Enhanced T cell activation in *Plasmodium falciparum* malaria-infected human immunodeficiency virus-1 patients from Mozambique

Helena Chavale[1,2], Joanna Reis Santos-Oliveira[3], Alda Maria Da-Cruz[3]/+, Sonia Enosse[1,2]

[1]Instituto Nacional de Saúde, Ministério da Saúde, Maputo, Moçambique [2]Laboratório de Biologia Molecular, Hospital Central de Maputo, Maputo, Moçambique [3]Laboratório Interdisciplinar de Pesquisas Médicas, Instituto Oswaldo Cruz-Fiocruz, Ministério de Saúde, Rio de Janeiro, RJ, Brasil

Human immunodeficiency virus (HIV)-1 infection has an important impact on malaria. Plasmodium falciparum *and HIV-1 co-infected patients (*Pf*/HIV) present with a high degree of anaemia, enhanced parasitaemia and decreased CD4^{+} T cell counts, which increase the risk of developing severe malaria. In addition, infection with either* Pf *or HIV-1 alone causes extensive immune activation. Our hypothesis was that lymphocyte activation is potentiated in* Pf*/HIV co-infected patients, consequently worsening their immunosuppressed state. To test this hypothesis, 22* Pf*/ HIV patients, 34 malaria patients, 29 HIV/AIDS patients and 10 healthy controls without malaria or HIV/acquired immune deficiency syndrome (AIDS) from Maputo/Mozambique were recruited for this study. As expected, anaemia was most prevalent in the* Pf*/HIV group. A significant variation in parasite density was observed in the* Pf*/HIV co-infected group (110-75,000 parasites/µL), although the median values were similar to those of the malaria only patients. The CD4^{+} T cell counts were significantly lower in the* Pf*/HIV group than in the HIV/AIDS only or malaria only patients. Lymphocyte activation was evaluated by the percentage of activation-associated molecules [CD38 expression on CD8^{+} and human leukocyte antigen-DR expression on CD3^{+} T cells]. The highest CD38 expression was detected in the* Pf*/HIV co-infected patients (median = 78.2%). The malaria only (median = 50%) and HIV/AIDS only (median = 52%) patients also exhibited elevated levels of these molecules, although the values were lower than those of the* Pf*/HIV co-infected cases. Our findings suggest that enhanced T-cell activation in co-infected patients can worsen the immune response to both diseases.*

Key words: malaria/HIV-AIDS - cellular activation - *Plasmodium* - co-infection - CD4^{+} T cells - Mozambique

As leading causes of morbidity and mortality, malaria and human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) are two of the most important diseases in Africa (Idemyor et al. 2007). *Plasmodium falciparum*-associated HIV-1 (*Pf*/HIV) infection has been described in many sub-Saharan Africa countries and data from these countries account for most of the available clinical data regarding this co-infection (Chalwe et al. 2009, Davenport et al. 2010, Kiyingi et al. 2010, Mills et al. 2010, Serna-Bolea et al. 2010, Naniche et al. 2011). A relevant issue in malaria-endemic regions is the identification of HIV/AIDS patients among suspected cases of acute malaria (Mills et al. 2010, Serna-Bolea et al. 2010). Furthermore, HIV-1 infection is a risk factor for receiving an incorrect diagnosis of malaria (Berg et al. 2008). Thus, surveillance health programs should invest considerable effort to minimise the misdiagnosis of febrile diseases.

In Mozambique (Mz), malaria is endemic throughout the country and is most prevalent after the rainy season, which lasts from December-April. Malaria and HIV/AIDS are the most important causes of mortality with rates of 29% and 27%, respectively (INS/INE/ICF 2010). According to the World Malaria Report (WHO 2011), approximately four million estimated cases and 3,300 deaths due to malaria were reported in Mz during 2010. Considering that 11.5% of population is estimated to be infected with HIV-1, the overlap of these dually endemic diseases increases for the risk of *Pf* and HIV-1 co-infection. Recently, a study of 136 HIV-1 infected individuals from southwest Mz revealed that 5.1% were also infected with *Pf* (Naniche et al. 2011).

