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Distinct systemic cytokine signatures define symptomatic malaria in children: insights from a longitudinal study in Togo

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Abstract

Background Malaria remains a significant public health challenge in sub-Saharan Africa (SSA), particularly among children, where immune responses and clinical manifestations to *Plasmodium falciparum* infection vary widely. Understanding the interplay between parasitemia, immune responses, and clinical outcomes is essential to improving case management. This study aimed to identify systemic cytokine signatures that define symptomatic malaria in children, by analyzing cytokine dynamics over time and their association with parasite burden and clinical status in children aged 6–10 years in Togo.

Methods A longitudinal cohort study was conducted in the Prefecture d'Agoè, Togo, from November 2023 to February 2024. Sixty children were enrolled and treated with artemether-lumefantrine and albendazole. Weekly monitoring was performed over 12 weeks using microscopy and qPCR for *P. falciparum* detection. Systemic cytokines, including IL-1β, IL-6, IFN-γ, IL-17 A, IL-5, and IL-10, were measured by Sandwich ELISA. Associations between cytokine concentrations, parasite densities, and cycle threshold (Ct) values were assessed using Spearman's correlation.

Results Of the 60 children, 25% ($n=15$) tested positive for *P. falciparum* by qPCR, while 15% ($n=9$) had microscopy-confirmed infections. Overall, 33.3% ($n=5$) progressed to symptomatic malaria; the remainder were asymptomatic carriers. Symptomatic cases exhibited significantly elevated IL-6 (vs. non-infected, $p=0.0004$; vs. asymptomatic, $p=0.0003$; vs. recovered, $p=0.0037$), IFN-γ (vs. non-infected, $p<0.0001$; vs. asymptomatic, $p=0.0048$), and IL-10 levels (vs. non-infected, $p<0.0001$; vs. recovered, $p=0.0004$). IL-10 levels were also higher in asymptomatic children compared to non-infected ($p=0.0013$), while IFN-γ was elevated in recovered cases (vs. non-infected, $p=0.0309$). In symptomatic children, IL-10 levels showed a strong negative correlation with Ct values ($r=-0.973$, $p=0.001$) and a

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strong positive correlation with parasite density ($r = 0.911$, $p = 0.001$). IFN-γ exhibited moderate correlations with both, Ct values ($r = -0.442$, $p = 0.037$) and parasite density ($r = 0.518$, $p = 0.027$).

Conclusion This study identifies distinct cytokine signatures, particularly elevated IL-10 and IFN-γ, that characterize symptomatic malaria in children and correlate with parasite burden. These cytokines may serve as prognostic biomarkers and offer insights for targeted interventions in paediatric malaria.

Keywords *Plasmodium falciparum*, Cytokines, IL-10, IFN-γ, Paediatric immune response, Malaria, Parasite burden

Introduction

Malaria remains a major global health challenge, with 282 million cases and over 610,000 deaths in 2024, predominantly in sub-Saharan Africa [1]. Children under five are the most affected, accounting for 75% of malaria-related deaths [1]. *Plasmodium falciparum*, the most virulent species, causes the most severe cases, especially in high-transmission areas like Togo [2]. Despite progress in malaria control, resistance to antimalarial drugs [3–5] and insecticides [6, 7] hinders further reductions in morbidity and mortality. This persistent burden highlights the complexity of malaria pathogenesis and the need for innovative treatment strategies.

Malaria can manifest along a clinical continuum, ranging from asymptomatic infection, with no clinical signs, to symptomatic disease, which may present as either uncomplicated or severe, life-threatening illness, shaped by host-parasite interactions [8]. Cytokines regulate immune responses, influencing disease outcomes [9]. Pro-inflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) contribute to parasite clearance but may also cause tissue damage if dysregulated [9], while interleukin-10 (IL-10) helps prevent excessive inflammation but may promote parasite survival [9, 10]. Cytokine profiles may determine clinical severity, particularly in children prone to severe complications such as cerebral malaria and severe malarial anaemia [9–12], yet longitudinal data on these immune responses remain scarce.

In Togo, malaria is the leading cause of morbidity and mortality in young children, with perennial transmission peaking during the rainy season [13, 14]. However, immunological biomarkers associated with malaria in this region are understudied. Subclinical malaria, characterized by low-density parasitaemia without symptoms, serves as a transmission reservoir and complicates disease control in endemic areas [15]. While these infections are undetectable by microscopy, quantitative polymerase chain reaction (qPCR) offers higher sensitivity, allowing the detection of low-level parasitemia [16, 17]. Such infections impair cognitive development, and contribute to chronic anaemia [18–21].

The interplay between parasitaemia and cytokine profiles in children remains an area of active research. Asymptomatic *P. falciparum* carriers may exhibit immune responses distinct from those of symptomatic individuals [9, 11], and there is evidence that these responses may be population specific. However, data specific to West African populations, particularly Togo, remain limited [14]. This knowledge gap underscores the need for population-specific studies to define immunological markers that differentiate malaria infection outcomes.

