# *Original Article*
# Pro- and anti-inflammatory cytokines in children with malaria in Franceville, Gabon

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**Abstract:** Severe *Plasmodium falciparum* malaria anemia (SMA) is a major cause of mortality in pediatric wards. Variations in inflammatory mediator production play an essential role in disease outcomes. Indeed, several studies have shown the involvement of pro- and anti-inflammatory cytokines such as IFN-γ, IL-6, TNF-α and IL-10 in malaria immunopathology. In other hand the exact role of Th17 cytokines such as IL-17, IL-22 and IL-21 in malaria remains poorly documented. Here, we investigated IFN-γ, TNF-α, IL-6, IL-12, IL-10, IL-4, IL-13, IL-17, IL-22 and IL-21 circulating levels and their association with malaria anemia and parasitemia in Gabonese children. Levels of IFN-γ (500 ± 100.2 pg/ml), IL-6 (64 ± 14.2 pg/ml), IL-10 (505 ± 35 pg/ml), IL-13 (30.6 ± 5.6 pg/ml) were significantly higher ($P < 0.03$) in infected children than in uninfected controls (210 ± 20 pg/ml, 17.5 pg/ml, 50 ± 25.9, pg/ml, 17.48 pg/ml, respectively). IFN-γ levels were significantly lower ($P = 0.04$) in children with SMA (400 ± 200 pg/ml) than in those with uncomplicated malaria (900 ± 450 pg/ml) and higher in those with parasitemia ($P = 0.019$). Levels of IL-6 and IL-10 were significantly higher in children with malarial anemia ($P < 0.001$) and hyperparasitemia ($P < 0.0001$). A significant association between IL-10 levels and parasite density was observed ($P < 0.00001$). IL-22 levels were significantly higher ($P = 0.01$) in infected children (72.57 ± 7.5 pg/ml) than in the controls (54.96 ± 1.93 pg/ml). IL-21 levels (44.46 ± 17.27 pg/ml) decreased with the severity of anemia ($P < 0.05$), whereas IL-17 levels increased in children with SMA (12.25 ± 1.25 pg/ml) than in those with mild malaria anemia (MMA: 6.2 ± 5.25 pg/ml, $P = 0.002$). Data suggest possible role of IFN-γ in the protection against SMA and parasite clearance. However, IL-6 and IL-10 could play a role in inflammatory response and pathophysiology of severe malaria anemia. Also, the role of IL-22 and IL-17 in *P. falciparum* malaria infection should be investigated.

**Keywords:** Severe *Plasmodium falciparum* malaria anemia (SMA), pro- and anti-inflammatory cytokines, children

## Introduction

*Plasmodium falciparum* malaria infection remains a major public health problem worldwide mostly in African regions [1]. Children aged under 5 years are the most susceptible population with anemia as the main severe complication [2, 3]. In Gabon, *P. falciparum* malaria is the main cause of neurological, hematological and infectious emergencies in healthcare structure [2, 4, 5]. In Franceville, southeast Gabon, where malaria is hyperendemic [6], a recrudescence of malaria infection has been observed between 2008 and 2012, accompanied by epidemiological modifications [7], and

higher incidence rates in older subjects, suggesting a decrease in acquired protective immunity [8, 9].

The pro-inflammatory cytokines seem to play an important role in malaria protection and parasite clearance. Also, the relative levels of pro- and anti-inflammatory cytokines are important mediators of development and outcomes of malarial anemia. Early production of pro-inflammatory T helper 1 (Th1) cytokines such as tumor necrosis factor (TNF)-alpha, interleukin (IL)-12 and interferon (IFN)-gamma may limit progression from uncomplicated malaria to severe complications [10, 11]. Indeed, they inhibit

**Table 1.** Demographical and hematological parameters of included patients (mean ± SD)

| | Children | |
|---|---|---|
| | Malaria-infected (n = 122) | Malaria-uninfected (n = 128) |
| Age mean (months) | 64.2 ± 2.8 | 60.5 ± 3.6 |
| Hemoglobin (g/dL) | 8.2 ± 0.2* | 8.9 ± 0.3 |
| Platelets (cells/mm$^3$) | 150,000 ± 10,000*** | 280,000 ± 10,000 |
| Red blood cells count (cells/mm$^3$) | 3,100,000 ± 100,000** | 4,800,000 ± 400,000 |
| White blood cells count (cells/mm$^3$) | 7700 ± 400 | 8400 ± 500 |
| Parasitemia (parasites/µL) | 46277 ± 8188 | 0 |

Age, leucocytes counts, hemoglobin concentrations and parasite densities in uninfected and infected children. *Plasmodium falciparum*-exposed children negative for parasites in thick blood smears and negative in rapid detection test kits for *P. falciparum* were defined as uninfected children. *$P < 0.05$; **$P < 0.0001$; ***$P < 0.000001$. SD = Standard deviation.

parasite growth and stimulate monocyte phagocytosis to enhance clearance of parasitized erythrocytes. Whereas, IL-6 cytokine is a major mediator of the acute phase response. Other pro-inflammatory cytokines such as IL-17 and IL-22, produced by other cell subtypes, including Th17 cells, are also involved in the immune response against *Plasmodium*. They contribute to inflammation by recruitment of neutrophils and induction of secretion of several pro-inflammatory cytokines. An increase in IL-17-producing CD4+ T cells in peripheral blood has been reported during *P. vivax* infection, along with the production of the pro-inflammatory cytokines IFN-γ, IL-10 and transforming growth factor (TGF)-beta [12]. Induction of IL-22 during murine infection protects against liver damage [13]. However, if these pro-inflammatory responses are not properly regulated during the acute infection, severe complications of malaria may ensue [14, 15]. Hence, the need for anti-inflammatory responses to control the production and possible cytopathic effects of pro-inflammatory cytokines. Regulatory cytokines such as IL-10 play an important role in *Plasmodium* infection, neutralizing excessive production of inflammatory Th1 cytokines [16, 17]. Anti-inflammatory Th2 cytokines including IL-4 and IL-13, regulate the humoral immune response, contributing to parasite clearance and inhibiting Th1 cytokine production [18, 19].