There is evidence that *Pf*/HIV co-infection enhances the spread of both *Pf* malaria and HIV-1 infection and may also influence the severity of the clinical manifestations of these diseases. Indeed, HIV infection has been considered an important risk factor for severe *Pf* malaria (Grimwade et al. 2004, Chalwe et al. 2009). Increased *Plasmodium* parasitaemia is most likely related to the impairment of parasite control caused by HIV-1-related immunosuppression (Whitworth et al. 2000, Patnaik et al. 2005). Patients with low CD4^{+} T cell counts of less than 350 cells/mm^{3} are more likely to exhibit complications arising malaria (Cohen et al. 2005, Mouala et al. 2009). Moreover, HIV-1 infection is associated with an increased prevalence of anaemia in *Pf* malaria (Otieno et al. 2006, Davenport et al. 2010). Malaria can also affect HIV-1 infection. HIV/AIDS pa-

tients with malaria can exhibit a transitory reduction in the number of CD4+ T cells, which may be partially reversible after successful antimalarial therapy (Van Geertruyden et al. 2006a). A causal relationship between malarial episodes and a decline in CD4+ T cell counts in HIV-1 patients remains to be established (Mermim et al. 2006). Acute malaria elevates the HIV viral load (VL), which in turn can enhance the risk for HIV transmission (Kublin et al. 2005). In addition, *Plasmodium* antigens lead to strong cellular activation (Worku et al. 1997) which may facilitate *de novo* HIV-1 infection and replication (Froebel et al. 2004). The preference of HIV-1 for infecting activated memory CD4+ T lymphocytes can increase cell death (Grossman et al. 2002). Consequently, it is possible that, in co-infected patients, *Pf*-specific T-cell clones are depleted by HIV-1 during each malaria episode (Whitworth & Hewitt 2005, Mermim et al. 2006). Therefore, these factors can decrease the immune response to both diseases and contribute to HIV disease progression.

Cell activation is one of the crucial mechanisms in the pathogenesis of both malaria (Worku et al. 1997) and HIV-1/AIDS (Douek et al. 2009). The release of pro-inflammatory cytokines due to increased T cell activation during acute malaria has been implicated in the clinical complications and contributes to a disruption in the balance of effector/regulatory immune state (Ayimba et al. 2011, Davenport et al. 2012). In HIV-1/AIDS, it is well established that the VL is directly associated with CD8+ T cell activation (Benito et al. 2004). However, the mechanism by which the association of these two pathogens can impact the immunopathogenesis of malaria/HIV-AIDS remains under discussion. Few studies addressing the compromised immunological state have been reported for malaria/HIV-AIDS co-infection (Naniche et al. 2011) and, to our knowledge, none has investigated the consequences of cellular activation in the immunosuppression state. Because both *Pf* and HIV alone can cause immune activation, our hypothesis was that lymphocyte activation is potentiated in co-infected patients. As a consequence, activation-induced lymphocyte death could aggravate the immunosuppressed state of co-infected patients. For test this hypothesis, we evaluated the severity of anaemia, *Plasmodium* parasite density (PD) and impairment of the cellular immune response in malaria/ HIV-1 co-infected adults from southwest Mz.

PATIENTS, MATERIALS AND METHODS

Study population - All patients were recruited from the Polana Caniço Health Centre (PCHC) in Maputo, the capital of Mz, between January-December 2010. PCHC is an outpatient health care centre situated in a suburb of Maputo and provides medical care for patients from three neighbourhoods: Maxaquene, Polana Caniço and Hulene. Maputo is an area of stable malaria transmission, with a rate of ≥ one case per 1,000 inhabitants and a high percentage of individuals have a past history of malaria (73-76%). HIV-1 prevalence in Mz is 11.5%, affecting approximately 9,000 individuals each year (INE 2009). In PCHC, the malaria care service is responsible for the diagnosis of malaria and the dispensation of antimalarial drugs according to the malaria management guidelines established by Ministry of Health (MISAU)/Mz. This health centre also attends to and counsels HIV/AIDS patients.

