

Immune dysregulation in malaria, typhoid fever, and co- infection: roles of cytokine and oxidative stress profiles in a case-control study

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Abstract

Background: Malaria and typhoid fever are among the most common causes of clinical consultations in tropical regions, posing significant diagnostic challenges. The role of physiological stress in immune modulation, as well as the relationship between oxidative stress and cytokine profiles during co-infection, remains poorly explored. This study aimed to evaluate cortisol levels, oxidative stress balance, and immune alterations during co-infection with *Plasmodium* and *Salmonella*, and to assess the diagnostic performance of selected biomarkers among febrile patients.

Methods: This cross-sectional observational study involved 81 participants with malaria and/or typhoid fever, as well as healthy individuals and febrile controls, recruited from patients attending the Etoug-Ebe Baptist Hospital in Yaoundé, Cameroon. The stress marker cortisol, malondialdehyde, superoxide dismutase and catalase activities and a panel of four cytokines (IL-4, IL-10, IFN- γ , and TNF- α) were measured using ELISA and spectrophotometric techniques. Statistical analyses were performed using SPSS version 26 and GraphPad Prism version 9. Principal component analysis and variable factor mapping were conducted using R software version 4.5.3. Receiver operating characteristic curve analysis was performed to discriminate between infection status and febrile conditions.

Results: Cortisol and Malondialdehyde levels were significantly elevated in co-infected individuals compared to controls ($p < 0.01$), accompanied by reduced superoxide dismutase and catalase activities. Cortisol showed significant positive correlations with body temperature and parasitemia, and negative correlations with antioxidant markers. Significant increases in IFN- γ ($p < 0.0001$), IL-10 ($p < 0.01$), and the IFN- γ /IL-10 ratio ($p < 0.0001$) were observed in infected patients compared to controls. IL-10 levels were significantly higher during malaria and typhoid fever mono-infections than during co-infection ($p < 0.0001$). Principal component analysis showed that co-infected patients were strongly associated with cortisol, Malondialdehyde, and IFN- γ . Receiver operating characteristic curve analysis demonstrated that cortisol, TNF- α , IL-4, and IL-10 exhibited good diagnostic performance.

Conclusion: Malaria and typhoid fever co-infection may lead to increased physiological stress, enhanced tissue damage, depletion of antioxidant defences, and an imbalance between Th1 and Th2 cytokine responses. Cortisol, IL-4, IL-10, and TNF- α demonstrated promising diagnostic potential. Further studies are required to validate their usefulness in the diagnosis of malaria and typhoid fever among febrile patients.

Clinical trial number Not applicable

1. Introduction

Fever has historically been perceived as a form of punishment from the gods and is one of the oldest recognised manifestations of disease [1]. In tropical regions, febrile illnesses represent a major cause of healthcare consultations, with poverty, poor sanitation, and underdevelopment playing significant roles in their spread [2, 3]. In Cameroon, malaria and typhoid fever are among the most commonly diagnosed

febrile diseases in patients attending health facilities [4]. Malaria is caused by protozoan parasites of the genus *Plasmodium*, transmitted to humans through the bite of an infected female *Anopheles* mosquito [5]. Five species of *Plasmodium* are known to infect humans: *Plasmodium falciparum* (the most virulent), *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* [6, 7]. According to the *World Malaria Report 2025*, there were an estimated 282 million malaria cases worldwide in 2024, with approximately 95% occurring in Africa [8]. In Cameroon, malaria accounts for about 2.7 million cases, with no significant change in incidence reported in 2024 [8]. Typhoid fever, on the other hand, is the most common form of enteric fever globally [9]. It is caused by bacteria of the genus *Salmonella* and is transmitted through the ingestion of contaminated food or water [10]. Four main *Salmonella* serotypes are responsible for enteric fever in humans: *Salmonella Typhi* (responsible for the majority of cases and deaths) and *Salmonella Paratyphi* A, B, and C [4]. According to the latest WHO report on typhoid fever (2019), there are an estimated 11 to 21 million cases worldwide each year, resulting in approximately 128,000 to 161,000 deaths [11].