Despite the importance of the pro and anti-inflammatory cytokines production in human immune responses to *Plasmodium falciparum* malaria infection, that is not well documented in Gabonese children. Hence, in this study, we investigate pro- and anti-inflammatory responses in Gabonese children according to their malarial disease status, by measuring selected cytokines in plasma from these children.

**Patients and methods**

*Study area and population*

This study was conducted at Amissa Bongo Regional Hospital, Franceville, southeastern Gabon. Between 2011 and 2014, children presenting to the pediatric ward with a febrile syndrome or a history of fever during the previous 48 h were included. The children were classified according to their hemoglobin (Hb) level: no malarial anemia (UMA ≥ 11.0 g/dl), mild malaria anemia (MMA: 5.0 to 10.9 g/dl) or severe malaria anemia (SMA: < 5.0 g/dl). *P. falciparum*-exposed children with negative thick blood smears and rapid *P. falciparum* detection kit results (Optimal-IT, Biorad, France) served as uninfected controls. The parents or legal guardians gave their written informed consent before each child's enrollment in the study, which was approved by the Gabonese National Research Ethics Committee (N°00370/MSP/CABMD).

*Blood samples*

Venous blood (2.0-5.0 ml) was collected in EDTA tubes. Blood smears were stained with Giemsa according to Lambaréné method for microscopic *P. falciparum* identification and quantification [20]. All slides were examined by two well-trained microscopists from the International Medical Research Center in Franceville, Gabon. Hemoglobin, red blood cells, white blood cells and platelets were measured with an automated device (Beckman Coulter AcT diff2, Beckman Coulter Corporation, Miami, FL).

*Cytokine assays*

Blood samples were immediately centrifuged, and plasma was aliquoted and stored at -80°C
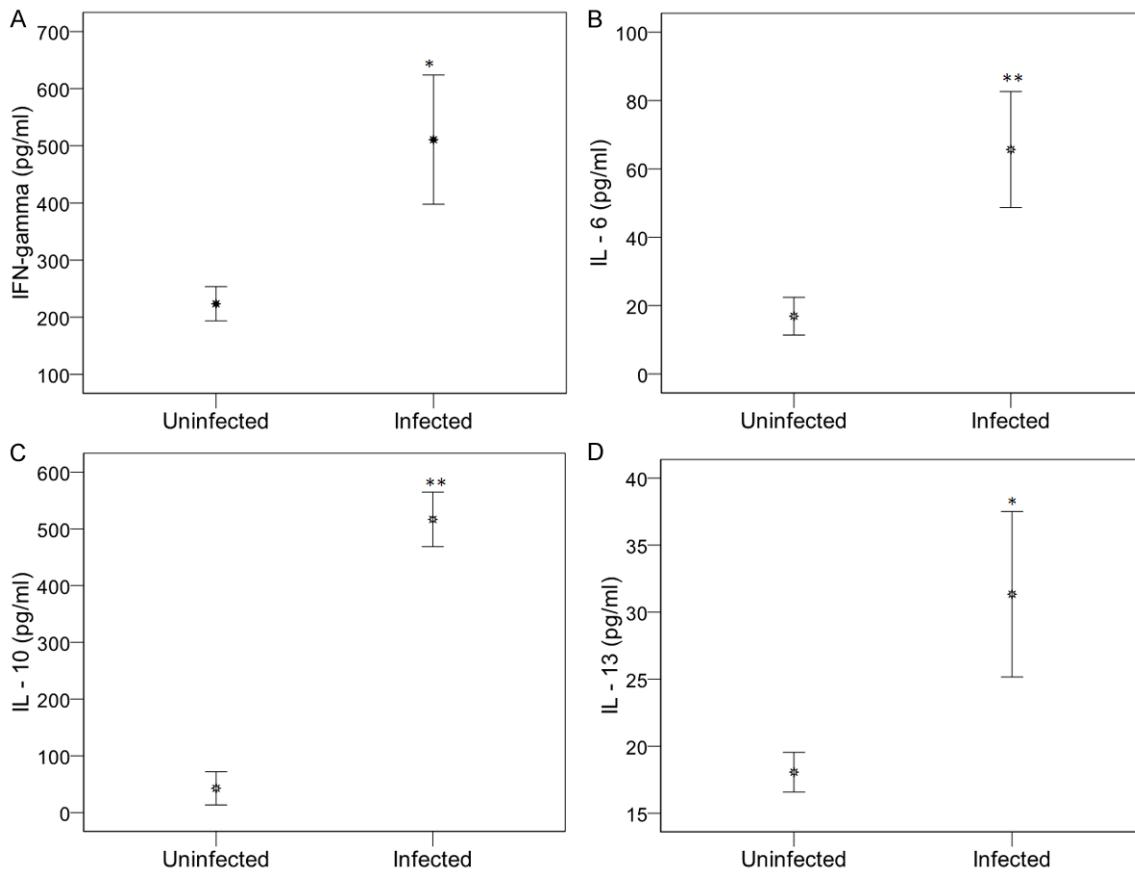
**Figure 1.** Levels of pro- and anti-inflammatory cytokines in plasma from uninfected and infected children. Plasma concentrations of cytokines IFN-γ (A), IL-6 (B), IL-10 (C) and IL-13 (D) were quantified in *Plasmodium falciparum* infected children and uninfected children. Data are represented with arithmetic means of cytokines concentrations in pg/ml, with the standard error. *P. falciparum*-exposed children negative for parasites in blood smears and in rapid detection test kits were uninfected children. Significant differences between uninfected and infected children are indicated, *$P < 0.05$, **$P < 0.00001$ compared to uninfected children.