A cross-sectional study was performed in which *Pf*/HIV-1 co-infected patients were recruited from patients presenting with clinical malaria. A total of 99 recruited participants were placed into the following four groups: 22 Pf/HIV, 29 patients infected with HIV-1 only (HIV-1/AIDS), 38 infected patients with *Pf* only (malaria) and a group of 10 subjects who were negative for both malaria and HIV-1 [healthy controls (HC)]. The demographic characteristics of the study subjects are shown in Table. Their ages ranged from 20-75 years (mean age - 38 ± 12.2; median - 37 years) and the majority of the participants were female (59.2%). Laboratory exams were performed to diagnose anaemia, malaria and HIV/AIDS according to the MISAU/Mz guidelines. The levels of haemoglobin (Hg) and white blood cells (WBC) were determined by a complete haemogram exam. The results were expressed as g/dL for Hg and $10^3/\mu L$ for WBC.

The patients were screened for malaria with a qualitative rapid diagnostic test (RDT) (SD Malaria Antigen Pf BIOLINE kit, Standard Diagnosis Inc, Giheung-ku,

TABLE

Demographic characteristics of study population

Cases/controls	Cases (n)	Age (years) mean ± SD[a] (median)	Distribution per gender	
			Male (40.7%) (%)	Female (59.2%) (%)
Pf/HIV co-infected	22	38.2 ± 15 (34)	36.4	63.6
Malaria	38	39.4 ± 13 (39)	31.6	68.4
HIV/AIDS	29	37 ± 10.8 (36)	51.7	48.3
Healthy controls[b]	10	34.5 ±7 (34)	50	50

a: no statistical difference among the four groups; *b*: subjects without malaria or human immunodeficiency virus (HIV)-1/acquired immune deficiency syndrome (AIDS); *Pf*/HIV: *Plasmodium falciparum* and HIV-1 co-infected patients; SD: standard deviation.

Republic of South Korea) to detect the histidine rich protein II of *Pf* in blood samples. After their consent, patients with a RDT positive test were screened for HIV according to the Mozambican National protocol. This protocol consists of a sequential algorithm of two immunochromatographic rapid tests. All individuals were first screened with the Determine HIV-1/2 test (Abbott Laboratories Co, Ltd, Tokyo Japan). All specimens that were reactive by this screening assay were further tested using the HIV test, Uni-gold™ Recombigen® (Trinity Biotech PLC, Bray Ireland). Patients over 18 years of age, who were infected with HIV-1, who did not receive antiretroviral (ARV) therapy and who had a positive RDT for malaria were recruited for the co-infected study group. Pregnancy tests were used to identify pregnant women that were excluded from the study. Patients who were positive for only malaria or HIV-1 infection were enrolled into either the malaria or HIV/AIDS groups, respectively. Volunteers who were negative for both tests comprised the HC group. HIV/AIDS patients were referred to the counselling program.

Determination of Plasmodium PD - The *Plasmodium* species identification and PD were determined in RDT positive patients. To determine the malarial parasite density, the finger was pricked and a thick blood smear was made. The blood smears were stained with Giemsa stain and were examined under oil-immersion microscope by trained laboratory technicians until had greater than 500 WBC or 500 parasites. The *Pf* PD was calculated after the actual leukocyte count was taken into consideration and was determined by the following formula: PD = (number of parasites/number WBC on each slide) x WBC per mL from each patient x 10^3. The results were expressed as the number of parasites/μL.

Determination of absolute T cells counts and lymphocyte activation levels - To quantify the number of CD4+ T and CD8+ T lymphocytes in the blood, BD Tritest® monoclonal antibodies specific for CD4, CD8 and CD3 conjugated to FITC, PE and PerCP, respectively and the BDTrue Count® reagent kit were used according to the manufacturer's instructions (BD Biosciences, Franklin Lakes, NJ, USA). In parallel, the blood cells were also stained with anti-CD8 FITC/anti-CD38 PE and anti-CD3 FITC/anti-human leukocyte antigen (HLA)-DR PE monoclonal antibodies (BD Simultest™, BD Biosciences, San Jose, CA, USA).

The samples were acquired using a FACSCalibur® flow cytometer (BD, USA) and analysed by Multiset® software for CD4 counting, whereas phenotypic analysis was performed with by CellQuest™ software. Both software programs were obtained from BD Biosciences. The experiments were performed at the centre of reference for AIDS diagnosis at the MISAU/Mz [Laboratory of Molecular Biology and Hematology at Maputo Central Hospital, Laboratory of Parasitology and Immunology National Institutes of Health (INS)] and in Brazil [Laboratory of Molecular Immunology and AIDS, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (IOC/FIOCRUZ), Ministry of Health].