To address this knowledge gap, we conducted this study to identify systemic cytokine signatures that define symptomatic malaria in children aged 6–10 years in Togo. By integrating longitudinal parasite monitoring with detailed cytokine profiling, we sought to uncover immune markers associated with malaria infection phenotypes. These findings may inform the development of prognostic biomarkers and guide future immunomodulatory strategies in paediatric malaria case management.

Methods

Study site and population

The study was conducted in Prefecture d'Agoè (Togo), from November 2023 to February 2024. This semi-urban area experiences perennial malaria transmission throughout the year, with incidence typically peaking during and shortly after the two annual rainy seasons, a long rainy season from approximately March to July and a shorter rainy season around October to December, when vector breeding conditions are most favorable [13, 14]. A total of 60 children aged 6 to 10 years, closely matched for gender, were enrolled into the study. This age group was selected based on the reported high susceptibility to clinical malaria and parasitaemia [22, 23] and inclusion criteria required that participants be ineligible for seasonal malaria chemoprevention (SMC), exhibit no vomiting during treatment, reside in the study area, be in good health with no chronic condition, be suitable for safe finger-prick blood sampling and be willing to provide assent and parental informed consent.

Study design and procedures

The study utilized a longitudinal active cohort design. The study team monitored the participants over 12 weeks (3 months), in three phases involving participant recruitment, baseline sample collection, and continuous monitoring for *Plasmodium falciparum* infection (Fig. 1).

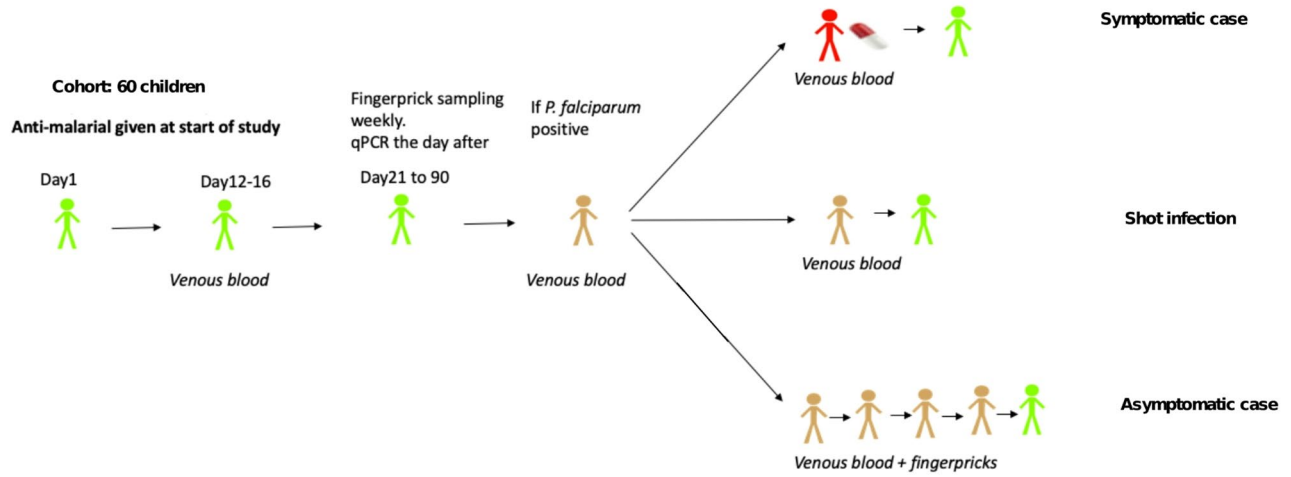

Fig. 1 Schematic overview of the study design. Phase 1 (Day 1): Participant recruitment and treatment. Phase 2 (Days 15–16): Baseline sample collection. Phase 3 (Days 21–90): Weekly fingerprick monitoring for *Plasmodium falciparum* detection

Phase 1: Recruitment and baseline treatment: children were recruited based on predefined eligibility criteria. At recruitment, data were collected on socio-demographic characteristics, sleeping under an insecticide-treated net, use of antibiotics, antimalarials, traditional remedies, and any history of fever or malaria-related symptoms over the past two weeks. All enrolled participants received a full 3-day, twice-daily course of artemether–lumefantrine and a single 400 mg dose of albendazole (UbiPharm Togo, Lomé, Togo) on Day 1 to ensure absence of malaria or worm infection before follow-up as worm infection is common and could influence immune responses during follow-up. Finger-prick blood samples were also collected for *Plasmodium falciparum* detection by qPCR and microscopy.

Phase 2: Baseline sample collection: on days 15 and 16 post-treatment, venous blood samples were collected to establish baseline plasma levels. This baseline sampling was to ensure that all children began the follow-up period at the same immunological and parasitological status. Blood samples were analyzed via microscopy and qPCR to confirm complete parasite clearance before initiating weekly monitoring.