until use. Circulating cytokine levels were determined with indirect enzyme-linked immunosorbent assays (ELISA) using the Ready-SET-Go!® kit (eBioscience®), according to the manufacturer's instructions. Optical densities (OD) were measured at 450 nm with a reference at 620 nm in an ELISA plate reader (Stat Fax 3200®, Bioblock Scientific; Fisher). The detection limits were as follows: 2 pg/ml for IL-6, IL-10 and IL-4; 4 pg/ml for IFN-γ, TNF-α, IL-13 and IL-17; 8 pg/ml for IL-22 and IL-21; and 31 pg/ml for IL-12p40. All samples were tested in duplicate, and the mean of the two OD values was used for analyses.

*Statistical analysis*

Variables were compared between the clinical subgroups (uninfected, UAM, MMA and SMA) by using the non-parametric measures. Differences between subgroups were analyzed for statistical significance using the Kruskal-Wallis non-parametric test. If significant differences were detected, the Mann-Whitney U non-parametric test was used for pairwise comparisons. Possible correlations between levels of cytokines and parasite density were identified using Spearman's rho test. All statistical tests used SPSS version 17.0 for Windows (SPSS Inc., Chicago, USA). *P* values below 0.05 were considered statistically significant.

**Results**

*Clinical and biological characteristics (Table 1)*

A total of 250 children aged between 6 to 168 months were included, of whom 122 had *P. falciparum* infection (21 with uncomplicated malaria, 86 with mild malarial anemia and 15
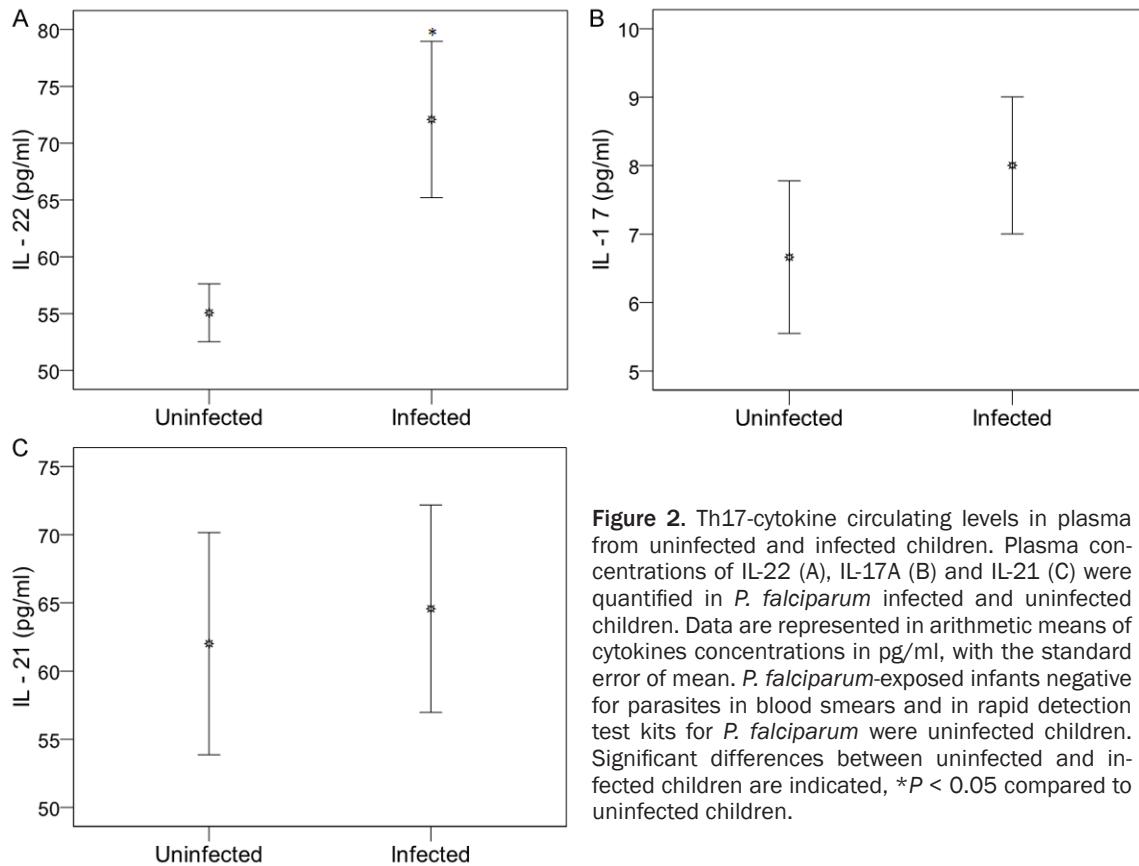
Figure 2. Th17-cytokine circulating levels in plasma from uninfected and infected children. Plasma concentrations of IL-22 (A), IL-17A (B) and IL-21 (C) were quantified in *P. falciparum* infected and uninfected children. Data are represented in arithmetic means of cytokines concentrations in pg/ml, with the standard error of mean. *P. falciparum*-exposed infants negative for parasites in blood smears and in rapid detection test kits for *P. falciparum* were uninfected children. Significant differences between uninfected and infected children are indicated, *$P < 0.05$ compared to uninfected children.