Although the validated marker of CD8+ T cell activation is the co-expression of CD38 and HLA-DR, activated cells were identified by detecting CD38 on CD8+ T cells and HLA-DR on CD3+ T cells. These molecules were used as surrogates for the validated marker (Douek et al. 2009). HLA-DR positivity was determined on the CD3+ T cell population and CD38 positivity was determined on the CD8+ T cell population, inside the gate previously established for the CD3+ T cell population. The results were expressed as the percentage of HLA-DR on CD3+ and CD38 on CD8high T cells.

Statistical analysis - The values were expressed as the median and interquartile range (IQR). The Mann-Whitney *U* test and the non-parametric Spearman's test were used for the comparison and correlation analysis of the data, respectively (GraphPad Prism, version 4.0, San Diego, CA, USA). The differences were considered significant when p < 0.05.

Ethics - The study was approved by the Mozambican Ethical Committee (National Bioethics Committee for Health), under protocol 194/CNBS and written informed consent was obtained from all participants.

RESULTS

Impact of malaria-associated HIV-1/AIDS on the severity of anaemia - Hg levels were used to evaluate the degree of anaemia (Fig. 1). Co-infected patients had lower Hg levels (median = 10.1 g/dL, IQR - 8.4-11.2 g/dL) than did patients with *Pf* only (median = 12g/dL, IQR - 10.2-13.3 g/dL, p < 0.001) or HIV-1/AIDS only (median = 11.4 g/dL, IQR - 11.2-12.6 g/dL, p < 0.05) or the HC group (median = 12.8, IQR - 11.5-14 g/dL n = 10, p < 0.01) (Fig. 1). No significant differences were observed between the malaria and HIV-1/AIDS only patients or between the HC and the malaria or HIV-1/AIDS only patients.
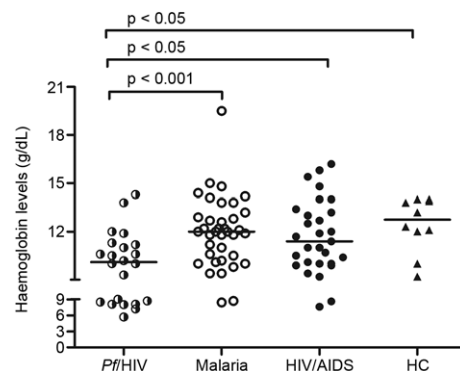

Fig. 1: anaemia severity estimated by haemoglobin concentration in *Plasmodium falciparum* and human immunodeficiency virus (HIV)-1 co-infected (*Pf*/HIV)/acquired immune deficiency syndrome (AIDS) patients. *Pf*/HIV co-infected patients (black and white circles), *Pf* malaria only patients (white circles) and HIV-1/AIDS only patients (black circles) and healthy controls (HC) without malaria or HIV-1/AIDS (solid triangles). Each point represents one subject. The horizontal bars express median.
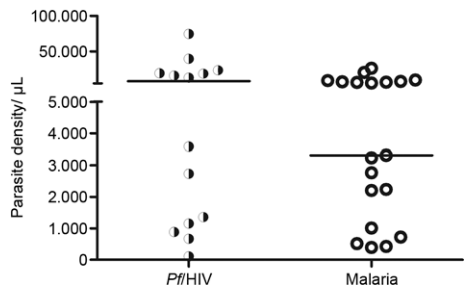
Fig. 2: determination of *Plasmodium falciparum* (*Pf*) parasite density (PD) in human immunodeficiency virus (HIV)-1 co-infected patients. The blood smears were stained with Giemsa stain and the parasites were counted until the observed fields had reached greater than 500 white blood cells or 500 parasites. The PD was calculated according to the formula shown in Patients, Materials and Methods section, considering individual actual leukocytes counts per mL. Black and white circles: *Pf*/HIV co-infected patients; white circles: *Pf* malaria only patients. Each point represents one subject. The horizontal bars express median. There was no statistic difference between groups.
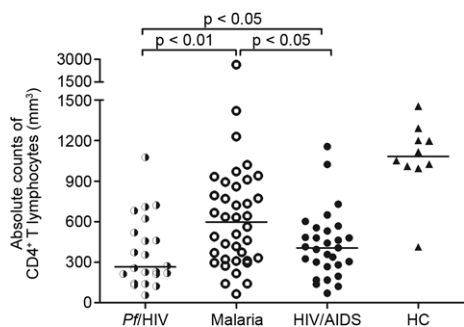