Phase 3: Weekly monitoring and follow-up: From day 21 to day 90, participants underwent weekly finger-prick sampling. Dried blood spot (DBS) samples and thick/thin blood smears were prepared for *P. falciparum* detection by qPCR and microscopy, respectively. Venous blood samples were collected whenever a child tested positive for malaria for the first time, and again if the same child later developed symptoms or remained asymptomatic. Participants with confirmed *P. falciparum* infection and elevated axillary temperatures (38–40 °C) were classified as symptomatic and referred immediately for clinical management. These children received a full 3-day course of artemether-lumefantrine and were deemed to have reached the study endpoint, although follow-up venous samples were collected two weeks' post-treatment once symptoms had resolved. In contrast, children who tested positive but remained afebrile, most of whom were detected only by qPCR, were not referred for treatment but were closely monitored throughout the study to observe the natural progression of asymptomatic infections.

Sample processing

Venous blood samples collected from participants were immediately processed to separate plasma. Briefly, the whole blood was centrifuged at 1200 g for 5 min at room temperature to isolate the plasma fraction, which was then aliquoted into sterile tubes. The plasma samples were stored at -20 °C for subsequent analyses.

Determination of blood group (ABO and Rh typing)

The ABO blood groups and Rh (D) factor of each participant were determined using a standard slide agglutination method based on the principle of antigen-antibody interaction using commercially available antisera. Briefly, an opaline plate was used as the reaction surface and divided into four sections labelled respectively Anti-A, Anti-B, Anti-AB and Anti-D reagents. One drop (~ 50 µL) of each antiserum (Anti-A, Anti-B, Anti-AB and Anti-D; Cypress Diagnostic Langdorp, Hulshout, Belgium) was placed on the corresponding areas of the plate. A drop of freshly collected whole blood was added to each reagent. Each mixture was gently stirred using sterile applicator sticks. The plate was then gently rocked for 1–2 min at room temperature. Agglutination was observed visually and recorded immediately. Agglutination with Anti-A

indicated the presence of A antigen (blood group A), with Anti-B indicated B antigen (blood group B), with both Anti-A and Anti-B indicated blood group AB, and absence of agglutination indicated blood group O. Similarly, agglutination with Anti-D serum confirmed Rh positivity, while its absence confirmed Rh negativity.

Determination of haemoglobin type

The haemoglobin (Hb) type of each participant was determined using cellulose acetate electrophoresis at alkaline pH. Briefly, hemolysates were prepared by mixing a small volume of whole blood with lysing reagent (Hemolysate Preparation Reagent; Cypress Diagnostic Langdorp). The resulting lysate was applied to a cellulose acetate strip (Cypress Diagnostic Langdorp) previously soaked in Tris-EDTA-borate buffer at pH 8.6. The strip was then placed in the electrophoresis chamber and subjected to an electric field at a constant voltage (200 volts) for 30 min. During electrophoresis, haemoglobin fractions migrate according to their net charge, allowing the separation of HbA, HbS, HbC, HbF, and other variants. After electrophoresis, the strip was removed, dried, and stained with a specific haemoglobin stain. The stained strip was then examined visually, and the migration patterns were compared with control samples to determine the haemoglobin genotype of each subject.

Microscopic parasite identification and quantification

Thick and thin blood smears were prepared and used for parasite detection and quantification and *Plasmodium* species confirmation, respectively. Thin smears were fixed in methanol (Honeywell, Seeize, Germany) and then air-dried, while thick smears were allowed to air-dry without fixation. Both smear types were stained with 10% Giemsa for 20 min (Cypress Diagnostics, Langdorp), air-dried, and examined under a light microscope (Olympus CH 30, Japan) at ×100 oil-immersion magnification. Parasite density was calculated as the number of parasites counted per 200 white blood cells (WBCs) and expressed as parasites/μL of blood, assuming a standard WBC count of 8,000 WBCs/μL. A slide was declared negative if no parasites were seen after examining at least 100 high-power fields. To ensure diagnostic accuracy, all slides were independently read by two trained microscopists. In cases of discordant results (species identification, parasite positivity, or density > 50% difference), a third senior microscopist reviewed the slide, and the final result was assigned based on consensus.

Table 1 Primer and probe sequences

Name	5′-3′ Sequence
Var forward	CCCATACACAACCAAYTGGA
Var reverse	TTCGCACATATCTCTATGTCTATCT
Var probe	6-FAM-TRTTCCATAAATGGT-NFQ-MGB

Sub-microscopic parasite detection and quantification by qPCR

DNA was extracted from fingerprick blood samples using the FavorPrep Column Kit (Thermo Scientific™, San Diego, USA) following the manufacturer's protocol. Briefly, the collected blood samples were lysed to release nucleic acids. The lysate was mixed with a binding buffer, ensuring DNA adherence to the silica membrane of the spin column. Following a series of washes to remove contaminants such as proteins and salts, the bound DNA was eluted in nuclease-free water.

