Cells and mediators of inflammation (C-reactive protein, nitric oxide, platelets and neutrophils) in the acute and convalescent phases of uncomplicated *Plasmodium vivax* and *Plasmodium falciparum* infection

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*The haematological changes and release of soluble mediators, particularly C-reactive protein (CRP) and nitric oxide (NO), during uncomplicated malaria have not been well studied, especially in Brazilian areas in which the disease is endemic. Therefore, the present study examined these factors in acute (day 0) and convalescent phase (day 13) patients infected with *Plasmodium falciparum* and *Plasmodium vivax* malaria in the Brazilian Amazon. Haematologic parameters were measured using automated cell counting, CRP levels were measured with ELISA and NO plasma levels were measured by the Griess reaction. Our data indicate that individuals with uncomplicated *P. vivax* and *P. falciparum* infection presented similar inflammatory profiles with respect to white blood cells, with high band cell production and a considerable degree of thrombocytopenia during the acute phase of infection. Higher CRP levels were detected in acute *P. vivax* infection than in acute *P. falciparum* infection, while higher NO was detected in patients with acute and convalescent *P. falciparum* infections. Although changes in these mediators cannot predict malaria infection, the haematological aspects associated with malaria infection, especially the roles of platelets and band cells, need to be investigated further.*

Key words: malaria - C-reactive protein - nitric oxide - band neutrophils

Haematologic changes, such as anaemia, thrombocytopenia and leucocytosis or leucopenia, are well recognised to be associated with malaria infection. The extent of these alterations varies with the level of malaria endemicity, patient genetic background (e.g., presence of haemoglobinopathies), nutritional status, demographic factors and malaria immunity (Price et al. 2001, Erhart et al. 2004). Changes in the total white cell (WC) and platelet (PLT) counts have been documented in several clinical *vivax* and *falciparum* malaria cases in immune and naïve patients of all ages (Martelo et al. 1969, Winters & Murray 1992, Ladhani et al. 2002, Taylor et al. 2008) and the disease is often associated with mild or moderate thrombocytopenia in adults and children from malaria-endemic areas. Profound thrombocytopenia is unusual and rarely associated with haemorrhagic manifestations (Casals-Pascual et al. 2006). In most clinical studies, thrombocytopenia is not associated with disease severity or mortality, but the combination of a low PLT count and high density of polymorphonuclear cells indicates a strong inflammatory profile. In this inflammatory milieu, neutrophils are one of the key players in the innate immune system and represent the first line of defence against invading microorganisms, mainly because they are the most numerous blood leucocyte cells (50-75%) and the most effective circulatory phagocytes. Neutrophils are generated in the bone marrow from myeloid human precursors cells by a highly coordinated differentiation process, which is characterised by significant morphological changes and the up-regulation of signalling pathways and receptors for inflammatory mediators (Mora-Jensen et al. 2011). Consequently, the neutrophil precursor count is also an important indicator of inflammation (Zhu et al. 2010, Corti et al. 2012) and variations in this parameter are routinely measured in the laboratory in malaria endemic areas as a sensitive, but nonspecific indicator of infection with malaria parasites (Maina et al. 2010, Habeeb et al. 2012).

In addition to cytokines and chemokines, other unspecific mediators of inflammation are also biomarkers in malaria infection. Several soluble mediators released during malaria infection, particularly C-reactive protein (CRP) and nitric oxide (NO), were identified as important inflammatory biomarkers (McGuire et al. 1996, Armah et al. 2007, Conroy et al. 2011). In fact, NO and CRP levels have proven to be valuable in assessing the severity of malaria and as prognostic tools in the follow-up re-
sponse to treatments (Gillespie et al. 1991, Nahreivanian et al. 2008). CRP is believed to play an important role as an early defence system against infection in the body; during acute inflammation, CRP levels increase to as much as 50,000 times above normal, typically within 6 h and peak at 48 h. The CRP level is an accurate indicator of inflammation and the only known factor to interfere with CRP production is liver failure. CRP is well characterised as a serum acute phase protein and has been shown to be involved in multiple immunoregulatory functions. For example, CRP activates the classical complement cascade, opsonises bacteria for phagocytosis and stimulates phagocytic cells (Dong & Wright 1996). Although CRP is predominantly produced and secreted by hepatocytes, other cells, including subsets of lymphocytes, Kupffer cells and blood monocytes, have also been shown to synthesise this protein (Dong & Wright 1996). The first identification of a role for CRP in defence against infection in humans was related to its capacity to bind to phosphocholine in membranes of microorganisms (Gotschlich & Edelman 1967). In malaria, CRP secretion is induced by proinflammatory cytokines that are secreted by host mononuclear cells (Harraz et al. 1992) and strong correlations have been found between CRP levels and parasitaemias. Meanwhile, NO, which is highly toxic to intra-erythrocytic malaria parasites, seems to be associated with the severity of Plasmodium falciparum malaria. Indeed, the high NO production by neutrophils has been correlated with fast parasite clearance in P. falciparum malaria (Greve et al. 1999), suggesting that the release of nonspecific inflammatory markers might be more important for immune protection from malaria than is generally appreciated.