Fig. 3: evaluation of immunosuppressed state in malaria associated human immunodeficiency virus (HIV)-1/acquired immune deficiency syndrome (AIDS) patients by the absolute number of CD4+ T lymphocytes. Black and white circles: *Plasmodium falciparum* (*Pf*)/HIV co-infected patients; black circles: HIV-1/AIDS only patients; solid triangles: healthy controls (HC) without malaria or HIV-1/AIDS; white circles: *Pf* malaria only patients. Each point represents one subject. The horizontal bars express median.

PD in Pf/HIV - Fifty-nine patients were positive for malaria by RDT; however the PD quantification was possible for only 33 of these patients (Fig. 2). There was no significant difference in the PD of the co-infected patients (median = 8,326 parasites/μL, IQR - 1,087-20,357 parasites/μL, n = 14) compared to the malaria only patients (median = 3,308 parasites/μL, IQR - 1,013-8,050 parasites/μL, n = 19). Although, high levels of parasitaemia were observed in both groups, a wider variation in the parasite load was observed in the co-infected patients (110-75,000 parasites/μL).

Malaria-associated HIV-1 co-infected patients present with low CD4+ T lymphocytes levels - To determine whether the malaria association may influence the immunosuppression caused by HIV-1 infection, the blood levels of CD4+ T lymphocytes were quantified (Fig. 3). Significantly lower CD4+ T values were observed in the Pf/HIV co-infected patients (median = 265 cells/mm³, IQR - 195-545 cells/mm³, n = 22) than in the HIV-1/AIDS patients (median = 406 cells/mm³, IQR-274-543 cells/mm³, n = 29, $p < 0.05$). These levels were also lower than those observed in the malaria patients (median = 597 cells/mm³, IQR - 318-868 cells/mm³, n = 38, $p < 0.01$) or the HC group (median = 1,084 cells/mm³, IQR - 1,007-1,224 cells/mm³, n = 10, $p < 0.001$). In addition, HIV-1/AIDS patients also exhibited reduced CD4+ T cell levels compared to malaria patients ($p < 0.05$) or HC ($p < 0.001$). A positive correlation between CD4+ T cell counts and Hg levels were observed when all groups were evaluated together (r = 0.40, $p < 0.001$) (data not shown).

The association of malaria plus HIV-1 enhances the T cell activation state - To determine whether *Pf* infection may contribute to augmentation T cell activation in Pf/HIV co-infected patients, the percentage of activated CD3+ and CD8+ T cells was measured by flow cytometry using the expression of the activation-associated membrane molecules HLA-DR and CD38, respectively.
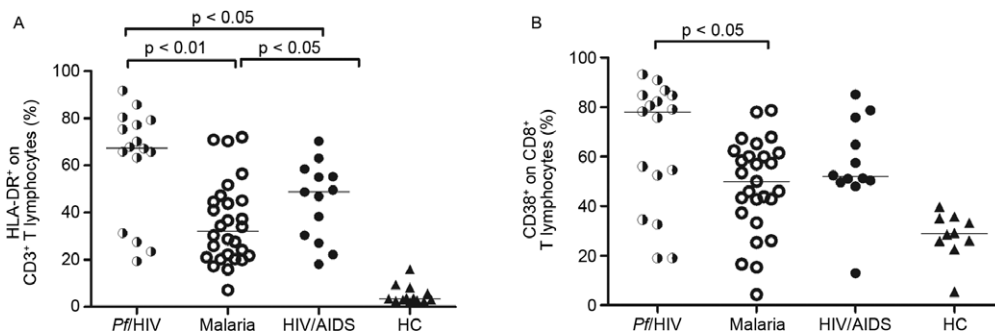

Fig. 4: T lymphocyte activation state in *Plasmodium falciparum* and human immunodeficiency virus (HIV)-1 co-infected (*Pf*/HIV)/acquired immune deficiency syndrome (AIDS) patients. Black and white circles: *Pf*/HIV co-infected patients; black circles: HIV-1/AIDS only patients; solid triangles: healthy controls (HC) without malaria or HIV-1/AIDS; white circles: *Pf* malaria only patients. The flow cytometry analysis of the percentage of human leukocyte antigen-DR on CD3+ T (A) and CD38 on CD8+ T (B) cells were determined in lymphocyte gates as described in Patients, Materials and Methods section. Each point represents one subject. The horizontal bars express median.

