Published in final edited form as:

Am J Hematol. 2010 April ; 85(4): 227–233. doi:10.1002/ajh.21653.

Hematological Predictors of Increased Severe Anemia in Kenyan Children Co-infected with *Plasmodium falciparum* and HIV-1

Gregory C. Davenport, M.S.¹, Collins Ouma, Ph.D.^{2,3}, James B. Hittner, Ph.D.⁴, Tom Were, Ph.D.², Yamo Ouma, M.S.², John M. Ong'echa, Ph.D.², and Douglas J. Perkins, Ph.D.^{2,5,*}

¹ Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

² University of New Mexico/KEMRI Laboratories of Parasitic and Viral Diseases, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

³ Department of Biomedical Sciences and Technology, Maseno University, Maseno, Kenya

⁴ Department of Psychology, College of Charleston, Charleston, SC, USA

⁵ Department of Internal Medicine, Division of Infectious Diseases, School of Medicine, University of New Mexico, Albuquerque, NM, USA

Abstract

Malaria and HIV-1 are co-endemic in many developing countries, with anemia being the most common pediatric hematological manifestation of each disease. Anemia is also one of the primary causes of mortality in children mono-infected with either malaria or HIV-1. Although our previous results showed HIV-1(+) children with acute *Plasmodium falciparum* malaria [Pf(+)] have more profound anemia, potential causes of severe anemia in co-infected children remain unknown. As such, children with *P. falciparum* malaria (aged 3-36 mos., n=542) from a holoendemic malaria transmission area of western Kenya were stratified into three groups: HIV-1 negative [HIV-1(-)/Pf(+)]; HIV-1 exposed [HIV-1(exp)/Pf(+)]; and HIV-1 infected [HIV-1(+)/Pf(+)]. Comprehensive clinical, parasitological, and hematological measures were determined upon enrollment. Univariate, correlational, and hierarchical regression analyses were used to determine differences among the groups and to define predictors of worsening anemia. HIV-1(+)/Pf(+) children had significantly more malarial pigment-containing neutrophils (PCN), monocytosis, increased severe anemia (Hb <6.0g/dL), and nearly ten-fold greater mortality within three months of enrollment. Common causes of anemia in malaria-infected children, such as increased parasitemia or reduced erythropoiesis, did not account for worsening anemia in the HIV-1(+)/Pf(+) group, nor did carriage of sickle cell trait or G6PD deficiency. Hierarchical multiple regression analysis revealed that more profound anemia was associated with elevated PCM, younger age, and increasing HIV-1 status ([HIV-1(-) → HIV-1(exp) → HIV-1(+)]. Thus, malaria/HIV-1 co-infection is characterized

*Corresponding Author: Douglas J. Perkins, Ph.D. Director, Global and Geographic Medicine Program University of New Mexico Division of Infectious Diseases MSC10-5550 1 University of New Mexico Albuquerque, NM 87131-0001 phone: 505-272-6867 fax: 505-272-8441 dperkins@salud.unm.edu.

COMPETING INTERESTS

None of the authors of the manuscript have a personal or financial competing interest that influences the interpretation of data or presentation of any information contained herein.

AUTHORS' CONTRIBUTIONS

GCD collected and analyzed the data and prepared the manuscript. CO conducted the HIV testing. JBH designed the data analysis strategy and provided statistical support. TW conducted the microbiological testing. YO analyzed the blood smears for parasitologic indices. JMO aided in the study design and interpretation of the results. DJP designed the study, supervised the activities in New Mexico, Pennsylvania, and Kenya, and co-wrote the manuscript with GCD.

by more profound anemia and increased mortality, with acquisition of monocytic pigment having the most detrimental impact on Hb levels.

Keywords

malaria; hemozoin; pediatric; holoendemic; erythropoiesis; severe malarial anemia

BACKGROUND

Plasmodium falciparum [Pf(+)] and human immunodeficiency virus-1 (HIV-1) are co-endemic in many tropical and sub-tropical countries, with the potential risk for enhanced clinical, hematological, and parasitological complications. Approximately 250 million malaria infections are reported annually, resulting in greater than one million deaths, primarily in African children less than five years of age (1). Sub-Saharan Africa also contains approximately 67% of the global HIV population, with an estimated 22 million HIV infections (2). In 2007 alone, this region accounted for 75% of the global AIDS deaths and over 1.9 million individuals were newly infected (2). Despite the geographical overlap between malaria and HIV, a number of earlier studies revealed little or no definitive interactions between the two infections (3-7). However, more recent literature shows that malaria and HIV-1 co-infection results in adverse pathological outcomes in both diseases, such as, increased HIV-1 viral loads following acute malaria, increased malaria episodes in HIV-1 positive [HIV-1(+)] individuals, reduced hemoglobin (Hb) concentrations during malaria and HIV-1 co-infection, and reduced efficacy of antimalarial drugs (8-16).

Mono-infection with either malaria or HIV-1 is associated with hematological complications, including anemia, monocytosis, and hemolysis (17-20). Additional hematological complications in malaria include splenomegaly, leukopenia, leukocytosis, eosinophilia, and thrombocytopenia (17), while HIV-1 infection is characterized by suppression of all three major blood cell lineages (20). Although most interactions between malaria and HIV-1 have been described in adults (reviewed in (21)), our recent investigation demonstrated that HIV-1(+) children had a significantly higher risk of developing severe anemia (SA) during acute falciparum malaria than HIV-1 negative [HIV-1(-)] children (22). In addition, enhanced pathogenesis has been extensively described in pregnant women with malaria and HIV co-infection (23,24).

Our previous findings (25,26), and those of others (27-29), revealed that pigment-containing monocytes (PCM) and pigment-containing neutrophils (PCN) are important markers of malaria disease severity. Malarial pigment (hemozoin, *pfHz*) is a crystalline structure formed from monomeric heme and released as a by-product of parasitic proteolysis of host Hb (30). Phagocytic cells acquire *pfHz* through ingestion of parasitized red blood cells (pRBC) (31) and by scavenging free *pfHz* released into circulation following rupture of pRBC (32). To date, the impact of naturally-acquired intraleukocytic *pfHz* on Hb concentrations in co-infected individuals has not been reported.

To investigate etiologies of worsening anemia in HIV-1(+)/Pf(+) children, we performed comprehensive hematological analyses and examined potential causes of anemia in three groups of children (n=542; 3-36 mos. of age): HIV-1(-)/Pf(+); HIV-1 exposed (exp)/Pf(+), and HIV-1(+)/Pf(+). The study was conducted in a holoendemic *P. falciparum* transmission area of western Kenya in which SA is the primary manifestation of severe malaria in children less than 48 mos. of age, with pediatric cerebral malaria occurring only in rare cases (33,34).