All DNA samples were analyzed in duplicate using a var acidic terminal sequence (varATS)-based qPCR assay for detecting *Plasmodium falciparum*. The qPCR reaction consisted of 5 μL of extracted DNA combined with 15 μL of mastermix, prepared as follows: 10 μL 2× TaqMan mix, 1.6 μL forward primer (10 μM), 1.6 μL reverse primer (10 μM), 0.8 μL probe (0.4 μM), and 1 μL PCR-grade water. The primer and probe sequences are provided in Table 1. The reaction mix was run for pre-treatment at 50 °C, for 2 min; initial denaturation at 95 °C for 5 min; denaturation at 95 °C for 15 s; and annealing and elongation at 55 °C for 60 s for 45 cycles using the Corbett Research Rotor Gene 3000 (Corbett Research, Sydney, Australia). Negative controls contained DNA extracted from blood samples of individuals living in non-endemic regions, while non-template controls (NTCs) contained only the mastermix. Positive controls were prepared using 3D7 DBS standard dilutions. The qPCR outputs were analyzed using the Corbett Rotor Gene 3000 manager software (Corbett Research, Sydney, Australia). The 3D7 standards were used to generate a quantification curve, which established the cut-off Ct value for positivity. Samples were classified as follows: Positive: Ct values ≤ the cut-off Ct (dilution 7, ~ 0.22 parasites/μL) for both duplicates with normal amplification curves. Probable Positive: Ct values between the cut-off and the 95% CI upper limit, provided both duplicates amplified, and the curves were sound. Negative or False Positive: Ct values above the 95% CI upper limit or inconsistent amplification in duplicates. This classification was based on the known sensitivity and limit of detection of the var acidic terminal sequence-based (varATS) qPCR assay under the specific extraction conditions.

Cytokines profile assessment

Type I or innate cytokines (IL-1β, IL-6) and type II or adaptive cytokines (IFN-γ, IL-17 A, IL-5, IL-10), using the ready-to-use Invitrogen kit (Thermo Fisher Scientific), were quantified in plasma samples using Sandwich ELISA according to the manufacturer's protocol and as previously described [24, 25]. The intensity of the colour change, proportional to cytokine concentrations, was

measured using an ELISA Humareader plate (Human, Wiesbaden, Germany) [24, 25].

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 21, Armonk, NY) for descriptive analyses and for correlation analyses between variables using Spearman correlation analysis to assess the relationships between cytokine levels, parasite density, and cycle threshold (Ct) values. Spearman's rank correlation coefficients (ρ) were calculated between the levels of each cytokine and parasite densities, as well as between cytokine levels and Ct values. Positive and negative ρ values were interpreted to indicate the direction and strength of the associations. The Spearman's ranked correlation is robust as it makes no assumptions about the data distribution can effectively deal with monotonic relationships, where variable such as cytokine, parasite density levels may change in the direction but at variable rates. GraphPad Prism version 9.01 (GraphPad Software, San Diego, CA, USA) was used to assess the normality of data distribution using the Kolmogorov-Smirnov test. Since the data were found to be non-parametric, group comparisons were conducted using the Kruskal-Wallis test, followed by Dunn's post hoc test. GraphPad was also used for data visualization (dot plots). Results were presented as medians with interquartile ranges (IQR). A p-value < 0.05 was considered statistically significant.

Table 2 Study population socio-demographic characteristics

Characteristics	Size (%)	*P*-value
Sex		0.0286
Male	**32 (53.33)**	
Female	28 (46.67)	
Age (completed years)		0.0053
6	**17 (28.34)**	
7	12 (20.00)	
8	11 (18.33)	
9	14 (23.33)	
10	6 (10.00)	
Sleep under mosquito net		< 0.0001
Yes	**50 (83.33)**	
No	10 (16.67)	
Antimalarial/Antibiotics/Traditional therapy		< 0.0001
Yes	00 (0)	
No	**60 (100)**	
Malaria infection status		< 0.0001
Positive	0 (0)	
Negative	**60 (100)**	
Haemoglobin variants		0.0003
AA	**51 (85.00)**	
AC	5 (8.00)	
AS	3 (5.00)	
SC	1 (2.00)	
Blood group		0.0079
O	**27 (45.00)**	
A	16 (27.00)	
B	15 (25.00)	
AB	2 (3.00)	
Rhesus		0.0003
Positive	**54 (90.00)**	
Negative	6 (10,00)	
Fever		< 0.0001
Yes	00 (0)	
No	60 (100)	
Axillary temperature (°C)	**36.8–37.4**	

Data are expressed as frequencies and percentages. Statistical comparisons of proportions were conducted using the Chi-square (χ^2) test. Statistical significance was set at $p < 0.05$

Results

Socio-demographic and clinical characteristics of the study population

Of the 60 children enrolled, 53.3% ($n = 32$) were male, corresponding to a sex ratio of 1.14 (male/female). Children aged 6 years represented the largest age group (28.8%, $n = 17$). Most children (83.3%, $n = 50$) reported sleeping under a mosquito net at enrolment, and none had received antimalarial drugs, antibiotics, or traditional therapies at least two weeks prior to inclusion in the study.

Regarding biological characteristics, the majority of participants had blood group O ($n = 27$), followed by groups A ($n = 16$) and B ($n = 15$), and were predominantly Rhesus positive ($n = 54$). Most children ($n = 51$) carried the HbAA haemoglobin genotype. Notably, all the nine participants with abnormal haemoglobin genotypes, haemoglobin SC ($n = 1$), AS ($n = 3$), and AC ($n = 5$), remained uninfected throughout the follow-up period.

At the start of the study (enrolment), no child was positive for malaria infection, and none presented with fever and the recorded axillary temperatures of participants ranged from 36.8 °C to 37.4 °C. Table 2 shows socio-demographic and clinical characteristics of the study population.