Although the basic haematologic changes in non-specific markers of infection and inflammation associated with malaria infections are not recent findings, their contributions during acute uncomplicated malaria and after treatment remain unclear. Furthermore, the diagnostic value of these haematological parameters has not been well established in Brazilian endemic areas, in which more than 80% of malaria cases are caused by Plasmodium vivax (Oliveira-Ferreira et al. 2010). Therefore, the present study examined the changes in blood PLTs, neutrophils, CRP and NO in adults from the Brazilian Amazon with uncomplicated P. falciparum and P. vivax malaria who were followed up during the acute and convalescent phases of infection.

SUBJECTS, MATERIALS AND METHODS

Study population - The studied population included rainforest natives and migrants from several non-endemic areas of Brazil who resided in the region. Samples and survey data were collected during the dry months of June-August in 2007, coinciding with the period of increased malaria transmission in the state of Rondônia. The individuals who sought malaria diagnosis at the Polyclinic Ana Adelaide outpatient clinic in Porto Velho and tested positive by thick blood smear were formally invited to participate in our study. A total of 71 symptomatic malaria patients were enrolled and blood samples were collected on the day of diagnosis before treatment (acute phase) and 15 days later (convalescent phase). Of the 71 initially enrolled patients, 55 returned for the 15-day follow-up. The patients were grouped by plasmodial species: P. vivax (n = 47) and P. falciparum (n = 24). Written informed consent was obtained from all adult donors or from the parents of underage donors. The study was reviewed and approved by the Oswaldo Cruz Foundation Ethical Committee and the National Ethical Committee of Brazil.

Clinical and epidemiological survey - To evaluate the epidemiological factors that may influence the variations in the inflammatory markers studied and the symptoms, all donors were interviewed after providing informed consent. The survey included questions related to demographics, time of residence in the endemic area, personal and family histories of malaria, use of malaria prophylaxis, presence of malaria symptoms and personal knowledge of malaria. The survey data were entered into a database created with Epi Info 2007 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Collection of human blood samples and malaria diagnosis - Venous peripheral blood (10 mL) was collected into ethylenediamine tetracetic acid tubes. Thin and thick blood smears from all donors were examined for malaria parasites at 1,000X magnification under oil immersion; all slides were examined by two researchers with expertise in malaria diagnosis. Donors who were positive for P. vivax and/or P. falciparum were subsequently treated with the chemotherapeutic regimen recommended by the Brazilian Ministry of Health. To confirm the parasitological diagnosis (day 0) and parasite clearance (day 15), we performed molecular analyses of all samples using primers specific for genus (Plasmodium sp.) and species (P. falciparum and P. vivax). The amplification protocols were described previously (Snounou et al. 1993).

PLT and neutrophil counts - Complete blood counts were performed using an automatic haematology analyser (ABX Pentra) and peripheral smears of blood samples were made for routine differential blood cellular quantification. A manual differential WC count was also performed to distinguish the immature neutrophils. Neutropenia was defined as a neutrophil count < 1.20 × 10⁹/L and thrombocytopenia was defined as a PLT count < 150 × 10⁹/L.