METHODS

Study site and participants

Children aged 3-36 months (n=542) with *P. falciparum* parasitemia (any density) were recruited at Siaya District Hospital, western Kenya, during their first hospital contact for malaria from March 2004 to January 2006. Siaya District is a holoendemic *P. falciparum* transmission area where residents receive up to 300 infective bites per annum (35). None of the children in the study had cerebral malaria or non-falciparum malarial infections. A detailed description of the study area and pediatric population can be found in our previous publication (36).

Children with *P. falciparum* malaria were divided into three groups: HIV-1 negative [HIV-1(−), negative HIV serological results by both Determine® and Uni-Gold™ assays]; HIV-1 exposed [HIV-1(exp), at least one (of two) positive serological tests with Determine® and Uni-Gold™ and negative HIV-1 DNA PCR]; and HIV-1 positive [HIV-1(+), at least one (of two) positive serological result with the Determine® and Uni-Gold™ tests, and positive HIV-1 DNA PCR results on two consecutive HIV-1 DNA PCR assays three months apart according to our previously published methods (22)]. For the PCR analyses, HIV-1 gp41 primers were selected for highly conserved HIV-1 group M, N, and O sequences for use in western Kenya (37,38). Although the maternal HIV-1 status was unknown, based on the approved informed consent process for enrollment into the study, HIV-1(exp) children presumably acquired HIV-1 antibodies from their mother during gestation and/or through breastfeeding. It is important to note that none of the children in the cohort had received prior blood transfusions. Pre- and post-test HIV counseling were provided for the parents/guardians of all participants. Children positive for one or both HIV-1 serological tests were prophylactically treated with trimethoprim-sulfamethoxazole from the time of enrollment onward. None of the children had been initiated on antiretroviral (ARV) therapy at the time of enrollment since ARVs were not available during the study period. Children in all three groups were followed for three months post-enrollment to examine mortality. The parents/guardians of all children were asked to report to the hospital three months post-enrollment. For those children that did not report, members of the study team traveled to the residence and inquired about the child's health status. In addition, parents/guardians were asked to return to the hospital during each febrile episode that their child experienced prior to the three month follow-up visit.

Severe anemia was categorized according to a geographically appropriate definition for this holoendemic region (i.e., Hb <6.0g/dL) based on previous longitudinal Hb measures in children (<48 months of age; n>14,000) (33). The WHO standard of SA (i.e., Hb <5.0g/dL) (39) was also utilized to frame the current findings into a broader global context. Children were treated according to Ministry of Health, Kenya, guidelines that included intravenous quinine for the treatment of severe malaria and oral Coartem™ for non-severe malaria. Written informed consent was obtained from the participants' parents/guardians. Approval for the study was granted by the ethical and scientific review committees at the University of Pittsburgh, University of New Mexico, and the Kenya Medical Research Institute.

Laboratory methods

Asexual malaria trophozoites were determined with thick and thin Giemsa-stained peripheral blood smears prepared from venous blood samples for malaria parasite identification and quantification according to our previous methods (22). Complete blood counts were performed with a Beckman Coulter® A^c-T diff2™ (Beckman Coulter, Inc.) on blood obtained prior to administration of antimalarials and/or antipyretics. Glucose-6-phosphate dehydrogenase (G6PD) deficiency was assessed using the G6PDH Screening Kit

(Trinity Biotech, PLC) according to the manufacturer's instructions. The presence of Hb variants in sample hemolysates was detected using a Hb electrophoresis kit, which allowed for detection of HbS, HbC, HbF, and HbA, in either the heterozygous or homozygous states (Helena laboratories). The reticulocyte count, absolute reticulocyte number (ARN), reticulocyte production index (RPI), and prevalence and quantity of pigment-containing neutrophils (PCN) and pigment-containing monocytes (PCM) were determined per our previous methods (40).

Presence of bacteremia was performed by blood culture according to our previous report (25). Since bacteremia is a common cause of anemia in African children (41,42), which may have potentially confounding effects on results presented here, all children found to have bacteremia were excluded from the current study.

Statistical analyses

Data were analyzed using SPSS (version 15.0). Inter-group clinical, demographic, and hematological measures were compared by either ANOVA or Kruskal-Wallis tests, followed by pair-wise post-hoc comparison with Student's t-test or Mann-Whitney U test, respectively. Pearson's Chi Square (χ^2) or Fisher's exact test was used for comparing proportions. The conventional level of statistical significance was set at $P \leq 0.05$. Pearson correlations were performed to select (i.e., $P \leq 0.05$) and prioritize potential predictors of Hb to be entered into a hierarchical multiple regression model.

RESULTS

Demographic, clinical, and hematological characteristics

Children were stratified into three categories: HIV-1(-)/Pf(+), n=406; HIV-1(exp)/Pf(+), n=112; and HIV-1(+)/Pf(+), n=24. The demographic, clinical, and hematological characteristics of the study participants are listed in Table 1. Age (mos.) differed across the groups ($P=0.055$) with the HIV-1(+) group being the oldest. Additional significant inter-group differences included absolute monocyte count ($\times 10^3/\mu\text{L}$; $P=0.007$), Hb concentration (g/dL; $P=0.009$), hematocrit (Hct, %; $P=0.012$), red blood cell (RBC) count ($\times 10^6/\mu\text{L}$, $P=0.022$), mean corpuscular Hb concentration (MCHC, g/dL, $P=0.069$), and RBC distribution width (RDW; $P=0.018$). Post-hoc testing of these significant values revealed that, relative to the HIV-1(-)/Pf(+) and HIV-1(exp)/Pf(+) groups, the HIV-1(+)/Pf(+) group had higher monocyte counts ($P=0.002$ and $P=0.006$, respectively), decreased Hb levels ($P=0.004$ and $P=0.012$, respectively), reduced RBC counts ($P=0.011$ and $P=0.045$, respectively), lower MCHCs ($P=0.066$ and $P=0.027$, respectively), and greater RDWs ($P=0.007$ and $P=0.041$, respectively). Evaluation of malaria parasitological indices revealed that median peripheral (μL) and geometric mean (μL) parasitemias were not significantly different across the groups ($P=0.205$ and $P=0.123$, respectively).