Pf/HIV co-infected patients presented with the highest levels of lymphocyte activation compared to the other groups (Fig. 4A). The levels of HLA-DR$^+$ on the CD3$^+$ T cells from the *Pf*/HIV patients were significantly higher (median = 67.5%, IQR - 39.3-77.8%, n = 17) than on T cells from the malaria only patients (median = 32.1%, IQR - 21.2-45%, n = 29, p < 0.001) or the HIV-AIDS only patients (median = 48.8%, IQR - 28.8-57%, n = 13, p < 0.05). Notably, the malaria patients also exhibited high levels of HLA-DR$^+$ on CD3$^+$ T cells, although the levels were lower than those observed on cells from HIV-1/AIDS patients (p < 0.05).

With regard to the percentage of CD38$^+$ on CD8$^+$ T lymphocytes (Fig. 4B), the state of cellular activation was also more elevated in the *Pf*/HIV co-infected group (median = 78.2%, IQR - 43.5-85%, n = 17) than in the malaria only patients (median = 50%, IQR - 37.3-61.5%, n = 29, p < 0.01). However, no significant difference was observed with regard to the HIV-1/AIDS only group (median = 52%, IQR - 49.8-73.1%, n = 13). Elevated levels of activation were observed in the malaria and HIV-1/AIDS only patients and there were no difference between these groups. As expected, the lowest levels of cellular activation were observed in the HC group (HLA-DR$^+$, median = 3.4%, IQR - 2.6-7% and CD38$^+$, median = 28.9%, IQR - 25-35.3%).

To determine if the parasitaemia is involved in the cellular activation of *Pf*/HIV co-infected patients, a correlation analysis was performed. For this analysis 17 *Pf*/HIV co-infected patients and 29 malaria only patients were studied. No significant correlation between PD and the percentage of HLA-DR$^+$ and CD38$^+$ on the T lymphocytes was observed for either group. By contrast, a negative correlation was observed between the CD4$^+$ T cell counts and HLA-DR on the CD3$^+$ T lymphocytes when malaria, HIV-AIDS, Pf/HIV co-infected patients and the HC group were considered together (r = -0.70, p < 0.01) (Fig. 5).
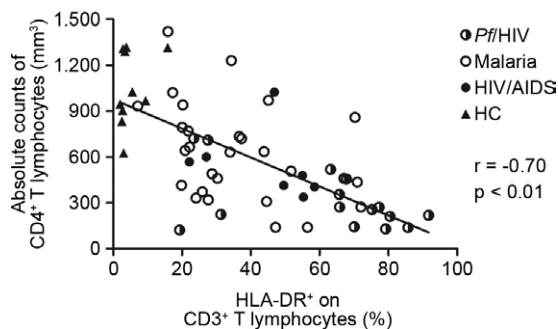

Fig. 5: correlation between CD4$^+$ T cell counts and human leukocyte antigen (HLA)-DR$^+$ on CD3$^+$ T lymphocytes in *Plasmodium falciparum* and human immunodeficiency virus (HIV)-1 co-infected (*Pf*/HIV)/acquired immune deficiency syndrome (AIDS) patients. Black and white circles: *Pf*/HIV co-infected patients; black circles: HIV-1/AIDS only patients; solid triangles: healthy controls (HC) without malaria or HIV-1/AIDS; white circles: *Pf* malaria only patients.