Participant with abnormal haemoglobin, blood group and malaria infection status

The majority of children had blood group O, followed closely by group A and B (Figure S1A), and a positive Rhesus factor (Figure S1B), and an AA haemoglobin (HbAA) genotype (Figure S1C). Interestingly, the 9 participants with abnormal haemoglobin genotypes, comprising haemoglobin SC ($n = 1$), haemoglobin AS ($n = 3$), and haemoglobin AC ($n = 5$), remained uninfected throughout the study.

Period prevalence and clinical outcomes of *Plasmodium falciparum* infection

The prevalence of malaria infection was relatively low in the cohort, with only 25% of children ($n = 15$) testing positive for *P. falciparum* by qPCR over the study period, yielding Ct values ranging from 12.06 to 28.11. The earliest infections were detected in week four post treatment. Of the 15 positive cases, parasites were detectable by microscopy for 60.0% ($n = 9$) of participants. Among the infected children, 5 (33.3%) developed symptoms, including three of the seven children who reported not regularly sleeping under a bednet indicative of increased risk of exposure to infective bites. Ironically, only one of them had an early symptomatic infection at week four. The other four children were asymptomatic at weeks 10, 11 and 12 post-treatments, suggesting other protective measures other than bednets might have prolonged their exposure to infective bites. Supplementary Figure S2 shows a flow diagram of infection dynamics within the cohort.

Cytokine expression patterns reveal differential immune response between clinical states

The analysis of cytokine profiles revealed distinct expression patterns associated with symptomatic malaria in the cohort (Fig. 2). Children with symptomatic malaria exhibited significantly higher levels of the pro-inflammatory cytokine IL-6 compared to non-infected ($p = 0.0004$), asymptomatic ($p = 0.0003$), and recovered ($p = 0.0037$) groups (Fig. 2A). Similarly, IFN-γ levels were significantly elevated in symptomatic children compared to the non-infected ($p < 0.0001$) and asymptomatic ($p = 0.0048$) groups. Notably, recovered children also showed significantly higher IFN-γ levels compared to their non-infected counterparts ($p = 0.0309$; Fig. 2C). In addition, the regulatory cytokine IL-10 was markedly increased in symptomatic children relative to non-infected ($p < 0.0001$) and recovered ($p = 0.0004$) individuals (Fig. 2F). Asymptomatic children also exhibited significantly elevated IL-10 levels compared to the non-infected group ($p = 0.0013$; Fig. 2F). Other cytokines measured showed some variation across clinical states; however, these differences were