Genetic variants

Certain genetic traits confer protection against severe malaria, namely sickle cell trait and G6PD deficiency (43,44). As shown in Table 1, HIV-1(+)/Pf(+) children had a lower incidence of sickle cell trait than either the HIV-1(-)/Pf(+) or HIV-1(exp)/Pf(+) children ($P=0.198$). In contrast, prevalence of G6PD deficiency increased across the groups, with the HIV-1(+)/Pf(+) group having the highest proportion of G6PD deficiency ($P=0.084$, Table 1).

Severe anemia distribution

Severe anemia is the primary manifestation of severe malaria and accounts for a substantial proportion of mortality in western Kenya, with the peak incidence occurring between ages

7-24 mos. (33,45). As such, children were classified according to a geographically appropriate definition of SA (i.e., Hb <6.0g/dL) (33) and the WHO definition (i.e., Hb <5.0g/dL) (39). The proportion of SA at Hb <6.0g/dL progressively increased with HIV-1 status ($P=0.020$), while proportion of SA at Hb <5.0g/dL was comparable in the HIV-1(-)/*Pf*(+) and HIV-1(exp)/*Pf*(+) groups, and highest in co-infected children ($P=0.026$, Fig. 1). Differences between the HIV-1(+)/*Pf*(+) and the HIV-1(-)/*Pf*(+) groups were significant for both Hb <6.0g/dL ($P=0.008$) and Hb <5.0g/dL ($P=0.025$), and between the HIV-1(+)/*Pf*(+) and HIV-1(exp)/*Pf*(+) groups ($P=0.016$) with the WHO standard (Fig. 1).

Mortality associated with co-infection

In holoendemic *P. falciparum* transmission areas such as Siaya District, approximately 30% of the mortality in children less than three years of age is due to *P. falciparum*-promoted SA (46). Examination of the three-month, post-enrollment mortality revealed that HIV-1(+)/*Pf*(+) children had significantly more mortality than both the HIV-1(-)/*Pf*(+) and HIV-1(exp)/*Pf*(+) groups ($P<0.001$, for both categories, Fig. 1). The number of children that died during the three-month follow-up period was 13 (3.2%) in HIV-1(-)/*Pf*(+) group, 6 (5.4%) in the HIV-1(exp)/*Pf*(+) group, and 8 (33.3%) in the HIV-1(+)/*Pf*(+) group.

Erythropoietic indices

To determine if altered erythropoietic responses were responsible for more profound anemia in co-infected children, measures of erythropoiesis were examined in the three groups. As shown in Table 2, the reticulocyte count and ARN did not significantly differ across the groups. We have previously shown that children with severe malarial anemia (SMA) have suppression of erythropoiesis (47), as evidenced by an RPI<2 (48). Although the majority of children in all three groups had suppression of erythropoiesis, the inter-group proportions were not significantly different ($P=0.766$, Table 2).

Intraleukocytic hemozoin

Previous investigations illustrated that intracellular *pf*Hz levels in circulating neutrophils and monocytes are associated with malaria disease severity (27,29,49). As shown in Fig. 2, the percentage of children with PCN was highest in the HIV-1(+)/*Pf*(+) group ($P=0.029$, inter-group difference). Post-hoc testing revealed that PCN was greater in the HIV-1(+)/*Pf*(+) group than the HIV-1(exp)/*Pf*(+) group ($P=0.016$), while the differences between the HIV-1(+)/*Pf*(+) and HIV-1(-)/*Pf*(+) groups, and HIV-1(exp)/*Pf*(+) and HIV-1(-)/*Pf*(+) groups were not significant ($P=0.079$ and $P=0.121$, respectively). In addition, the median concentration of PCN (μ L) was highest in children co-infected with malaria and HIV-1, but the across-group differences were not significant ($P=0.249$, Fig. 2). Examination of intra-monocytic *pf*Hz revealed that the percentage of children with PCM and the median concentration of PCM (μ L) were similar across the groups ($P=0.852$ and $P=0.456$, respectively, Fig. 2).

Linear correlation analyses

As an initial step to explore variables that were potentially important in predicting Hb levels, Pearson correlations were performed in the full sample ($n=542$) between the following measures: HIV-1 status [i.e., HIV-1(-), HIV-1(exp), and HIV-1(+)] age; PCM; PCN; and Hb. There were significant correlations between progressing HIV-1 status [HIV-1(-) $\rightarrow$ HIV-1(exp) $\rightarrow$ HIV-1(+)] and age ($r=0.091$, $P=0.034$) as well as Hb ($r=-0.105$, $P=0.017$). Significant relationships with age were also identified for PCM ($r=-0.099$, $P=0.021$) and Hb ($r=0.193$, $P<0.001$). The strongest relationship observed was between PCM and Hb ($r=-0.413$, $P<0.001$).

Predictors of Hb concentrations

Following the bivariate correlation analyses, a hierarchical multiple regression analysis was performed to identify predictors of Hb concentrations (Table 3). The influence of HIV-1 was determined by entering HIV-1 status [HIV-1(-) → HIV-1(exp) → HIV-1(+)] into the model. Variables were entered as predictors in two sequential blocks with Hb as the dependent variable. Block 1 consisted of age and HIV-1 status [HIV-1(-) → HIV-1(exp) → HIV-1(+)] as covariates, while block 2 was comprised of PCN and PCM. Prior to performing the regression, both PCM and PCN were inverse-transformed to approximate univariate normality. The hierarchical multiple regression model demonstrated that both age ($P<0.001$) and HIV-1 status ($P=0.011$) were significant predictors of Hb with block 1 being highly significant ($P<0.001$) and accounting for 4.4% of the variability in Hb. For block 2, PCM was also a significant predictor of Hb ($P<0.001$), whereas PCN did not significantly influence Hb levels ($P=0.138$). This second block was highly significant ($P<0.001$) and accounted for 14.8% of the variability in Hb. Examination of the squared semipartial correlations indicated that age, HIV-1 status, and PCM accounted for 2.8%, 1.2%, and 14.4% of the unique variance in Hb, respectively.

DISCUSSION

The current investigation presents a comprehensive examination of the hematological factors that contribute to worsening anemia in Kenyan children residing in a holoendemic *P. falciparum* environment with a high prevalence of HIV-1 infection. Co-infection with malaria and HIV-1 was associated with significantly higher rates of SA, regardless of whether the geographically-relevant definition (Hb $<6.0\text{g/dL}$) of anemia, or the WHO definition (Hb $<5.0\text{g/dL}$), was applied. Results presented here show that two prominent causes of anemia (i.e., malaria parasitemia and reduced erythropoiesis) were *not* responsible for worsening anemia in co-infected children. However, results presented here show that acquisition of *pfHz* by monocytes appears central to the pathogenesis underlying more profound anemia in HIV-1(+) children with falciparum malaria. This study is also the first investigation showing that malaria and HIV-1 co-infection is associated with significantly higher rates of pediatric mortality.