DISCUSSION

Most of the available data regarding malaria-associated HIV-AIDS is related to the consequences of HIV infection on the degree of anaemia, *Plasmodium* PD and CD4$^+$ T lymphocyte counts in *Pf* malaria patients, particularly in sub-Saharan Africa countries (Whitworth et al. 2000, Cohen et al. 2005, Patnaik et al. 2005, Mouala et al. 2009, Davenport et al. 2010). However, the impact of both infections on the state of immune cellular activation is largely unknown. This study investigated whether co-infection had a synergistic effect on the immune activation induced by *Plasmodium* or HIV-1 alone. Our results suggest that *Pf* malaria enhances T lymphocyte activation in individuals concomitantly infected with HIV-1. This phenomenon can affect immune effector function and may constitute an additional mechanism that contributes to the pathogenesis of malaria-associated HIV-1/AIDS disease.

Anaemia is a major symptom of malaria and can be caused by several mechanisms, including the destruction of infected erythrocytes, antibody-mediated lysis, hypersplenism, impaired erythropoiesis and cytokines (Idemyor et al. 2007, Haldar & Mohandas 2009, Quintero et al. 2011). In a previous study, 10% of adults with severe malaria had Hg levels of 7 g/dL or less, which is much lower than expected for a healthy individual (WHO 2000). Moreover, HIV-1 infection itself can also cause anaemia (Belperio & Rhew 2004) and constitutes an important risk factor for severe malaria in adults (Grimwade et al. 2004, Chalwe et al. 2009). In our study, anaemia was present at a higher degree in *Pf*/HIV co-infected patients, since they had lower Hg levels than the malaria only patients. This difference does not seem to be related to gender as the percentage of females was higher than males in both groups. In addition, the Hg concentration and PD are not correlated in the *Pf*/HIV co-infected patients or the malaria only patients. These results are consistent with other studies of malaria transmission in sub-Saharan Africa areas, such as Cameroon (Nkuo Akenji et al. 2002) and Kenya (Davenport et al. 2010). However, conflicting results regarding anaemia have also been observed in Kenya (Mc Elroy et al. 2000) and Tanzania (Ekval et al. 2001). As has been shown for other conditions, such as malnutrition, ARV drugs use or infection with intestinal parasites, co-infection with HIV and malaria can worsen the anaemic condition, particularly in low income regions.

Immunosuppression caused by HIV infection appears to impair the ability to control the parasitaemia, resulting in an enhancement of the *Plasmodium* PD (Van Geertruyden et al. 2006b). Despite reports that HIV individuals are more likely to be parasitaemic (Whitworth et al. 2000, Patnaik et al. 2005), there was no significant difference in the parasite load between the *Pf*/HIV co-infected patients and the malaria only patients is this study. It is important to note that, in our study, the parasitaemia intensity calculation was based on the individual leukocyte count per mL instead of a fixed number of leukocytes per mL. Notably, a wide range of PD levels was detected in the *Pf*/HIV co-infected group, varying from 110-75,000 parasites/µL.

Depletion of CD4$^+$ T cells is a common consequence of HIV-1 infection (Grossman et al. 2002). However, acute malaria only also reduces the number CD4$^+$ T lymphocytes (Chougnet et al. 1992, Kassa et al. 2006). Among the patients evaluated in this study, the lowest CD4$^+$ T values were observed in the co-infected patients. The number of CD4$^+$ T cells in the malaria or HIV-1/AIDS only patients was also lower than in the HC group. These results suggest a heightened degree of immunosuppression in malaria-associated HIV-1/AIDS. Interestingly, this reduction in CD4$^+$ T cells is transient and the levels of these cells increase after successful antimalarial therapy, although the number of CD4$^+$ T cells does not reach the previous counts (Kassa et al. 2006, Van Geertruyden et al. 2006a. Therefore, each episode of acute malaria causes an excess decrease in the CD4$^+$ T cell numbers (Mermim et al. 2006), which aggravates the immunosuppressed state. These facts highlight the importance of assessing any malaria-induced disturbances in the laboratory prior to deciding how to manage HIV positive individuals. This approach can avoid the misdiagnosis of ARV failure or even the premature initiation of therapy (Brentlinger et al. 2007). Unfortunately, access to ARV is not yet universal, either in Mz (INSIDA 2009) or in other African countries, whereas antimalarials drugs are currently accessible for the population.