CRP levels - The CRP levels were determined in all plasma samples using an in-house ELISA. Microtitre plates (Nunc/MaxiSorp, Rochester, NY, USA) were coated with a goat anti-human-CRP antibody (Sigma, USA; catalogue C8284) in carbonate-bicarbonate buffer (TCO₂) overnight at 4°C. The plates were then washed three times with phosphate-buffered saline-0.05% Tween 20 (PBST) and plasma samples diluted 1:500 in PBST were incubated with the plates for 1 h at 37°C. The plates were then washed three times and incubated with rabbit anti-human-CRP antibody (Sigma, USA; catalogue C3527) in PBST for 1 h at 37°C. The plates were washed three times and peroxidase-conjugated goat anti-rabbit-IgG antibodies (Sigma, USA; A0545) were added.
The wells were thoroughly washed to remove all unbound horseradish peroxidase (HRP)-conjugated antibodies and an o-phenylenediamine substrate solution was added to each well. The enzyme (HRP) and substrate were allowed to react for a short incubation period. The enzyme-substrate reaction was terminated by the addition of 2 N H₂SO₄ and the degree of colour change was measured at 492 nm ± 2 nm in a spectrophotometer (SpectraMax 250; Molecular Devices, Sunnyvale, CA). The plasma concentration of CRP was determined by comparison to standard concentrations of purified human CRP (Sigma, St. Louis, USA). The range of detection of CRP was 0.01-320 µg/mL. Sera from non-infected individuals were used on every plate as negative controls. Specific CRP optical density values were converted to concentration values (µg/mL) using sigmoidal curve-fit equations derived from CRP standard curves.

**Griess microassay detection of NO** - A modified Griess reaction was used to detect NO (Rockett et al. 1994, modified by Nahrevanian & Dascombe 2001). The NO levels in samples were indirectly measured after first converting nitrates to nitrates with a nitrate reductase treatment (Aspergillus species NAD[P]H, Sigma, UK) and NADPH β-nicotinamide adenine dinucleotide phosphate (Sigma Diagnostics, St. Louis, USA). Griess reagent [5% phosphoric acid, 1% sulphanilic acid and 0.1% N-(1-naphthyl-1)-ethylenediamine dihydrochloride, all from Sigma, UK, dissolved in 100 mL deionised water] was added and proteins were subsequently precipitated by trichloroacetic acid (BDH, England). The tube contents were mixed and centrifuged (Eppendorf centrifuge 5415 C, Germany); two samples of each supernatant were transferred to a flat-bottomed microplate and their absorbencies were read at 520 nm using a microplate reader (SpectraMax, Molecular Devices Inc). NO values were calculated from standard calibration plots (Nahrevanian & Dascombe 2001).

**RESULTS**

**Epidemiological and clinical characteristics of the studied population** - The mean age, length of time living in the endemic area, number of previous malaria infections and parasite density were similar between the *P. vivax* and *P. falciparum* malaria patient groups (Table). There were no significant differences in the general features of malaria exposure in the patient groups and malaria-endemic control group (group C), except for the number of months since the last malaria episode (p < 0.0001). All patients in the acute phase of infection (day 0) presented symptomatic uncomplicated malaria. Fever and headache were the most frequent symptoms reported by patients infected with either parasite, indicating that the plasmodial species did not show differences in the clinical manifestation of disease (p > 0.05 for all reported symptoms). Among the patients who returned at day 15, 87.5% had been diagnosed with *P. vivax* at day 0 and 62% had been diagnosed with *P. falciparum*. At day 15, no patients presented with symptoms and no parasites were detected by microscopic examination or polymerase chain reaction.

**PLTs** - The PLT counts were significantly lower in the acute phase of malaria infection than in the convalescent phase [day 0 vs. day 15; t = 10.74, degrees of freedom = 54, p < 0.001] or in the control group (p < 0.0001). Indeed, thrombocytopenia (less than 150,000 PLTs/mm³) was observed in 44% of studied individuals in the acute phase, regardless of *Plasmodium* species (47% for *P. falciparum* and 42.6% for *P. vivax*). After treatment, the mean PLT levels returned to normal (292,208/mm³), with levels similar to that of the control group (Fig. 1). The PLT number did not correlate with any epidemiological or clinical parameter evaluated in our survey; however, the mean parasitaemia of individuals who presented thrombocytopenia (5,077 parasites/mL) was significantly higher than in individuals without thrombocytopenia (3,331 parasites/mL; t = 2.271, p = 0.0422). Interestingly, this phenomenon was observed only in *P. vivax*-infected individuals (6,328 vs. 3,326 parasites/mL; t = 2.297, p = 0.0263) and not in *P. falciparum*-infected individuals (2802 vs. 3343; t = 0.3659, p = 0.8171).