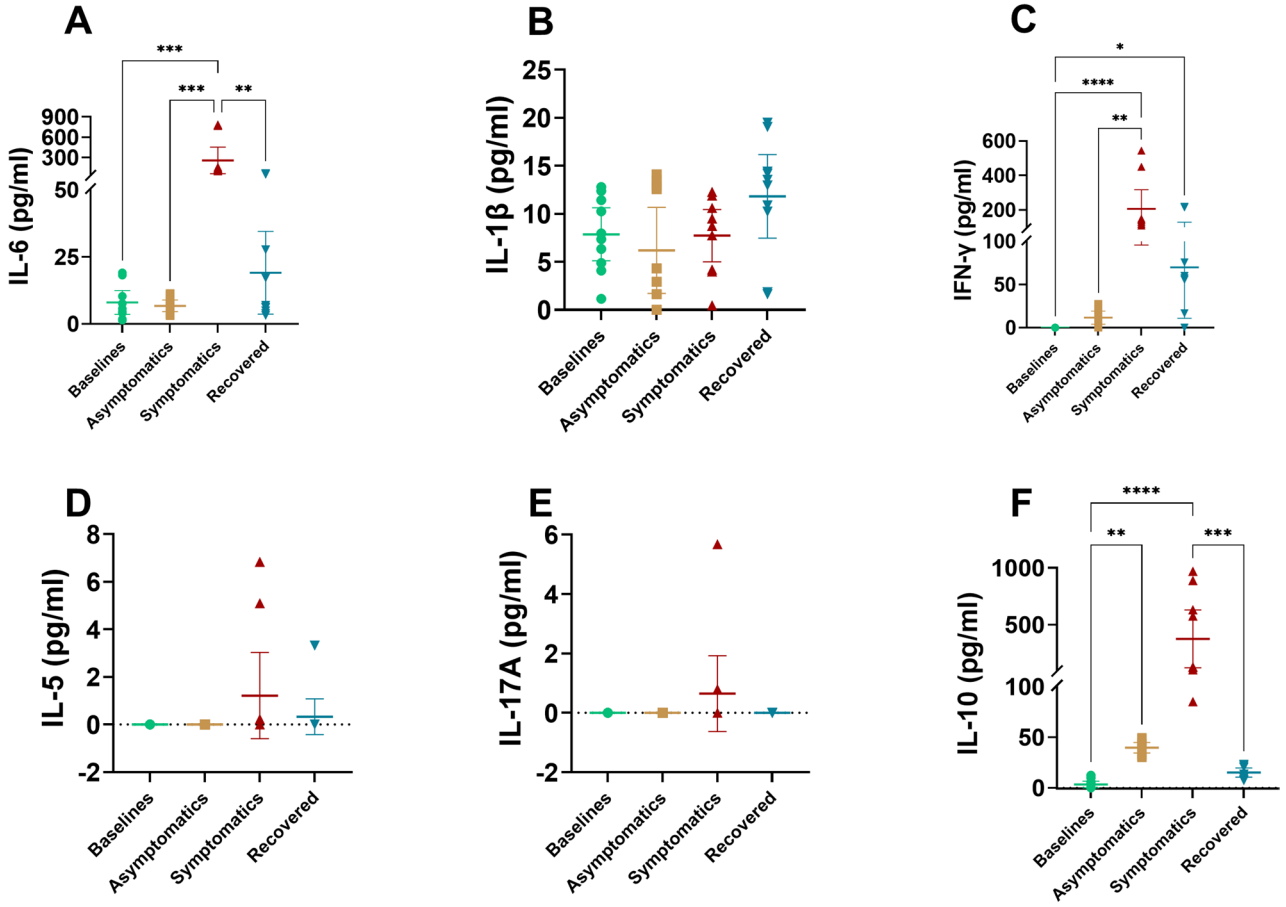

Fig. 2 Plasma cytokine dynamics across clinical malaria states. Dot plots show plasma concentrations (pg/mL) of key cytokines measured across clinical groups: **(Panel: A)** IL-6, **(Panel: B)** IL-1β, **(Panel: C)** IFN-γ, **(Panel: D)** IL-5, **(Panel: E)** IL-17 A, and **(Panel: F)** IL-10. Bars represent the median ± interquartile range (IQR). Each dot corresponds to an individual cytokine measurement. Comparisons were performed using the Kruskal-Wallis test followed by Dunn's post hoc test. Asterisks denote significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Table 3 Correlation analysis between cytokines and Ct values or parasite densities in symptomatic and asymptomatic children

Spearman correlations				
	Ct values		**Parasite densities**	
	***r* (rho)**	***p*-value**	***r* (rho)**	***p*-value**
Symptomatic children	(***n* = 5**)		(***n* = 5**)	
IL-1β	0.096	0.568	0.094	0.587
IL-6	0.162	0.332	0.038	0.825
IL-17 A	-0.137	0.412	-0.078	0.651
IL-10	**-0.973**	**0.001**	**0.911**	**0.001**
IL-5	-0.084	0.614	0.063	0.717
IFN-γ	**-0.442**	**0.037**	**0.518**	**0.022**
Asymptomatic children	(***n* = 5**)		(***n* = 5**)	
IL-1β	-0.232	0.235	0.078	0.705
IL-6	0.260	0.182	-0.351	0.079
IL-17 A	0.470	0.512	0.117	0.386
IL-10	0.063	0.750	-0.019	0.926
IL-5	-0.068	0.729	0.098	0.635
IFN-γ	0.103	0.601	0.138	0.501

not statistically significant between symptomatic, non-infected, asymptomatic, or recovered groups (Fig. 2B, D and E).

IL-10 and IFN-γ correlate with parasite density and qPCR Ct values in symptomatic malaria

Spearman correlation analysis revealed a strong negative correlation between IL-10 and Ct values (r = -0.973, $p = 0.001$), and a moderate negative correlation between IFN-γ and Ct values (r = -0.442, $p = 0.037$). Conversely, IL-10 showed a strong positive correlation with parasite density ($r = 0.911$, $p = 0.001$), while IFN-γ exhibited a moderate positive correlation ($r = 0.518$, $p = 0.022$). Notably, these associations were exclusive to symptomatic children (Table 3). No significant correlations were observed between cytokine levels and Ct values in the baseline (non-infected) samples or recovered individuals (supplementary Table S1).

Discussion

As targeted malaria interventions such as seasonal and/or perennial malaria chemoprevention (SMC) in young children are scaled up across West Africa, strong immunity from repeated infections is likely to be delayed in this group. Our longitudinal surveillance revealed a relatively low overall prevalence of *P. falciparum* malaria infection within a cohort emerging from the SMC target group (< 5years) aged 6 to 10 years in the Prefecture d'Agoè, Togo. In the present study, 25% of participants tested positive by qPCR over the three-month monitoring period during the high malaria season. This aligns with recent national trends showing a decline in malaria prevalence in Togo, attributed largely to targeted interventions such as SMC and extensive vector control interventions, particularly the widespread use of long-lasting insecticide-treated nets (LLITNs) [26]. Notably, 83.3% of the children reported sleeping under ITNs, suggesting that ITNs are still playing a critical role in disrupting malaria transmission [27–29]. Despite this, the high proportion (25%) of children testing positive for *P. falciparum* by qPCR is indicative of a reasonably large reservoir of infection that continues to fuel malaria transmission. These observations highlight the need for sensitive and scalable diagnostic tools that can detect low-level asymptomatic infections for treatment in order to shrink the infection reservoir towards malaria elimination; however, in high-transmission and resource-limited settings, population-wide screening is not currently feasible. Such approaches are more realistically applicable when transmission declines to low levels, particularly for targeted hotspot interventions or for preventing reintroduction in post-elimination contexts. It also highlights the importance of additional high-impact interventions, such as indoor residual spraying and larval source management, to push the malaria burden further down [27].