Results presented here demonstrate a progressive decline in Hb levels and increased SA in the HIV-1(exp)/*Pf*(+) and HIV-1(+)/*Pf*(+) groups, with HIV-1(+)/*Pf*(+) children having the greatest degree of SA. Malaria contributes to anemia through a number of different mechanisms, including RBC lysis, organ sequestration and destruction of erythrocytes, phagocytosis of uninfected and infected RBCs, and dyserythropoiesis [reviewed in (50)]. Although all three groups in this study had decreased MCV, normal MCH values, and MCHC in the low normal range, none of these values were significantly different across the groups, with the exception of decreased MCHC values in the HIV-1(+)/*Pf*(+) group. This hematological profile is suggestive of a microcytic hypochromic anemia, a finding typically attributed to iron deficiency, which may be a consequence of sequestered iron due to elevated levels of IL-6, and consequently hepcidin (51-53). Studies are currently ongoing in our laboratory to confirm this hypothesis. The RDW was substantially greater in the HIV-1(+)/*Pf*(+) group, suggesting that, despite the relative consistency in the RPIs across the groups, children with the greatest anemia also had the greatest compensation for their anemia, as indicated by variability in RBC size. Calis *et al.* (42) reported similar RDWs in their study participants, but concluded that folate and iron deficiency were not contributing factors in Malawian children with SA. However, it remains to be determined if folate and iron deficiency are important contributors to worsening anemia in malaria and HIV-1 co-infected children.

It is estimated that 15 to 20% of untreated HIV-1(+) infants will progress to AIDS, and ultimately death, by four years of age in developed nations (54), although the rate is likely much higher in African children. A study with participants from the U.S. and Puerto Rico reported 32% had progressed to CDC Clinical Category C or had died by 18 months (55). Consistent with these data, the HIV-1(+)/*Pf*(+) group in our study had nearly ten times more mortality (33.3%) over the three month follow-up period. However, while the proportion of SA cases and mortality was significantly increased in the HIV-1(+)/*Pf*(+) children, there was no correlation between death and Hb. Furthermore, upon longitudinally examining deceased children from the three groups, there were no significant differences between acute (febrile, non-parasitemic) or parasitemic visits, median Hb over those visits, or age at which children died. These data may be explained by the fact that substantially more children died at home versus at hospital, and therefore, cause of death, parasitemia status, and Hb levels were not available.

While not reaching statistical significance, there was a progressive increase in G6PD deficiency across the groups. The 7.5% prevalence of G6PD deficiency in our overall study population was similar to the 8.5% reported in a meta-analysis of two previous Kenyan studies (56). Interestingly, the HIV-1(exp)/*Pf*(+) and HIV-1(+)/*Pf*(+) individuals in the present study had greater than twice the frequency of G6PD deficiency than the HIV-1(-)/*Pf*(+) children. This X-linked deficiency provides mild protection against malaria infection (37,38), due to stunted parasite growth inside the G6PD-deficient RBC (57,58). However, oxidative stress caused by infections and various drugs can result in acute hemolysis and subsequent chronic anemia (43). Therefore, while this deficiency may provide some protection against acquisition of malarial infection and hyperparasitemia, anemia may be exacerbated once the host becomes infected and treatment ensues.

Although the proportion of children with sickle cell trait was not significant across the groups, there was a three-fold decrease in carriage of HbAS in HIV-1(+)/*Pf*(+) children. Failure to achieve statistical significance may be due to the sample size in the HIV-1(+)/*Pf*(+) group. Since HbAS confers 90% protection against severe malaria and mortality (59,60), sickle cell trait may be bolstering the more favorable outcomes (i.e., less severe anemia and fewer fatalities) seen in the HIV-1(-)/*Pf*(+) and HIV-1(exp)/*Pf*(+) groups. However, when G6PD deficiency and sickle cell status were entered into the multiple regression analysis, neither of these factors significantly predicted Hb levels.

Our previous study showed that SMA was associated with suppression of erythropoiesis (40). However, the erythropoietic response was not significantly different between the groups examined here, suggesting that neither HIV-1 exposure nor HIV-1 positivity antagonized erythropoiesis beyond that observed in malaria mono-infection. In addition, parasitemia levels were 46% and 37% lower in the HIV-1(exp)/*Pf*(+) and HIV-1(+)/*Pf*(+) groups, respectively, than in the HIV-1(-)/*Pf*(+) children, demonstrating that the degree of parasitemia does not correlate with the severity of anemia, as we (25) and others (46) have previously reported. However, the fact that the erythropoietic indices are nearly identical, while the degree of anemia is substantially more severe across the groups, indicates that a proportional response to the worsening anemia had not been achieved in the HIV-1(+)/*Pf*(+) group.

By examining *pf*H₂ burden in neutrophils and monocytes, it is possible to determine if individuals suffer from recent (acute) versus prolonged (chronic) malaria infection, since monocytes remain in circulation longer than neutrophils (61). Our previous study in Kenya demonstrated that SMA is characterized by increased chronicity of infection, resulting in higher levels of PCM and lower levels of PCN (25). However, our present data show that co-infection is associated with a higher percentage of PCN, suggesting that HIV-1(+)

children suffer from more acute malaria. Although the reason for this finding remains unclear, HIV-1(+)/*Pf*(+) children presumably had worse overall health status, which may result in more rapid treatment-seeking behavior by their caregiver. Additional longitudinal follow-up studies with active surveillance are required to confirm this hypothesis.

To investigate predictors of Hb levels, we first performed Pearson correlations followed by a hierarchical multiple regression analysis to determine those variables with the greatest influence on Hb. Results of the regression model indicated that increasing age was significantly associated with higher Hb levels. In contrast, increasing HIV-1 status [i.e., HIV-1(-) → HIV-1(exp) → HIV-1(+)] in children with falciparum malaria was a significant predictor of worsening anemia. This finding supports the higher proportion of SA found in HIV-1(+)/*Pf*(+) children. Increasing PCM was associated with worsening anemia, and of all the factors examined, was the strongest predictor of Hb. The negative correlation between PCM and Hb supports our previous findings (25,26) and those of others (28) illustrating that PCM is a significant predictor of anemia. Moreover, results presented here extend previous findings by demonstrating that monocytic acquisition of *p*/Hz is also a significant predictor of Hb in children with malaria and HIV-1 co-infection.