with severe malarial anemia) and 128 were free of malaria parasites. Mean parasitemia was 46277 ± 8188 parasites/µl. The mean age of the infected and uninfected children was 64.2 ± 2.8 and 60.5 ± 3.6 months, respectively (no significant difference). Infected children had significantly lower hemoglobin levels (8.2 ± 0.2 g/dL) than uninfected children (8.9 ± 0.3 g/dL; $P = 0.04$). Red blood cells (3.1 ± 0.1 × $10^6$ cells/mm³) and platelets (1.5 ± 0.1 × $10^5$ cells/mm³) counts were also significantly lower in infected children than in uninfected children (4.8 ± 0.4 × $10^6$ cells/mm³ and 2.8 ± 0.1 × $10^5$ cells/mm³, $P = 0.0001$ and $P = 0.00001$ respectively). White blood cells counts were lower in infected children (7.7 ± 0.4 × $10^3$ cells/mm³) than in uninfected children (8.4 ± 0.5 × $10^3$ cells/mm³) but the difference was not statistically significant.

*Increased plasma levels of pro- (IFN-γ and IL-6) and anti-inflammatory (IL-10 and IL-13) cytokines in infected children*

Levels of pro- (IFN-γ, IL-6) and anti-inflammatory (IL-13 and IL-10) cytokines were significantly

higher in *P. falciparum*-infected children than in uninfected children (**Figure 1**). The mean IFN-γ concentration was respectively 500 ± 100 and 210 ± 20 pg/ml ($P = 0.02$, **Figure 1A**), and the mean IL-6 concentration was respectively 64 ± 14.2 and 17.5 ± 2.5 pg/ml ($P < 0.00001$, **Figure 1B**). The mean IL-10 concentration was respectively 505 ± 35 and 50 ± 25.9 pg/ml ($P < 0.00001$, **Figure 1C**) and the mean IL-13 concentrations was respectively 30.6 ± 5.6 and 17.48 ± 1.58 pg/ml ($P = 0.03$, **Figure 1D**). The TNF-α concentration was significantly lower in infected children than in uninfected children ($P = 0.002$, data not shown). No significant difference in the mean IL-12p40 concentration was observed (384 ± 39.2 and 344.8 ± 29.8 pg/ml in infected and uninfected children, respectively). IL-4 levels were usually under the detection limit and were therefore excluded from the analysis.