Understanding the relationships between circulatory pro-inflammatory and anti-inflammatory cytokines, parasitological characteristics, and clinical outcomes in this SMC-emergent cohort is essential for assessing how naturally occurring immune protective mechanisms may lower the risk of symptomatic malaria, as well as for identifying potential risks associated with inadequate or dysregulated immune responses that fail to control parasitaemia or contribute to clinical disease. Although host-parasite interactions that trigger immune responses and their impact on *falciparum* malaria pathogenesis remain actively researched, studies have demonstrated that immune responses contribute to both protection and disease pathogenesis [30]. We examined cytokine correlates during *P. falciparum* infection states, and replicated the role of IL-6 and IFN-γ [31, 32]. Pro-inflammatory cytokines are important in modulating symptomatic malaria through parasite growth control and elimination [33]. This is in the context of a large body of strong evidence supporting the role of TNF-alpha (not measured in the present study) in the pathogenesis of malaria and other infectious pathogens [34, 35]. Also, findings from the present study highlight the regulatory role of IL-10 in maintaining the balance between pro- and anti-inflammatory cytokines in disease progression.

We observed that symptomatic children with *P. falciparum* infection had significantly elevated levels of IL-6 and IFN-γ than baseline levels or asymptomatic or uninfected children. Thus, suggesting a signature uniquely associated with mild (or uncomplicated) malaria disease, consistent with results of previous studies [36, 37]. IL-6 is known to be up-regulated by TNF-alpha, and acts

with other inflammatory mediators to control parasite growth [38]. IFN-γ secreted by $CD4^+$ Th1 cells is critical for optimal activation of $CD8^+$ T cells, B cells, and macrophages, all of which perform vital roles in the control of *Plasmodium* infection. The primary immune effector mechanisms by which IFN-γ can influence destruction of *Plasmodium*-infected cells include increasing the cytotoxic potential of $CD8^+$ T cells, inducing production of cytophilic antibodies by B cells and enhancing phagocytic abilities of immune cells such as macrophages [39] that aid in parasite opsonization and clearance. In the liver stage, IFN-γ binds to its receptor (IFNGR) and activates the JAK-STAT1 pathway leading to downstream signaling that induces the expression of inducible nitric oxide synthase in hepatocytes, leading to the production of nitric oxide, which either kills or inhibits intrahepatic parasites (sporozoites) and prevents establishment of infection.

High, early levels of IFN-γ are associated with protection against severe malaria [40]. However, excessive or late production of IFN-γ, particularly in susceptible young children (naïve returning travelers), contributes to immunopathology, such as the brain inflammation seen in cerebral malaria, by inducing adhesion molecules (e.g. ICAM-1, VCAM-1) that activate the vascular endothelium and cause infected red blood cells to sequester in the microvasculature [41]. IFN-γ and IL-6 interact as part of a complex regulatory network. IL-6 plays a broader role in systemic inflammation and adaptive immune responses. C-reactive protein (CRP), one of the main acute phase proteins, is modulated mainly by IL-6, TNF-α and IFN-γ. Previous studies demonstrated that these proteins were released during malaria infection [42, 43]. A strong binding of CRP with infected erythrocytes that activate the complement pathway [44] leading to infected erythrocyte clearance and hemolysis, a contributory factor to severe malarial anemia. IL-6 has been characterized to use trans-signalling (binding to soluble receptors, sIL-6R) to act on a wider range of non-immune cells (e.g., endothelial cells), leading to widespread inflammation and contributing significantly to the organ pathology seen in severe malaria. Inhibition of this trans-signalling pathway has been shown to protect mice from lethal malaria and members of these signalling pathway have been explored as drug targets [45]. The outburst of these cytokines is manifested in clinical symptoms such as fever, fatigue, and inflammation-related pathology [9, 10, 12]. Therefore, concomitant elevated levels of IL-10 in symptomatic children likely reflect a compensatory anti-inflammatory mechanism, aimed at optimizing the immune response to curtail sustained inflammation and prevent immunopathology [9, 10, 12].

In this light, the strong negative correlation between IL-10 in symptomatic children and qPCR Ct values (r = -0.973) and positive correlation with parasite density ($r = 0.911$) further support its role as a potential biomarker for high parasite burden and disease progression.

In asymptomatic children, cytokine levels were generally lower, with IL-10 being the only cytokine significantly elevated compared to uninfected children. This may reflect an immune-tolerant state, where IL-10 modulates inflammation sufficiently to prevent clinical symptoms, despite low-level parasitaemia. These findings reinforce the role of IL-10 as a key immunoregulatory cytokine that works in concert with other immune mediators to either result in parasite clearance or parasite persistence in low density chronic state. Similarly, IFN-γ may play a dual role: aiding parasite clearance while potentially exacerbating clinical symptoms when overexpressed. The differential expression and correlation patterns of IL-10 and IFN-γ between symptomatic and asymptomatic children support the idea that these cytokines are central to malaria pathophysiology, not only as markers of immune activation but also as potential modulators of infection outcome [46, 47].