Taken together, results presented here demonstrate that children with malaria and HIV-1 co-infection have more profound anemia and increased mortality relative to HIV-1(-) children. These findings also demonstrate that unlike parasitemia levels and suppression of erythropoiesis, *p*/Hz may play a central role in the pathogenesis of anemia in children co-infected with malaria and HIV-1. Since phagocytosis of *p*/Hz by monocytes and neutrophils promotes dysregulation in inflammatory mediators known to cause inflammatory-derived anemia (62-65), it will be important to determine if altered expression of cytokines, chemokines, and hematopoietic growth factors are responsible for more profound anemia in co-infected children.

Acknowledgments

This work was supported by the National Institute of Health grants RO1 AI51305 (DJP), D43 TW05884 (DJP), and the NIH/NIAD 5T32AI065380-03 Pitt AIDS Research Training Program (GCD). We are grateful to the parents, guardians, and children from the Siaya District community for their participation in the study. We also thank all the University of New Mexico-KEMRI staff and the Siaya District Hospital staff for their support during this study.

REFERENCES

1. World Malaria Report 2008. World Health Organization; Geneva: 2008.
2. HIV/AIDS JUNPo. 2008 Report on the Global AIDS Epidemic. UNAIDS; Geneva: 2008.
3. Muller O, Musoke P, Sen G, Moser R. Pediatric HIV-1 disease in a Kampala Hospital. *J Trop Pediatr*. 1990; 36:283–286. [PubMed: 2280434]
4. Kalyesubula I, Musoke-Mudido P, Marum L, Bagenda D, Aceng E, Ndugwa C, Olness K. Effects of malaria infection in human immunodeficiency virus type 1-infected Ugandan children. *Pediatr Infect Dis J*. 1997; 16:876–881. [PubMed: 9306483]
5. Greenberg AE, Nsa W, Ryder RW, Medi M, Nzeza M, Kitadi N, Baangi M, Malanda N, Davachi F, Hassig SE. *Plasmodium falciparum* malaria and perinatally acquired human immunodeficiency virus type 1 infection in Kinshasa, Zaire. A prospective, longitudinal cohort study of 587 children. *N Engl J Med*. 1991; 325:105–109. [PubMed: 2052043]
6. Colebunders R, Bahwe Y, Nekwei W, Ryder R, Perriens J, Nsimba K, Turner A, Francis H, Lebughe I, Van der Stuyft P, et al. Incidence of malaria and efficacy of oral quinine in patients recently infected with human immunodeficiency virus in Kinshasa, Zaire. *J Infect*. 1990; 21:167–173. [PubMed: 2230175]
7. Nguyen-Dinh P, Greenberg AE, Mann JM, Kabote N, Francis H, Colebunders RL, Huong AY, Quinn TC, Davachi F, Lyamba B, et al. Absence of association between *Plasmodium falciparum*

- malaria and human immunodeficiency virus infection in children in Kinshasa, Zaire. *Bull World Health Organ.* 1987; 65:607–613. [PubMed: 3322600]
8. Whitworth JA, Hewitt KA. Effect of malaria on HIV-1 progression and transmission. *Lancet.* 2005; 365:196–197. [PubMed: 15652587]
  9. Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S, Ojwiya A. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet.* 2000; 356:1051–1056. [PubMed: 11009139]
  10. van Eijk AM, Ayisi JG, Ter Kuile FO, Misore AO, Otieno JA, Kolczak MS, Kager PA, Steketee RW, Nahlen BL. Malaria and human immunodeficiency virus infection as risk factors for anemia in infants in Kisumu, western Kenya. *Am J Trop Med Hyg.* 2002; 67:44–53. [PubMed: 12363063]
  11. Kublin JG, Patnaik P, Jere CS, Miller WC, Hoffman IF, Chimbiya N, Pendame R, Taylor TE, Molyneux ME. Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet.* 2005; 365:233–240. [PubMed: 15652606]
  12. Grimwade K, Swingle G. Cotrimoxazole prophylaxis for opportunistic infections in children with HIV infection. *Cochrane Database Syst Rev.* 2003;CD003508. [PubMed: 12804472]
  13. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF. HIV infection as a cofactor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *Aids.* 2004; 18:547–554. [PubMed: 15090809]
  14. Cohen C, Karstaedt A, Frean J, Thomas J, Govender N, Prentice E, Dini L, Galpin J, Crewe-Brown H. Increased prevalence of severe malaria in HIV-infected adults in South Africa. *Clin Infect Dis.* 2005; 41:1631–1637. [PubMed: 16267737]
  15. Hoffman IF, Jere CS, Taylor TE, Munthali P, Dyer JR, Wirima JJ, Rogerson SJ, Kumwenda N, Eron JJ, Fiscus SA, Chakraborty H, Taha TE, Cohen MS, Molyneux ME. The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration. *AIDS.* 1999; 13:487–494. [PubMed: 10197377]
  16. Mwapasa V, Rogerson SJ, Molyneux ME, Abrams ET, Kamwendo DD, Lema VM, Tadesse E, Chaluluka E, Wilson PE, Meshnick SR. The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. *AIDS.* 2004; 18:1051–1059. [PubMed: 15096809]
  17. Abdalla SH. Hematopoiesis in human malaria. *Blood Cells.* 1990; 16:401–416. discussion 417–409. [PubMed: 2257320]
  18. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. *Am J Med.* 2004; 116(Suppl 7A): 27S–43S. [PubMed: 15050884]
  19. Sen R, Tewari AD, Sehgal PK, Singh U, Sikka R, Sen J. Clinico-haematological profile in acute and chronic *Plasmodium falciparum* malaria in children. *J Commun Dis.* 1994; 26:31–38. [PubMed: 7963380]
  20. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol.* 2003; 112:973–980. [PubMed: 14610491]
  21. Brentlinger PE, Behrens CB, Kublin JG. Challenges in the prevention, diagnosis, and treatment of malaria in human immunodeficiency virus infected adults in sub-Saharan Africa. *Arch Intern Med.* 2007; 167:1827–1836. [PubMed: 17893303]
  22. Otieno RO, Ouma C, Ong'echa JM, Keller CC, Were T, Waindi EN, Michaels MG, Day RD, Vulule JM, Perkins DJ. Increased severe anemia in HIV-1-exposed and HIV-1-positive infants and children during acute malaria. *AIDS.* 2006; 20:275–280. [PubMed: 16511422]
  23. Briand V, Badaut C, Cot M. Placental malaria, maternal HIV infection and infant morbidity. *Ann Trop Paediatr.* 2009; 29:71–83. [PubMed: 19460261]
  24. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, Rogerson SJ, Steketee RW. The burden of co-infection with human immunodeficiency virus type 1 and malaria