**Neutrophil and band cell variations in the acute and convalescent phases of malaria** - The mean neutrophil count in the control group (3.825 ± 1.081) was similar to the acute (3.503 ± 1.515; p = 0.3350) and convalescent phases (3.035 ± 1.433; p = 0.3111). The absolute number of neutrophils was not correlated with any epidemiological factor, but it was inversely correlated with parasitaemia in both *P. falciparum* and *P. vivax*-infected individuals (r = -0.284, p = 0.017, respectively). Interestingly, 71% of the studied individuals presented high numbers of band cells (immature neutrophils). Indeed, the absolute number of band neutrophils was markedly high in the acute phases of the *P. vivax* (207.2 ± 326.4/µL) and *P. falciparum* (284.1 ± 390.0/µL) groups; in the convalescent phase, the band cell number returned to a level similar to that of the control group (12.1 ± 28.0/µL) (Fig. 2B). The number of band cells was not correlated with parasitaemia in the acute phase. However, band cell number was inversely correlated with the number of previous malaria infections (r = -0.258; p = 0.0310) and directly correlated with the number of months since the last malaria infection (r = 0.265; p = 0.039).
CRP - During the acute phase, an increased concentration of CRP was observed in 87% of studied individuals compared to the control group (cut-off = mean + 3 standard deviation). The overall mean concentration during the acute phase of infection (day 0) was 27-times higher (27.82 ± 30.94 µg/mL) than that in the control group (2.2 ± 4.49 µg/mL, p < 0.0001), whereas in the convalescent phase (day 15), the CRP concentration dropped to basal levels (3.0 ± 2.53 µg/mL) (p < 0.0001) comparable to those of the control group (p = 0.826) (Fig. 3A). Interestingly, acute phase CRP levels were also higher in P. vivax patients (mean ± standard error of the means; 30.56 ± 4.5 µg/mL) than in P. falciparum patients (18.2 ± 4.5 µg/mL; p = 0.0273). However, CRP levels were not correlated with any of the clinical or epidemiological factors considered in our analysis.

NO - The overall median levels of plasma nitrite in the acute phase were significantly higher in P. falciparum-infected individuals (7.01 ± 5.90 µM) than in P. vivax-infected individuals (3.1 ± 2.4 µM; p = 0.0014) or the control group (2.99 ± 2.60 µM; p = 0.0292). No differences in the nitrite levels of all groups were observed between the convalescent phase and acute phase. However, the levels were higher in the P. falciparum group than the P. vivax (p = 0.0003) and control (p = 0.0029) groups. We did not observe any correlation between the nitrite plasma levels and symptoms, number of past malaria episodes, time of residence in the endemic area or number of months since the last malaria infection. However, the serum nitrite concentration was inversely correlated with the number of malaria infections in the past six months (r = -0.390 p = 0.030).

DISCUSSION

In the present work, we detected classical inflammatory biomarkers (cells and mediators) in uncomplicated malaria patients infected with P. falciparum or P. vivax during the acute (day 0) and convalescent phases (day 15). In our cohort, the P. vivax and P. falciparum groups were comparable in gender, age, time of malaria exposure and number of past malaria infections. The cohort included rainforest region natives and transmigrants from non-endemic areas of Brazil who had lived in the region for more than 10 years and the majority of studied individuals reported a prior experience with P. vivax or P. falciparum malaria. All patients presented uncomplicated malaria and exhibited general clinical symptoms, such as a history of fever and headache, regardless of the Plasmodium species detected. Indeed, our control group, which was composed of exposed individuals who reported no malaria episodes in the last five years, was similar to the infected cohorts. The selection of these individuals as controls was ideal to distinguish whether the alterations found in cells and mediators were related to malaria infection or the social and health conditions of the native Amazon population.