In general, only 60% of infections were detectable by microscopy compared to 100% by qPCR. The discrepancy between infections detected by qPCR and those identified via microscopy is not surprising and underscores the superior sensitivity of molecular diagnostics in detecting sub-microscopic parasitemia. This, however, brings into focus the need to address the diagnostic gap in detecting low-level malaria infections using easily deployable and scalable molecular-based point-of-care diagnostics [48], particularly in low-transmission or elimination settings where traditional microscopy may underestimate low-level infections, which can still contribute to disease transmission and adverse health outcomes [17]. However, the cost of qPCR reagents plus skilled personnel makes this option prohibitive for resource poor malaria endemic settings, highlighting the need for cheaper but efficacious tools such as digital diagnostic point-of-care assays based on the loop-mediated isothermal amplification (LAMP) techniques [49], including EasyNAT and Alethia [50]. These newer highly sensitive tools have the potential to revolutionise malaria diagnoses across *Plasmodium* species at very low infection levels accurately and reliably [49], and would key for malaria surveillance at the household level for asymptomatic infections, returning travellers from endemic populations, and post-treatment monitoring in resource poor settings.

The study also highlights the role of genetic and hematological factors that appear to influence falciparum malaria susceptibility and thus the infection outcomes. The absence of infections in children carrying HbAS, HbSC, or HbAC genotypes during the study period is consistent with the protective effect of these hemoglobin variants due to poor parasite growth reported in previous studies [51–53]. There is also evidence that differential

expression of CXCL10, TNF-α, CCL2, IL-8, and IL-6 pro-inflammatory cytokines may be linked to hemoglobin genotype and falciparum malaria susceptibility [54]. Additionally, the predominance of blood group O in this cohort may have conferred partial protection, as this blood group has been associated with reduced rosetting and cytoadherence, which are key mechanisms involved in severe malaria pathogenesis [55, 56].

The longitudinal nature of this study allowed for real-time monitoring of infection outcomes and immune responses, thereby offering a comprehensive view of malaria dynamics in this paediatric cohort. The finding that only a third of infected children transitioned to symptomatic malaria, despite similar environmental exposure, highlights the role of host-specific factors, such as genetic background, prior immunity, and immune-regulatory mechanisms, in modulating disease expression.

While the study provides meaningful insights, certain limitations must be acknowledged. The relatively small sample size limited our ability to detect immune correlates with less strong effects, potentially missing subtle associations. Nevertheless, the observed cytokine patterns are consistent with findings from larger, more powered studies, supporting the robustness of our results. However, caution should still be exercised when generalizing these findings beyond the studied cohort or region, as local epidemiological, genetic, and environmental factors may influence immune responses. Additionally, cytokine quantification by ELISA, while robust, may not capture the full complexity of immune responses, particularly at the cellular and functional levels. Future research employing high-dimensional techniques such as flow cytometry, single-cell transcriptomics, or multiplex cytokine profiling could provide a more nuanced understanding of immune heterogeneity in malaria.

Conclusion

This study reveals that distinct systemic cytokine signatures, particularly elevated IL-6, IFN-γ, and IL-10, define symptomatic *Plasmodium falciparum* malaria in children aged 6 to 10 years in Togo. These cytokines were significantly associated with parasite burden and clinical symptoms, distinguishing symptomatic cases from asymptomatic or uninfected states. The findings underscore the value of IL-10 and IFN-γ as potential immunological biomarkers of disease progression and immune activation. This highlights the need for molecular diagnostics to detect low-density infections, including sub-microscopic cases that would likely be missed by traditional microscopy. Further research into the mechanistic regulation of cytokines, other immune factors and the interplay with host genetic factors is essential to guide targeted interventions and improve malaria treatment outcomes in vulnerable paediatric populations.

Abbreviations

ELISA	Enzyme-Linked Immunosorbent Assay
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DBS	Dried blood spot
HbA	Hemoglobin A
HbS	Hemoglobin S
HbC	Hemoglobin C
HbF	Hemoglobin F
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IFN-γ	Interferon gamma
IL-17A	Interleukin-17
IL-5	Interleukin-5
IL-10	Interleukin-10
PBMCs	Peripheral blood mononuclear cells
qPCR	Quantitative polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-12499-6.

Supplementary Material 1

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Author contributions

L.A., G.K., M.R. and G.A.A. contributed to the design and conceptualization of the study; F.D.B., G.K., H.C.S., T.K., S.B., M.S., A.J.K., C.M., E.D., and F.M. contributed to sample collection and processing. F.D.B. performed the ELISA, data analysis and drafted the manuscript. L.A., G.K., and M.R. provided resources and contributed to data interpretation, writing, review and editing of the manuscript. All authors read and approved the final version.

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Data availability

The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the "Comité de Bioéthique pour la Recherche en Santé (CBRS)", Lomé, Togo, under the Ministry of Health, Public Hygiene, and Universal Access to Care (Opinion No 045/2023/CBRS, dated November 2, 2023). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participating children, and verbal assent was obtained from the children prior to their inclusion in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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