*IL-22 levels highest in malaria children (**Figure 2**)*

The mean plasma IL-22 concentration was 72.57 ± 7.5 pg/ml and 54.96 ± 1.93 pg/ml in
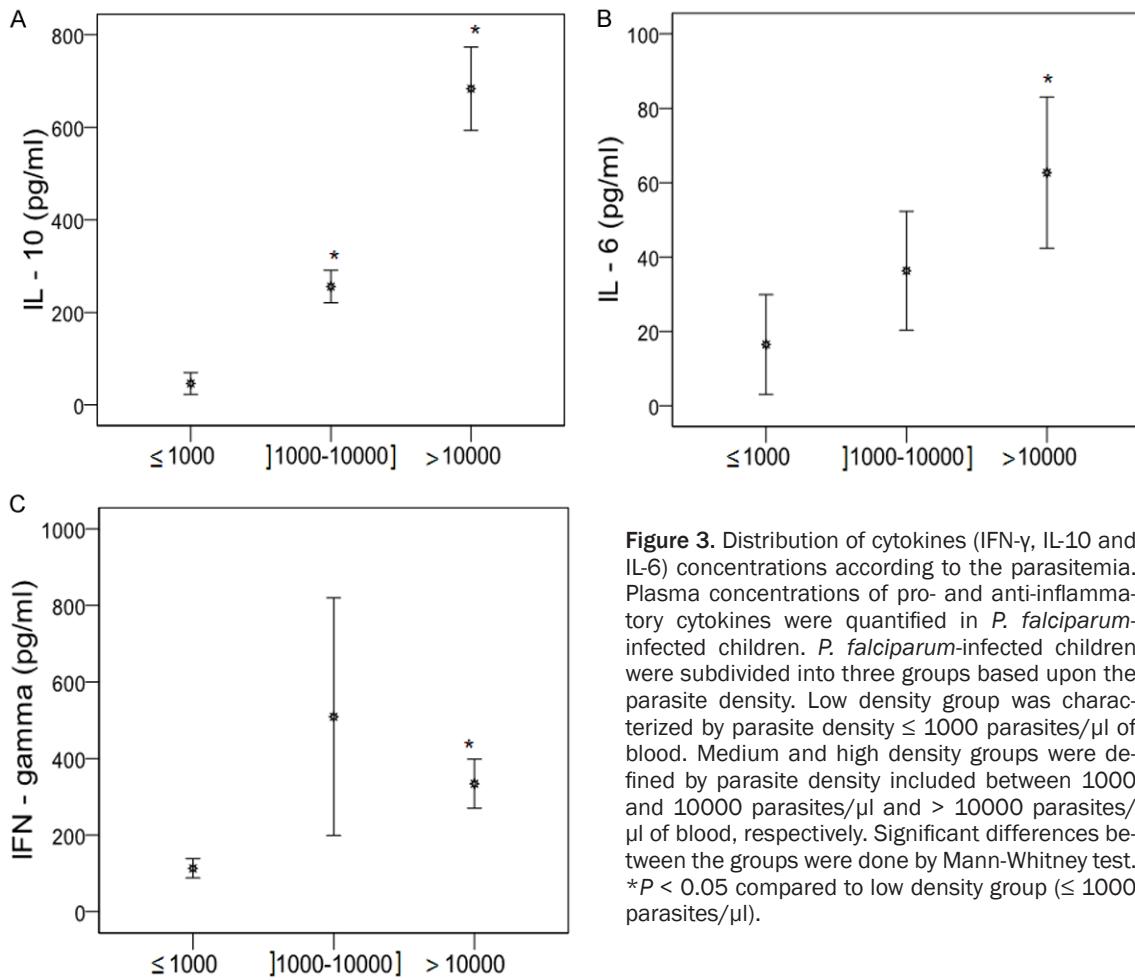
**Figure 3.** Distribution of cytokines (IFN-γ, IL-10 and IL-6) concentrations according to the parasitemia. Plasma concentrations of pro- and anti-inflammatory cytokines were quantified in *P. falciparum*-infected children. *P. falciparum*-infected children were subdivided into three groups based upon the parasite density. Low density group was characterized by parasite density ≤ 1000 parasites/μl of blood. Medium and high density groups were defined by parasite density included between 1000 and 10000 parasites/μl and > 10000 parasites/μl of blood, respectively. Significant differences between the groups were done by Mann-Whitney test. *$P < 0.05$ compared to low density group (≤ 1000 parasites/μl).

children with and without *P. falciparum* infection ($P = 0.01$, **Figure 2A**). IL-17 and IL-21 levels were not significantly different (**Figure 2B**, **2C**).

*Plasmodium falciparum parasitemia is strongly associated with IL-10 levels (Figure 3)*

Infected children were divided into three groups according to mean *P. falciparum* density: low (≤ 1000), medium (]1000-10000]) and high (> 10000). IL-10 levels increased significantly with the degree of parasitemia (49.6 ± 5.3 pg/ml, 298.4 ± 22.4 pg/ml and 720.0 ± 50.0 pg/ml for low, medium and high parasitemia; $P < 0.0001$, **Figure 3A**). Likewise, IL-6 levels were significantly higher in children with medium and high parasite density than in those with low parasite density (36.3 ± 15.9 and 62.7 ± 20.7 pg/ml versus 16.5 ± 13.4 pg/ml; $P = 0.05$; **Figure 3B**). IL-22 and IFN-γ concentrations were higher in children with medium parasite density (76.82 ± 15.1 pg/ml and 509.5 ± 300 pg/ml

for IL-22 and IFN-γ) than in those with low parasite density (51.5 ± 9.7 pg/ml and 113.37 ± 25.3 pg/ml) or high parasite density (69.5 ± 8.9 pg/ml and 334.4 ± 63.9 pg/ml), although the difference was only significant for IFN-γ (< 1000 vs > 10000 parasites/μl, $P = 0.03$, **Figure 3C**). By contrast, IL-13 levels decreased as parasitemia increased, although the difference was not significant ($P > 0.05$, data not shown).

Thus, IL-10 (rho = 0.49, $P ≤ 0.000001$), IFN-γ (rho = 0.23, $P = 0.019$) and IL-6 (rho = 0.24, $P = 0.05$) levels correlated with parasite density. The strongest association with parasite density was with IL-10 (**Table 2**).

*Cytokine levels according to anemia*

We then analyzed pro- and anti-inflammatory cytokine profiles in children with unanemic malaria (UAM), mild malarial anemia (MMA), and severe malarial anemia (SMA). As shown

**Table 2.** Correlation between plasma levels of inflammatory cytokines and parasitemia

| Cytokines | P. falciparum infected children | |
| --- | --- | --- |
| | rho | p |
| IFN-γ | 0.231 | 0.019 |
| TNF-α | 0.017 | 0.868 |
| IL-6 | 0.242 | 0.05 |
| IL12p40 | 0.023 | 0.897 |
| IL-17 | 0.081 | 0.515 |
| IL-22 | 0.032 | 0.784 |
| IL-21 | 0.099 | 0.459 |
| IL-10 | 0.412 | 0.00001 |
| IL-4 | 0.055 | 0.714 |
| IL-13 | 0.082 | 0.546 |

Spearman Rank Correlation analysis was carried between cytokines and parasitemia in *P. falciparum*-infected children. Correlation coefficient (rho) is given. Correlation is significant at $P < 0.05$.