Malaria is a complex multi-system disorder involving several inflammatory mediators. Monocytes, macrophages, endothelial cells, neutrophils and PLTs all express pattern recognition receptors, primarily toll-like receptors (Akira & Takeda 2004). Of these, PLTs and neutrophils are the most numerous, mobile and likely to encounter merozoites and parasite-altered erythrocytes early in infection. Therefore, one of the haematological hallmarks of acute malaria is thrombocytopenia. Despite the frequent occurrence of severe bleeding in severe malaria or co-infection (Abbasi et al. 2009), malaria-related thrombocytopenia is usually observed at frequencies ranging
from 24-94% (Lacerda et al. 2011). Moreover, it is unclear whether this haematological complication is more frequent in *P. vivax* or *P. falciparum* malaria. In our study, low PLT counts were present in a large portion of acute phase patients independent of the plasmodial species, but PLTs returned to basal levels in the convalescent phase. Among *P. vivax*-infected individuals, parasitaemia was significantly higher in individuals with confirmed thrombocytopenia than in non-thrombocytopenic individuals. The trend of decreasing PLT count with increasing levels of parasitaemia observed in this study has been extensively described in *P. falciparum* over the past several decades (Perrin et al. 1982, Rojanasthien et al. 1992, Richards et al. 1998), but it is less documented in the literature for *P. vivax* (Erhart et al. 2004). Thrombocytopenia in uncomplicated malaria appears to be immune mediated; the immune complexes circulating in malaria-infected patients may play a role in the peripheral destruction of PLTs and RBCs. Indeed, the phagocytosis of PLTs has also been reported in malaria patients. However, electron microscopy analyses have also demonstrated a direct interaction between plasmidium and PLTs in patients with malaria (McMorran et al. 2009).

Several reports indicate that neutrophils may be involved in the early control of blood parasites (Appelberg 2007). The activation of neutrophils in the initial stages of malaria infection is well supported (Graca-Souza et al. 2010, Mohammed et al. 2003) and transcriptome analyses of whole blood from humans infected with *P. falciparum* also revealed a unique gene expression profile related to neutrophil activity during early infection (Griffiths et al. 2005). Although low neutrophil count has been linked with acute *P. falciparum* malaria (Ladhani et al. 2002) and the contributions of neutrophils to host defence have been long investigated (Brown & Smalley 1981, Kharazmi & Jepsen 1984), their roles in uncomplicated (*P. vivax* and *P. falciparum*) malaria remain unclear. Our data indicate that malaria-infected individuals had neither an increased frequency of neutropaenia nor a low neutrophil count relative to the control group. However, the high band neutrophil count observed in the acute phase of infection and the return to normal levels in the convalescent phase suggest an accelerated production and/or release of neutrophils during malaria infection. Indeed, the inverse correlation of band count with the number of past malaria infections and the direct correlation of band count with the number of months since the last malaria infection suggest that less exposed individuals respond to parasite stimulus with a greater inflammatory profile and, consequently, increased neutrophil production.

Therefore, to evaluate the inflammatory profile associated with uncomplicated malaria infections, we investigated the plasma levels of two inflammatory mediators, CRP and NO, in patients with *P. falciparum* and *P. vivax* infections. As expected, we observed high levels of CRP in the acute phase of malaria infection (Pepys & Balfz 1983). However, the role of CRP in malaria pathogenesis or protection is not well established and the majority of data available concerns *P. falciparum* infections in African populations. In Brazilian populations, possible roles for CRP and other inflammation markers were reported in severe vivax malaria patients (Andrade et al. 2010); otherwise, the literature lacks data for CRP levels in patients with uncomplicated *P. falciparum* and *P. vivax* malaria in Brazilian endemic areas. In our work, higher CRP levels were found in the plasma of *P. vivax*-infected individuals compared to *P. falciparum*-infected

