.42	<0.001	−0.61	<0.001
Parasite load ($\log_{10}$ parasites/μL)*	−0.59	<0.001	−0.51	<0.001	0.61	<0.001	0.59	<0.001	0.01	0.93	—	—
Male (vs female)	0.004	0.97	—	—	0.34	0.045	0.54	0.005	0.27	0.12	1.27	<0.001
Age (yr)	−0.01	0.08	—	—	−0.03	0.001	−0.02	0.04	−0.04	<0.001	−0.03	0.002
Body weight (kg)	−0.01	0.05	—	—	−0.01	0.348	—	—	−0.01	0.13	—	—
HIV-1*	−0.28	0.24	−0.65	<0.001	−0.37	0.04	−0.40	0.048	−0.47	0.01	−0.88	<0.001
SP vs AL†	−0.41	0.001	−0.45	<0.001	0.08	0.62	—	—	0.32	0.07	−0.27	0.08

*Patients with Hb <7.0 g/L, hyperparasitemia were excluded at enrollment.
†SP vs AL treatment.

during a malaria episode remain active at least 14 days after successful treatment. Furthermore, compared with an efficacious and rapidly acting drug such as AL, the slower acting SP was a risk factor for a greater Hb decline observed at day 14. After adjustment, HIV-1 infection was retained as an independent risk factor for a greater Hb decline. As the degree of immune suppression did not influence the Hb decline, the mechanisms behind these greater decline in HIV infected are unclear. In this phase, the major contributor to the hemolytic anemia is the accelerated destruction of uninfected RBC,[15] probably linked to immune mechanisms.[1,16] Considering that there is a chronic activation of the immune system by the HIV infection, this may explain the probable accelerated destruction of uninfected RBC in this group of patients.

From 14 days onward, when the malaria-related factors contributing to hemolytic anemia have presumably abated, HIV-1 infection remained, next to higher Hb, lower parasitemia at day 0, and female gender and age, an independent risk factor for a slower hematological recovery. The slow hematological recovery was also not associated with the degree of immune suppression and could be explained by the impairment of the erythropoiesis and of the iron mobilization.[17] It is unclear if the myelosuppressive effect of HIV-1 on the host erythropoietic system was enhanced due to a synergistic interaction between HIV-1 and malaria. The drug regimen did not have any influence on the hematological recovery, obviously because patients with recurrent parasitemia were excluded. The administration of the longer acting SP has been associated with lower rates of new infections, and this effect could provide an additional protection against anemia (compared with AL). However, this may be true when SP resistance is low. In this study, there was no difference in the occurrence of new infections between the 2 study arms, whereas recrudescences were much more frequent in the SP arm (reported in[18]). Such a little or no prophylactic effect related to SP indicates that resistance to this treatment was high with recurrent infections further decreasing Hb.

A faster Hb decline at day 14 and a better recovery afterward were both related to higher parasitemia levels. The faster decline was expected, but the physiopathological mechanisms behind the faster recovery are unclear.

Our data add to the evidence that HIV-1–infected patients represent, next to children and pregnant women, an additional vulnerable group for malaria.[19,20] Female gender and age are well-known risk factors for both a slower Hb recovery and for HIV-1 infection. Furthermore, HIV-1 immune suppressed individuals have a higher risk of malaria infection and disease, a higher parasite load during a clinical malaria episode, and more likely to fail the treatment.[8,19] This could rapidly lead to a vicious circle in which frequent malaria infections, even if successfully treated, may prevent a full hematological recovery and further worsen the anemia in a modest but stepwise manner. This is well illustrated in our patients with recurrent parasitemia at day 45, who had a lower Hb level at day 45 than at enrollment. This is worrying for all HIV–malaria-coinfected patients as there was no correlation with the degree of immune suppression. Furthermore, in immune suppressed patients, anemia is an independent prognostic marker of HIV disease progression.[21] Therefore, HIV treatment guidelines recommend to start antiretroviral treatment for any "persistent unexplained anemia," operationally defined as anemia that does not have any other apparent cause and fails to respond to the combination of anthelmintics, hematinics, and antimalarials after 1 month.[12] Considering that in successfully treated patients Hb had not increased after 45 days, the diagnosis of persistent unexplained anemia should be done only after a longer period, possibly 2 or 3 months, from the clinical attack. Furthermore, as reported before, the absolute CD4 count, the chosen marker to monitor the progression of HIV-1 infection, is also altered during or just after a clinical malaria episode.[22] Finally, patients with a low CD4 count or clinical stage 4, and eventually stage 3 or stage 4, should be given antiretroviral treatment or cotrimoxazole prophylaxis, both of them influencing the hematological status and possibly causing anemia.[23] Therefore, this subgroup should be carefully evaluated.

Recent estimates show almost half of the world's malaria is in holoendemic areas.[24] Clinicians and program managers working in areas where both diseases are prevalent should be aware that malaria and HIV-1 infection have specific and different but synergistic hematological effects. Prompt diagnosis and effective treatment is the backbone of malaria

control, but in this highly vulnerable group of HIV infected, malaria prevention should be prioritized. Co-trimoxazole, an antifolate with antimalarial activities, is currently recommended as preventive therapy in immune suppressed HIV-1–infected patients.[25] Furthermore, the combination of co-trimoxazole, insecticide-treated bed nets substantially reduced the frequency of malaria in adults with HIV.[26] These policies, if widely implemented with other preventive measures, might decrease the malaria burden in HIV-1–infected individuals, including the anemia burden associated with repeated malaria episodes.[27]

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