in **Figure 4**, IFN-γ, IL-17 and IL-6 levels differed significantly across the three subgroups. IFN-γ levels decreased with increasing anemia severity, with mean concentrations of 954 ± 546 pg/ml in the UAM group, 442.12 ± 100 pg/ml in the MMA group and 200 ± 49.15 pg/ml in the SMA group ($P = 0.05$, **Figure 4A**). In contrast, IL-6 levels increased with increasing anemia severity: respectively 18.3 ± 6.7 pg/ml, 75.2 ± 20.2 pg/ml and 98.9 ± 38.1 pg/ml ($P = 0.03$, **Figure 4B**). IL-17 concentrations were highest in the UAM and SMA groups (12.7 ± 4 pg/ml and 11 ± 3.6 pg/ml, respectively), and lowest in the MMA group (6.2 ± 0.8 pg/ml), ($P = 0.012$, **Figure 4C**). IL-21 levels fell significantly with increasing anemia severity: 71.2 ± 12.2, 55.7 ± 11.2 and 44.5 ± 17.3 pg/ml respectively; with significant differences between the UAM and MMA groups ($P = 0.05$) and between the UAM and SMA groups ($P = 0.028$) (**Figure 4D**). IL-10 levels differed significantly across the groups ($P < 0.01$, **Figure 4E**), being highest in the MMA group (580 ± 80 pg/ml), followed by the SMA (430 ± 60 pg/ml) and UAM groups (400 ± 250 pg/ml). IL-13 levels were significantly higher in the MMA group (37.37 ± 10.4 pg/ml) than in the UAM (21 ± 4 pg/ml) and SMA groups (11.2 ± 2.8 pg/ml), ($P = 0.03$; **Figure 4F**).

### Discussion

We analyzed differences in cytokine responses between *P. falciparum*-infected and uninfected children living in the same area with similar socioeconomic and environmental conditions. All the infected children were free of cerebral malaria, anemia being the most frequent clinical manifestation in children under the age of 5 years [2]. We found that infected children had significantly higher IFN-γ levels than uninfected children, suggesting IFN-γ induction by *P. falciparum*. IFN-γ levels also fell significantly with the severity of malarial anemia, suggesting a protective role. This is consistent with previous data showing that IFN-γ controls parasite multiplication, promotes parasite clearance and protects against severe malaria [21-24]. Previous studies have shown a protective effect of IFN-γ against clinical malarial anemia among young children living in a holoendemic area [25]. IFN-γ production in response to pre-erythrocytic antigens was also associated with high hemoglobin concentrations and a reduced prevalence of malarial anemia in these children. Our data thus confirm the role of IFN-γ as a key mediator of the protective immune response against *P. falciparum* and its role in the pathophysiology of severe malarial anemia.