### TABLE

Clinical and epidemiological characteristics of the studied population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 19)</th>
<th>Malaria (n = 71)</th>
<th>Plasmodium vivax (n = 47)</th>
<th>Plasmodium falciparum (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male [nº (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age [median of years (IQ)]</td>
<td>29 (29-40)</td>
<td>28 (22-40)</td>
<td>28 (22-38)</td>
<td>28.5 (23-41)</td>
</tr>
<tr>
<td>Years of residence in endemic area [median (IQ)]</td>
<td>29 (18-32)</td>
<td>25 (20-36)</td>
<td>24 (21-36)</td>
<td>27 (19-37)</td>
</tr>
<tr>
<td>Previous malaria episodes [median (IQ)]</td>
<td>1 (0-3)</td>
<td>3 (1-10)</td>
<td>3 (1-8)</td>
<td>4 (2-10)</td>
</tr>
<tr>
<td>Months since the last malaria episode [median (IQ)]</td>
<td>60 (20-180)</td>
<td>11 (2-34)</td>
<td>10 (2-24)</td>
<td>13 (2-102)</td>
</tr>
<tr>
<td>Elapsed days since symptoms onset [median (IQ)]</td>
<td>NA</td>
<td>3 (2-10)</td>
<td>3 (2-7)</td>
<td>3 (2-10)</td>
</tr>
<tr>
<td>Symptoms (frequency) [nº (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>61 (85)</td>
<td>39 (83)</td>
<td>22 (91.7)</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>60 (84)</td>
<td>39 (83)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0</td>
<td>52 (73)</td>
<td>32 (68.1)</td>
<td>20 (83.3)</td>
</tr>
<tr>
<td>Shiver</td>
<td>0</td>
<td>52 (73)</td>
<td>31 (66)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>39 (69)</td>
<td>23 (48.9)</td>
<td>16 (66.7)</td>
</tr>
<tr>
<td>Parasitaemia [parasites (µL)]</td>
<td>2.051</td>
<td>1.256</td>
<td>2.293</td>
<td></td>
</tr>
<tr>
<td>Median (IQ)</td>
<td>0 (874-6.614)</td>
<td>(697-5.222)</td>
<td>(874-7.481)</td>
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</tr>
</tbody>
</table>

IQ: interquartile range; NA: non-applicable.
individuals, suggesting a more robust inflammatory profile for \textit{P. vivax} infection in the studied population. Due to a number of conflicting publications, the roles of CRP and NO in the immune response to \textit{Plasmodium} remain uncertain (Kremsner et al. 1996, Gyan et al. 2002, Awasthi et al. 2003, Clark & Cowden 2003). NO, which is produced by various cells in the liver and peripheral blood, could serve as a defence against invading microorganisms and parasites (Ellis et al. 1998). In animal models, NO regulated by CD8 T cells can prevent the development of the exo-erythrocytic stage in the liver. The NO pathway may be a necessary component for the inhibition of sporozoite development and the elimination of infected hepatocytes (Seguin et al. 1994). It is not known whether the induction of NO activity is sufficient for protection against sporozoite development in the liver or necessary for CRP and/or other inflammatory mediators to induce pathogenic processes or protective responses against human malaria parasites.

In our cohort, the plasma levels of NO in the acute and convalescent phases were higher in \textit{P. falciparum} patients than in controls and \textit{P. vivax} patients. The presence of elevated NO in \textit{P. falciparum} individuals was reported in uncomplicated and complicated \textit{P. falciparum} malaria cases (el-Nashar et al. 2002, Gyan et al. 2002, Nahreinan 2006, Yeo et al. 2007). Finally, we cannot rule out the possibility that genetic polymorphisms in the parasite and/or in the human genes encoding CRP (Israelsson et al. 2009, Giha et al. 2010, 2011) and NO (Kun et al. 2001, Hobbs et al. 2002, Levesque et al. 2010), which have been reported in different regions, could be responsible for the differences between the levels of these molecules in our population and those reported in other endemic areas.

In conclusion, our data indicate that individuals with uncomplicated \textit{P. vivax} and \textit{P. falciparum} infection presented similar white blood cell inflammatory profiles, with high band cell production and a considerable degree of thrombocytopenia during the acute phase of infection. Higher CRP levels were detected in acute \textit{P. vivax} infection, while higher NO levels were detected in acute and convalescent \textit{P. falciparum} infections. Although changes in these mediators are not diagnostic of malaria infection, the haematological parameters associated with malaria infection, particularly the role of PLTs and band cells, require further investigation.

REFERENCES