- in pregnant women in sub-saharan Africa. *Am J Trop Med Hyg.* 2004; 71:41–54. [PubMed: 15331818]
25. Awandare GA, Ouma Y, Ouma C, Were T, Otieno R, Keller CC, Davenport GC, Hittner JB, Vulule J, Ferrell R, Ong'echa JM, Perkins DJ. Role of monocyte-acquired hemozoin in suppression of macrophage migration inhibitory factor in children with severe malarial anemia. *Infect Immun.* 2007; 75:201–210. [PubMed: 17060471]
  26. Keller CC, Yamo O, Ouma C, Ong'echa JM, Ounah D, Hittner JB, Vulule JM, Perkins DJ. Acquisition of hemozoin by monocytes down-regulates interleukin-12 p40 (IL-12p40) transcripts and circulating IL-12p70 through an IL-10-dependent mechanism: *in vivo* and *in vitro* findings in severe malarial anemia. *Infect Immun.* 2006; 74:5249–5260. [PubMed: 16926419]
  27. Amodu OK, Adeyemo AA, Olumese PE, Gbadegesin RA. Intraleucocytic malaria pigment and clinical severity of malaria in children. *Trans R Soc Trop Med Hyg.* 1998; 92:54–56. [PubMed: 9692152]
  28. Lyke KE, Diallo DA, Dicko A, Kone A, Coulibaly D, Guindo A, Cissoko Y, Sangare L, Coulibaly S, Dakouo B, Taylor TE, Doumbo OK, Plowe CV. Association of intraleukocytic *Plasmodium falciparum* malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria. *Am J Trop Med Hyg.* 2003; 69:253–259. [PubMed: 14628940]
  29. Nguyen PH, Day N, Pram TD, Ferguson DJ, White NJ. Intraleucocytic malaria pigment and prognosis in severe malaria. *Trans R Soc Trop Med Hyg.* 1995; 89:200–204. [PubMed: 7778149]
  30. Egan TJ. Physico-chemical aspects of hemozoin (malaria pigment) structure and formation. *J Inorg Biochem.* 2002; 91:19–26. [PubMed: 12121758]
  31. Arese P, Schwarzer E. Malarial pigment (haemozoin): a very active 'inert' substance. *Ann Trop Med Parasitol.* 1997; 91:501–516. [PubMed: 9329987]
  32. Schwarzer E, Alessio M, Ulliers D, Arese P. Phagocytosis of the malarial pigment, hemozoin, impairs expression of major histocompatibility complex class II antigen, CD54, and CD11c in human monocytes. *Infect Immun.* 1998; 66:1601–1606. [PubMed: 9529087]
  33. McElroy PD, Lal AA, Hawley WA, Bloland PB, Kuile FO, Oloo AJ, Harlow SD, Lin X, Nahlen BL. Analysis of repeated hemoglobin measures in full-term, normal birth weight Kenyan children between birth and four years of age. III. The Asemobo Bay Cohort Project. *Am J Trop Med Hyg.* 1999; 61:932–940. [PubMed: 10674673]
  34. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, Weber MW, Pinder M, Nahlen B, Obonyo C, Newbold C, Gupta S, Marsh K. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet.* 1997; 349:1650–1654. [PubMed: 9186382]
  35. Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, et al. *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am J Trop Med Hyg.* 1994; 50:529–536. [PubMed: 7911282]
  36. Ong'echa JM, Keller CC, Were T, Ouma C, Otieno RO, Landis-Lewis Z, Ochiel D, Slingluff JL, Mogere S, Ogonji GA, Orago AS, Vulule JM, Kaplan SS, Day RD, Perkins DJ. Parasitemia, anemia, and malarial anemia in infants and young children in a rural holoendemic *Plasmodium falciparum* transmission area. *Am J Trop Med Hyg.* 2006; 74:376–385. [PubMed: 16525094]
  37. Cappadoro M, Giribaldi G, O'Brien E, Turrini F, Mannu F, Ulliers D, Simula G, Luzzatto L, Arese P. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. *Blood.* 1998; 92:2527–2534. [PubMed: 9746794]
  38. Ruwende C, Hill A. Glucose-6-phosphate dehydrogenase deficiency and malaria. *J Mol Med.* 1998; 76:581–588. [PubMed: 9694435]
  39. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg.* 2000; 94(Suppl 1):S1–90. [PubMed: 11103309]
  40. Were T, Hittner JB, Ouma C, Otieno RO, Orago AS, Ong'echa JM, Vulule JM, Keller CC, Perkins DJ. Suppression of RANTES in children with *Plasmodium falciparum* malaria. *Haematologica.* 2006; 91:1396–1399. [PubMed: 17018392]

41. Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B, Berkley JA. Salmonella bacteremia in Kenyan children. *Pediatr Infect Dis J*. 2006; 25:230–236. [PubMed: 16511385]
42. Calis JC, Phiri KS, Faragher EB, Brabin BJ, Bates I, Cuevas LE, de Haan RJ, Phiri AI, Malange P, Khoka M, Hulshof PJ, van Lieshout L, Beld MG, Teo YY, Rockett KA, Richardson A, Kwiatkowski DP, Molyneux ME, van Hensbroek MB. Severe anemia in Malawian children. *N Engl J Med*. 2008; 358:888–899. [PubMed: 18305266]
43. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet*. 2008; 371:64–74. [PubMed: 18177777]
44. Min-Oo G, Gros P. Erythrocyte variants and the nature of their malaria protective effect. *Cell Microbiol*. 2005; 7:753–763. [PubMed: 15888079]
45. Bloland PB, Ruebush TK, McCormick JB, Ayisi J, Boriga DA, Oloo AJ, Beach R, Hawley W, Lal A, Nahlen B, Udhayakumar V, Campbell CC. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission I. Description of study site, general methodology, and study population. *Am J Trop Med Hyg*. 1999; 60:635–640. [PubMed: 10348240]
46. Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. Red cell surface changes and erythrophagocytosis in children with severe *Plasmodium falciparum* anemia. *Blood*. 2000; 95:1481–1486. [PubMed: 10666228]
47. Awandare GA, Goka B, Boeuf P, Tetteh JK, Kurtzhals JA, Behr C, Akanmori BD. Increased levels of inflammatory mediators in children with severe *Plasmodium falciparum* malaria with respiratory distress. *J Infect Dis*. 2006; 194:1438–1446. [PubMed: 17054074]
48. Lee, GR. Anemia: General Aspects. Williams & Wilkins; Baltimore: 1999.
49. Luty AJ, Perkins DJ, Lell B, Schmidt-Ott R, Lehman LG, Luckner D, Greve B, Matousek P, Herbig K, Schmid D, Weinberg JB, Kremsner PG. Low interleukin-12 activity in severe *Plasmodium falciparum* malaria. *Infect Immun*. 2000; 68:3909–3915. [PubMed: 10858202]
50. Ghosh K. Pathogenesis of anemia in malaria: a concise review. *Parasitol Res*. 2007; 101:1463–1469. [PubMed: 17874326]
51. Ganz T. Molecular pathogenesis of anemia of chronic disease. *Pediatr Blood Cancer*. 2006; 46:554–557. [PubMed: 16261603]
52. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004; 113:1271–1276. [PubMed: 15124018]
53. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood*. 2003; 101:2461–2463. [PubMed: 12433676]
54. Mayaux MJ, Burgard M, Teglas JP, Cottalorda J, Krivine A, Simon F, Puel J, Tamalet C, Dormont D, Masquelier B, Doussin A, Rouzioux C, Blanche S. Neonatal characteristics in rapidly progressive perinatally acquired HIV-1 disease. The French Pediatric HIV Infection Study Group. *JAMA*. 1996; 275:606–610. [PubMed: 8594241]
55. Rich KC, Fowler MG, Mofenson LM, Abboud R, Pitt J, Diaz C, Hanson IC, Cooper E, Mendez H. Maternal and infant factors predicting disease progression in human immunodeficiency virus type 1-infected infants. Women and Infants Transmission Study Group. *Pediatrics*. 2000; 105:e8. [PubMed: 10617745]
56. Nkhoma ET, Poole C, Vannappagari V, Hall SA, Beutler E. The global prevalence of glucose-6-phosphate dehydrogenase deficiency: a systematic review and meta-analysis. *Blood Cells Mol Dis*. 2009; 42:267–278. [PubMed: 19233695]
57. Clark IA, Hunt NH. Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infect Immun*. 1983; 39:1–6. [PubMed: 6822409]
58. Miller J, Golenser J, Spira DT, Kosower NS. *Plasmodium falciparum*: thiol status and growth in normal and glucose-6-phosphate dehydrogenase deficient human erythrocytes. *Exp Parasitol*. 1984; 57:239–247. [PubMed: 6373352]
59. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA, Udhayakumar V. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet*. 2002; 359:1311–1312. [PubMed: 11965279]

60. Williams TN, Mwangi TW, Wambua S, Alexander ND, Kortok M, Snow RW, Marsh K. Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. *J Infect Dis.* 2005; 192:178–186. [PubMed: 15942909]
61. Tomas Ganz, RIL. William's Hematology. McGraw-Hill; New York: 1995. p. 875-878. William's Hematology
62. Sherry BA, Alava G, Tracey KJ, Martiney J, Cerami A, Slater AF. Malaria-specific metabolite hemozoin mediates the release of several potent endogenous pyrogens (TNF, MIP-1 alpha, and MIP-1 beta) *in vitro*, and altered thermoregulation *in vivo*. *J Inflamm.* 1995; 45:85–96. [PubMed: 7583361]
63. Perkins DJ, Moore JM, Otieno J, Shi YP, Nahlen BL, Udhayakumar V, Lal AA. *In vivo* acquisition of hemozoin by placental blood mononuclear cells suppresses PGE2, TNF-alpha, and IL-10. *Biochem Biophys Res Commun.* 2003; 311:839–846. [PubMed: 14623257]
64. Keller CC, Kremsner PG, Hittner JB, Misukonis MA, Weinberg JB, Perkins DJ. Elevated nitric oxide production in children with malarial anemia: hemozoin-induced nitric oxide synthase type 2 transcripts and nitric oxide in blood mononuclear cells. *Infect Immun.* 2004; 72:4868–4873. [PubMed: 15271950]
65. Casals-Pascual C, Kai O, Cheung JO, Williams S, Lowe B, Nyanoti M, Williams TN, Maitland K, Molyneux M, Newton CR, Peshu N, Watt SM, Roberts DJ. Suppression of erythropoiesis in malarial anemia is associated with hemozoin *in vitro* and *in vivo*. *Blood.* 2006; 108:2569–2577. [PubMed: 16804108]

Figure 1. Increased Anemia and Mortality in HIV-1(+) Children

Bars represent percentage of children with SA (left y-axis), while lines depicts mortality percentage (right y-axis) in each HIV status. Differences in the proportion of individuals with SA ($P=0.020$) and those deceased ($P<0.001$) were compared using Pearson's χ^2 test. Pair-wise comparison revealed significant differences between proportions of children with SA and mortality in the HIV-1(-)/Pf(+) vs. HIV-1(exp)/Pf(+) groups ($P=0.045$ and $P<0.001$, respectively) and HIV-1(-)/Pf(+) vs. HIV-1(+)/Pf(+) groups ($P=0.008$ and $P<0.001$, respectively). There were 13 (3.2%) HIV-1(-)/Pf(+), 6 (5.4%) HIV-1(exp)/Pf(+), and 8 (33.3%) HIV-1(+)/Pf(+) children that died within the three-month follow-up period after enrollment. SA (Hb <6.0g/dL) cases were as follows: 137 (35.2%) HIV-1(-)/Pf(+); 44 (41.1%) HIV-1(exp)/Pf(+); and 14 (63.6%) HIV-1(+)/Pf(+).

Figure 2. Increased Intraleukocytic pfHz in HIV-1(+) Children

Bars represent mean PCN/μL (black) and PCM/μL (grey) concentrations and are associated with the left y-axis, while percentage of children with intraleukocytic *pfHz* are depicted with a broken line (PCN) and grey line (PCM) and associated with the right y-axis. Differences in the proportion of individuals with PCN [9.4% HIV-1(-)/*Pf*(+), 4.5% HIV-(exp)/*Pf*(+), 20.8% HIV-1(+)/*Pf*(+); $P=0.029$] and PCM [48.3% HIV-1(-)/*Pf*(+), 48.2% HIV-(exp)/*Pf*(+), 54.2% HIV-1(+)/*Pf*(+); $P=0.852$] were compared using Pearson's χ^2 test, while PCN [HIV-1(-)/*Pf*(+), 0.24/μL; HIV-1(exp)/*Pf*(+), 0.16/μL; HIV-1(+)/*Pf*(+), 0.54/μL; $P=0.249$] and PCM concentrations [HIV-1(-)/*Pf*(+), 3.09/μL; HIV-1(exp)/*Pf*(+), 2.75/μL; HIV-1(+)/*Pf*(+), 4.13/μL; $P=0.456$] were compared across the groups using the Kruskal-Wallis test. Pair-wise comparisons were performed using either Pearson's χ^2 test, for categorical variables, or Mann-Whitney U, for continuous variables. A total of 30 monocytes and 100 neutrophils were examined per slide and expressed as a percentage of the counted monocytes and neutrophils, and then PCM and PCN concentrations were derived by multiplying the percentages by the total absolute monocyte and neutrophil counts, respectively.