Proinflammatory cytokines play an important role in immune responses to *P. falciparum*, and IL-6 was significantly produced in our infected children. Increased levels of IL-6 were also been detected in plasma from children with severe malarial anemia, in keeping with data on Mozambican children showing that severe malaria is associated with high IL-6 levels [26]. Our data demonstrate an association between high IL-6 levels and a high parasite burden. IL-6 does not protect against parasite multiplication. Other studies have shown an association between low IL-6 and hyperparasitemia [27]. These results suggest that strong IL-6 production could contribute to the pathogenesis of severe malarial anemia. A study of Malawian children showed that chronic iron deficiency was strongly associated with higher production of IL-6 [28]. IL-6 induces an increase in transferrin receptor density on hepatocytes [29, 30], an increase in ferritin synthesis, and a decrease of transferrin synthesis [29]. This pro-inflammatory response is modulated by IL-10. Indeed, initial production of IL-12 and TFN-α enhanced IL-6 production, followed by an increase in IL-10 production as a counter-regulatory mechanism inhibiting the production of pro-inflammatory cytokines such as TNF-α
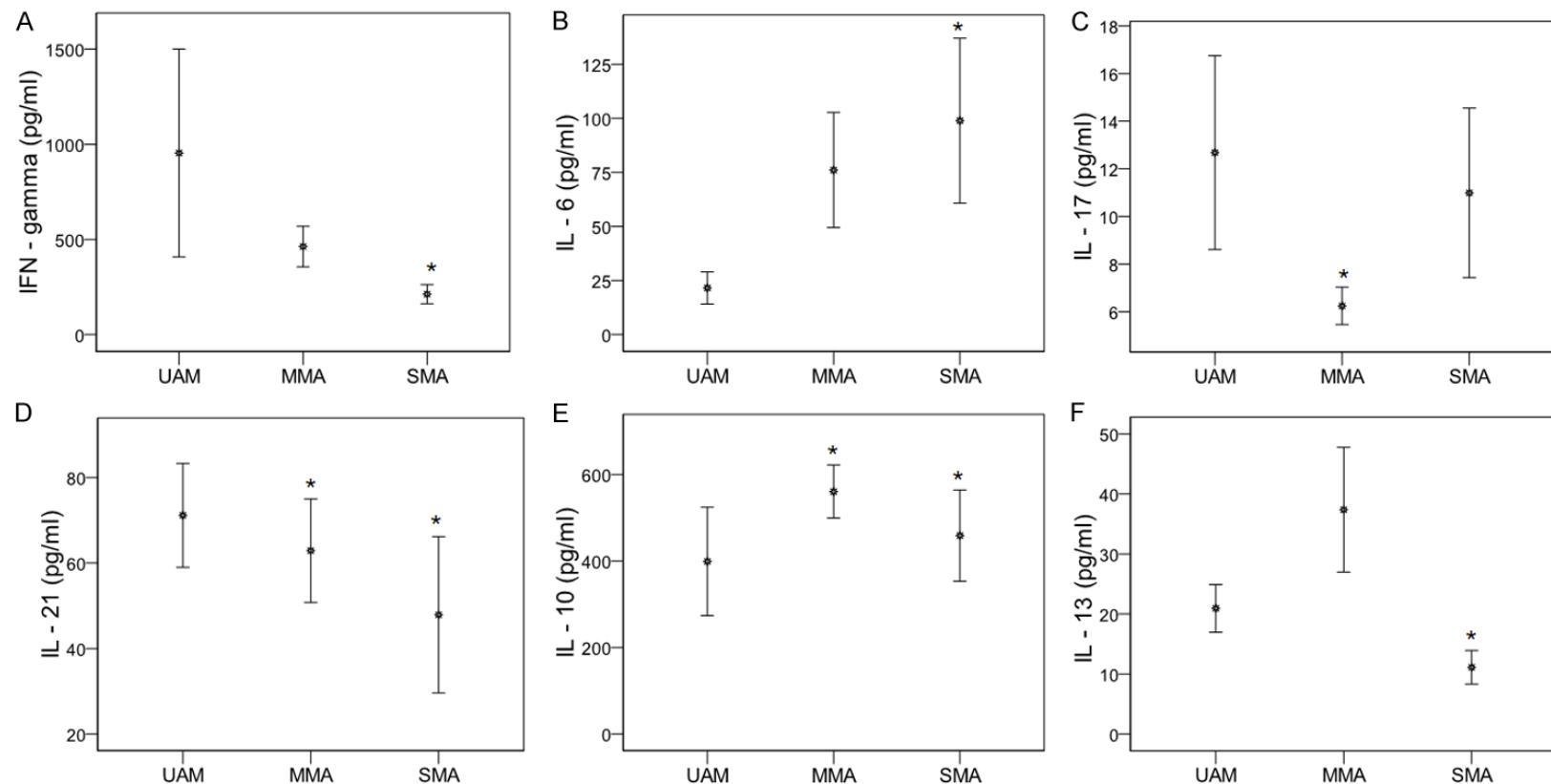
**Figure 4.** Pro- and anti-inflammatory cytokines profiles according to malaria anaemia statue. Plasma concentrations of cytokines IFN-γ (A) and interleukin (IL)-6 (B), IL-17A (C), IL-21 (D), IL-10 (E) and IL-13 (F) were quantified in *P. falciparum* infected and uninfected children. Results represent the arithmetic means of cytokines concentrations in pg/ml, with the standard error of mean. Children were subdivided into severe malaria anaemia (SMA: Hb < 5.0 g/dL), mild malaria anaemia (MMA: $5 \leq Hb < 11$ g/dL) and unanaemia malaria (UAM: Hb ≥ 11 g/dL). Kruskall-Wallis tests and pairwise comparisons using Mann-Whitney U tests were used. Differences were significant at *p*-value < 0.05. *$P$ < 0.05 compared to unanaemia malaria (UAM).

and IL-12. Although high levels of TFN-α have been shown to correlate with malaria severity in several studies [16, 27, 31], low TNF-α levels were observed in our study. This could be explained by the negative action of IL-10 on pro-inflammatory responses. IL-10 completely abolishes TNF-α production in response to malarial antigens [32]. IL-12 plays an important role in the adaptive immune response to malaria. Also in this study, plasma levels of IL12p40, the common subunit of IL12p70 and IL-23, were detected in both infected and uninfected children, with no significant difference. This could be explained by the high production of IL-10 in infected children, with a suppressive effect on IL-12 production. Indeed IL-12 levels were paradoxically lower in African children with severe malaria [33-35]. Likewise, a study of Kenyan children showed that those with malarial anemia had low circulating levels of IL-12p70 and down-regulated IL-12p40 mRNA levels, caused by phagocytosis of malarial pigment by monocytes and induction of the IL-10 overproduction by malarial pigment [36].

IL-10 is an important anti-inflammatory cytokine [37, 38]. We found increased plasma levels of IL-10 in infected children compared with uninfected children, especially in those with malarial anemia. We also found that IL-10 levels increased with increasing parasitemia. These results are consistent with a study of Kenyan children in whom anemia severity and elevated parasitemia were associated with increased IL-10 concentrations [39]. This suggests that *P. falciparum* stimulates IL-10 production in dose-dependent manner. IL-10 is used by regulatory T cells to control the immune response. Indeed, *P. falciparum*-infected erythrocytes induce regulatory T cell expansion, followed by increased IL-10 and IL-6 production [22, 40]. In addition to a direct correlation between T regulatory cell numbers and IL-10 plasma levels in infected patients [41, 42], it has been shown that CD4+CD25+Foxp3+ T cells induced during malaria upregulate their IL-10 expression [42, 43]. Other authors also report that regulatory T cells are associated with higher rates of parasite growth, suggesting that *P. falciparum*-mediated induction of regulatory T cells might be a virulence factor [44].