CD4$^+$ T cell counts have been implicated in *Plasmodium* control (Brentlinger et al. 2007, Idemyor 2007), because these cells are negatively correlated with PD (Withworth et al. 2000, Patnaik et al. 2005). However, we did not observe any association between these parameters, perhaps because of the study sample size. Moreover, because the adult population evaluated in this study is from a stable transmission area, these adults are considered immune and therefore, the level of parasitaemia should be lower than in children from the same area, potentially explaining the lack of correlation between the PD and immunosuppression.

Chronic immune activation is the strongest predictor of the progression rate to AIDS in HIV-1 infected individuals (Douek et al. 2009). Acute malaria infection may also be associated with an enhanced cellular activation state. Co-expression of CD38/HLA-DR on T cell subsets has previously been studied in both HIV-1/AIDS or malaria alone (Hunt et al. 2003, Eggena et al. 2006, Naniche et al. 2011) and immune activation was considered a predictor of treatment failure in malaria patients (Eggena et al. 2006). Malaria infection can present with a pro-inflammatory response and cytokine imbalance (Ayimba et al. 2011, Davenport et al. 2012). In this study, *Pf*/HIV co-infected patients exhibited the highest percentages of HLA-DR and CD38 on the CD3$^+$ and CD8$^+$ T lymphocytes, respectively. Indeed, malaria patients had higher percentages of CD38 on CD8$^+$ and HLA-DR on CD3$^+$ T cells than did the healthy subjects, confirming previous data that malaria itself contributes to activation (Worku et al. 1997). Although dual-positive CD38/HLA-DR on CD8$^+$ T cells is a more accurate measure of the activation state, when both molecules were analysed separately, they displayed the same profile. Similar to that observed for the CD4$^+$ T counts, the viral or parasite antigens ap-

pear to act together to boost cell activation, either directly or by inducing the release of soluble factors. HIV-1 co-infection with other diseases, such as leishmaniasis (Santos-Oliveira et al. 2010), tuberculosis (Rodrigues et al. 2003) or leprosy (Giacoia-Gripp et al. 2011), has previously been demonstrated to increase cellular activation. A previous study conducted in Mz did not identify a significant correlation between the "malaria infection" parameter and the levels of HLA-DR/CD38 on activated CD4$^+$ and CD8$^+$ T cells (Naniche et al. 2011). However, the authors of this study did not compare *Pf*/HIV co-infected patients to malaria or HIV/AIDS only patients. We cannot exclude the possibility that dual-infected *Pf*-HIV patients could be in more advanced stages of HIV/AIDS and/or have more opportunistic infections, which could contribute to decreased CD4$^+$ T cell counts and increased T cell activation.

Viral replication is a well-known factor that contributes to lymphocyte activation and is considered a predictor parameter for HIV VL (Benito et al. 2004). Nevertheless, one limitation of our study is that we did not determine the HIV-1 VL, because this assessment is not routinely available for African public health systems (INSIDA 2009). This study did not find a causal relationship between *Pf* PD and T cell activation. However, malarial antigens cannot be ruled out as an important factor for immune activation. Therefore, the role of each infection in this exacerbated activation state and the additional implications for the pathogenesis and management of malaria/HIV-1 co-infection must be clarified. In addition, we did not follow-up the *Pf*/HIV co-infected patients during malaria, their convalescence to evaluate whether the T-cell activation state decreased and achieved the activation levels observed in the malaria or HIV-1/AIDS only patients.

Our results demonstrate that the anaemia, *Pf* parasitaemia and CD4$^+$ T counts profiles of malaria-HIV-1 infected patients from southwest Mz are similar to those observed in other sub-Saharan Africa studies (Patnaik et al. 2005, Van Geertruyden et al. 2006a, Davenport et al. 2010). Complementary to these studies, our findings suggest that malaria/HIV-1-AIDS association may contribute to enhanced T cell activation in co-infected patients. This phenomenon is of utmost importance because these cells can (i) augment the release of proinflammatory cytokines causing systemic damage and (ii) be more susceptible to die by activation-induced cell death (Kemp et al. 2002). Finally, potential regional differences are expected to occur depending on the malaria endemicity. Additional studies in sub-Saharan Africa countries, including prospective follow-up are of extreme importance to better understand the relationship between malaria and HIV-1 infection and the consequences of these infections on the immunopathogenesis of malaria/HIV-1 co-infection.

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