Table 1

Clinical, demographic, and hematological characteristics of the study participants.

Characteristic	HIV-1(-)/Pf(+)	HIV-1(exp)/Pf(+)	HIV-1(+)/Pf(+)	P
Number of subjects	406	112	24	N/A
Age (mos.)	10.5 (10.5)	9.0 (6.2)	12.1 (9.6)	0.055
Gender, male/female ^a	219/187	57/55	14/10	0.755
Axillary temperature (°C)	37.5 (1.7)	37.5 (1.6)	37.6 (1.0)	0.913
Glucose (mMol/L)	5.1 (1.3)	4.9 (1.5)	4.8 (1.2)	0.544
Hematological Indices				
WBC (×10 ⁹ /μL)	11.2 (6.6)	11.7 (6.7)	13.3 (8.4)	0.144
Lymphocytes (×10 ³ /μL)	50.0 (19.2)	51.9 (17.4)	49.4 (16.7)	0.163
Monocytes (×10 ³ /μL)	8.9 (5.8)	8.8 (5.2)	13.0 (6.3)	0.007
Granulocytes (×10 ³ /μL)	41.0 (22.8)	38.0 (18.6)	35.6 (21.1)	0.124
Platelets (×10 ³ /μL)	163 (124)	140 (108)	158 (82)	0.129
Hemoglobin (g/dL)	6.9 (3.5)	6.2 (2.7)	5.2 (2.9)	0.009
Hematocrit (%)	22.0 (10.4)	20.5 (7.8)	17.7 (6.5)	0.012
RBC (×10 ⁶ /μL)	3.2 (1.8)	3.1 (1.5)	2.5 (1.6)	0.022
MCV (fL)	70.3 (12.3)	68.8 (11.8)	73.3 (11.4)	0.361
MCH (fL/cell)	22.6 (4.4)	22.2 (3.5)	22.1 (4.1)	0.899
MCHC (g/dL)	32.1 (2.6)	32.3 (2.3)	30.5 (4.1)	0.069
RDW	21.3 (4.7)	22.2 (5.2)	23.5 (7.6)	0.018
Parasitological Indices				
Parasitemia (/μL)	22,281 (51,064)	15,299 (37,040)	16,220 (43,127)	0.205
Geometric mean parasitemia (/μL) ^b	15,739	12,736	11,850	0.123
Genetic Variants				
Sickle cell trait, n (%) ^c	57 (14.1)	18 (16.1)	1 (4.3)	0.198
G6PD deficiency, n (%) ^{c,d}	25 (6.7)	13 (12.5)	3 (15.0)	0.084

Data are presented as median (interquartile range) and compared using the Kruskal-Wallis test unless stated otherwise.

^aDifferences in the proportion of gender were compared using Pearson's χ^2 test.^bGeometric mean parasitemia was compared using ANOVA.^cDifferences in the proportion of individuals with sickle cell trait (HbAS) and G6PD deficiency were compared using Pearson's χ^2 test.

^dG6PD deficiency was defined as hemizygous in males and homozygous in females, since it is an X-linked gene. Children were grouped as follows: HIV-1 (-)/Pf(+), negative reaction on the Determine® and Uni-Gold™ HIV-1 serology tests; HIV-1(exp)/Pf(+), positive reaction on one or both serology tests and a negative reaction for HIV-1 by PCR; HIV-1(+)/Pf(+), positive reaction on one or both serology tests and a positive reaction on two consecutive HIV-1 PCR assays three months apart. The number and species of asexual *Plasmodium* organisms per 300 white blood cells (WBC) were determined using Giemsa-stained thin and thick smear venous blood. Complete blood counts were determined in venous blood using a Coulter® AcT diff2™ (Beckman Coulter Corp.). Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC); and Red Blood Cell Distribution Width (RDW).

Table 2

Erythropoietic indices.

Characteristic	HIV-1(-)/Pf(+)	HIV-1(exp)/Pf(+)	HIV-1(+)/Pf(+)	P
Number of subjects	406	112	24	N/A
Reticulocyte Count (%) ^b	2.7 (4.1)	3.0 (3.9)	3.3 (6.7)	0.319
Absolute Reticulocyte Number ($\times 10^{12}/L$) ^a	0.065 (0.08)	0.064 (0.08)	0.066 (0.10)	0.966
RPI (μL) ^a	1.45 (1.90)	1.45 (1.94)	1.76 (2.66)	0.974
RPI<2, n (%) ^b	234 (61.6)	66 (62.9)	12 (54.5)	0.766

^aData are presented as median (interquartile range) and differences between the three groups were compared using Kruskal-Wallis test.

^bDifferences in the reticulocyte count (%) were compared using Pearson's χ^2 test. Reticulocyte production index (RPI) and absolute reticulocyte number (ARN) were calculated as follows: Reticulocyte Index (RI) = reticulocyte count $\times$ hematocrit / 30.7 (average hematocrit of children <5yrs of age in Siaya district); Maturation Factor (MF) = $1 + 0.05 (30.7 - \text{hematocrit})$; RPI = RI / MF; ARN = (RI $\times$ RBC count) / 100.

Table 3

Predictors of hemoglobin concentrations.

Variable	β -weight	Semipartial r^2	<i>P</i>	Block Δ statistics
Age	0.133	0.017	0.004	
HIV-1 Status	-0.113	0.013	0.014	
Block 1 Summary:				$R^2=0.033$ $P<0.001$
PCM	-0.415	0.155	<0.001	
PCN	0.071	0.005	0.102	
Block 2 Summary:				$R^2=0.159$ $P<0.001$

Hierarchical multiple regression analysis was performed to determine predictors of Hb levels. Age (mos.) and HIV-1 status [i.e., HIV-1(-) $\rightarrow$ HIV-1(exp) $\rightarrow$ and HIV-1(+)] were entered in block 1; and pigment-containing monocytes (PCM) and pigment-containing neutrophils (PCN) were entered into block 2. The full model was significant at $F(5, 512)= 18.748$, $P<0.001$, $R=0.441$, $R^2=0.194$.