In protozoan infections, a role of Th17 cytokines has been reported in inflammatory responses and host defense mechanisms. Indeed, IL-17 is important for the resolution of *Trypanosoma cruzi* infection [45] and may mediate protection from *Toxoplasma gondii* infection [46, 47]. On the other hand, *T. gondii* infection enhances IL-17 expression, which contributes to the inflammatory response, and IL-17 promotes fatal *T. gondii* infection [48] and the inflammatory reaction in human leishmaniasis [49]. Patients with acute *P. vivax* infection showed a significant increase in circulating Tregs producing anti-inflammatory as well as pro-inflammatory cytokines such as IL-17 [43, 50]. In our study, IL-17 levels were similar in infected and uninfected children. Analysis of cytokine production according to anemic status showed significantly higher IL-17 production in children with severe malarial anemia than in those with mild malarial anemia. In contrast, Ong'echa and collaborators noted significantly low levels of IL-17 in subjects with uncomplicated malaria and severe malarial anemia in western Kenya [51]. IL-17 might play a dual role in malaria. In the early phase of infection, high IL-17 production may prevent severe malaria. However, in acute malaria, the overproduction of IL-17 induces SMA. IL-17 levels increase significantly in patients with autoimmune hemolytic anemia and correlated with the degree of anemia [52]. Also IL-17 level was more important in patients with autoimmune hemolytic anemia to red blood cells and significantly associated with more severe anemia [52]. Unlike IL-17, IL-22 levels were elevated. This is consistent with work from Mastelic and collaborators who showed a role of IL-22 in protection against liver damage during *P. chabaudi* infection [13]. The high production of IL-22 observed here suggests the involvement of this cytokine in protective immunity to *P. falciparum*. In addition, induction of IL-22 depends on IL-6, IL-23 and IL-1β. Also IL-6 production in infected children could suggest that IL-22 could be produce by Th17 cells in *P. falciparum* malaria infection. However, other cell subtypes such as Th22, NK and NKT are also known to produce IL-22 [53-55]. Further studies are therefore needed to determine the cellular source and role of IL-22 in protection or pathology induced during *P. falciparum* malaria infection.

IL-21 levels, although not significantly different between infected and uninfected subjects, fell significantly with the severity of anemia, suggesting that IL-21 plays a role in the protective immune response against *P. falciparum*. Our

results are in agreement with data on young Gabonese children infected with *P. falciparum*, which showed a strong correlation of IL-21 levels with anti-EBA-175 peptide 4 IgG1 levels. Furthermore, IL-21 production is associated with low parasite burdens and normal hemoglobin levels [56].

Given the importance of the antibody response in protective immunity to malaria, we evaluated the production of Th2 cytokines, which promote immunoglobulin production and class switching. IL-4 production was detected in few of the infected children. A significant difference in IL-13 levels was observed between infected and uninfected children. Levels of IL-13 were higher in children with mild malarial anemia than in those with severe malarial anemia, which could indicate a protective role of IL-13 in severe malarial anemia. In addition, IL-13 production decreased with increasing parasitemia. The presence of IL-13 in infected children could suggest a Th2 (anti-inflammatory) response. Indeed, Th2 cytokines stimulate B cell activation producing *P. falciparum*-specific antibodies and downregulate the Th1 cytokine response. The absence of IL-4 did not prevent B cell responses in our subjects. Indeed, during malaria, the frequency of IL-4-producing CD4+ T cells decreases while the frequency other CD4+ T-cells able to help B-cells to produce *Plasmodium*-specific antibodies is high [57, 58]. Moreover, control of infection and specific IgG antibody responses is possible in the complete absence of IL-4 [59]. Recent studies describe a subset of T helper cell, Tfh cells, producing IL-21 and other cytokines such as IFN-γ and IL-4, which are important for B cell help and activation of protective B cell responses against malaria [60, 61]. Our results do not support the direct involvement of IL-4 in parasite clearance.

In conclusion, our findings show that malaria infected children present the significant increased levels as well as of pro- and anti-inflammatory cytokines. These children have shown especially high levels of INF-γ, which decreases with increased malarial anemia severity and high parasite density. These results suggest that INF-γ cytokine may contribute to protection against severe malaria anemia and parasite clearance. In addition, the results showed that the infected children had higher levels of IL-6

cytokine which increases with increased malarial anemia severity and high parasite density. Also increased IL-6 levels in *P. falciparum* infection could be associated with pathogenesis of malaria anemia. In other hand, the infected children were shown to have higher levels of IL-10 and IL-13, which decreases in children with severe malaria anemia, furthermore IL-10 levels correlated with parasite density. These findings suggest that higher levels of anti-inflammatory cytokines, especially IL-10 levels may contribute to pathogenesis of complicated malaria by inhibiting the INF-γ production. The findings presented here showed the increased IL-22 levels in infected children, the increased IL-17 levels in unanemic malaria and severe malaria anemia. In contrast, decreased IL-21 levels were associated with increased malaria anemia severity. Further studies are needed to evaluate the implication of IL-17, IL-22 and Il-21 cytokines in protection and/or pathogenesis of malaria.

## Disclosure of conflict of interest

None.

## Authors' contribution

SLOL conceived, designed and coordinated the study, performed statistical analysis and wrote the paper. BTAG participated in sample collection and carried out the immunoassays. HN, LCK, SMN and IPM collected clinical samples and provided parasitological and hematological data. NTM participated in immunoassays.

JBLD designed and coordinated the study, performed statistical analysis, and participated in writing the paper. All the authors read and approved the final manuscript.

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