When the body feels threatened during an infection, stress occurs [12]. It can lead to both physical and psychological health issues, with a rapid, proportional response associated with the degree of infection [13]. The physiological stress response during malaria and typhoid fever has attracted considerable research interest, with evidence indicating that cortisol plays an important role in modulating immune responses against *Plasmodium* and *Salmonella* infections [14–17]. Cortisol, one key stress molecule, is secreted through activation of the Hypothalamic-Pituitary-Adrenal Axis (HPA axis), which is stimulated by pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α [18]. It exerts immunoregulatory effects through a negative feedback mechanism, inhibiting the production of pro-inflammatory cytokines such as IL-2, IFN- γ , IL-1, IL-6, IL-8, IL-12, and IL-1 β , while promoting the production of anti-inflammatory cytokines such as IL-4, IL-5, IL-10, and IL-13 [19]. However, some studies suggest that cortisol may exert a broader suppressive effect, inhibiting both pro- and anti-inflammatory cytokine production [20]. Another particular mechanism in the body has been seen to be associated with the production of cytokines during an infection.

Oxidative stress is a well-established phenomenon linked to the pathogenesis of both malaria and typhoid fever, as well as to associated immune responses [21]. During malaria infection, oxidative stress can be both beneficial and pathological. On one hand, it plays a crucial role in parasite clearance by innate immune cells; on the other hand, excessive oxidative stress contributes to tissue damage [22, 23]. In malaria, classically activated (M1) macrophages generate high levels of reactive oxygen species (ROS) and produce pro-inflammatory cytokines such as TNF- α , IFN- γ , and IL-6. In contrast, alternatively activated (M2) macrophages secrete anti-inflammatory cytokines, including IL-4, IL-10, and IL-13 [24]. Monocytes also contribute to the inflammatory response by producing mediators such as IL-6, nitric oxide (NO), and TNF- α , facilitating the phagocytosis of both infected and uninfected erythrocytes [25]. To regulate this pro-inflammatory response, the host immune system induces anti-inflammatory cytokines, particularly IL-10, IL-4, and transforming growth factor-beta (TGF- β) [17, 26]. Similarly, oxidative stress is observed during *Salmonella* infection and contributes to bacterial clearance while also serving as an indicator of disease status [17, 27, 28]. The NADPH oxidase system plays a key role in the generation of ROS, alongside nitric oxide synthase (NOS) [29]. NOS catalyses the oxidation of L-arginine to NO, a process

that is upregulated in response to microbial products or pro-inflammatory cytokines such as IL-1 β , TNF- α , and IFN- γ [30]. Cell-mediated immunity further enhances antimicrobial defense through T helper 1 (Th1) cells, which produce cytokines such as IFN- γ and TNF- α , promoting the microbicidal activity of macrophages via the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [29]. In contrast, T helper 2 (Th2) cells secrete IL-4, IL-5, IL-10, and TGF- β , supporting B-cell proliferation, differentiation, and the humoral immune response [29].

Studies investigating malaria and typhoid fever co-infection, particularly in relation to oxidative stress and immune alterations, remain limited [3, 17, 31]. Despite the high prevalence of both diseases in endemic regions, their combined effects on host immunity, oxidative stress balance, and inflammatory responses are still poorly understood. In particular, the immunological mechanisms underlying co-infection, including cytokine interactions and inflammatory responses, have not been extensively elucidated. Furthermore, although several studies have highlighted the diagnostic potential of cytokines as biomarkers for infectious diseases [32], including some evidence of malaria [33]. Little is known about their diagnostic performance and clinically relevant cut-off values during malaria and typhoid fever co-infection. In addition, the relationship between oxidative stress, cytokine responses, and physiological stress during co-infection remains largely unexplored. Therefore, this study aimed to evaluate inflammatory cytokine profiles, cortisol levels, and oxidative stress parameters among febrile patients positive for malaria and/or typhoid fever. The study also assessed the relationships between stress response and cytokine balance, as well as the diagnostic potential of selected biomarkers using the Receiver Operating Characteristic (ROC) curve analysis.

2. Methodology

2.1 Study Site

This study was conducted at the Etoug-Ebe Baptist Yaoundé, Cameroon (Fig. 1). This hospital is one reference centre in the city of Yaoundé, situated at 3°50'58.6"N and 11°28'59.6"E (latitude: 3.8496017, longitude: 11.4832077) with a high daily patient intake.

2.2. Study population and design

A cross-sectional study was conducted from June to August 2023, involving 288 febrile patients aged 2 years or older who presented with a body temperature of 37.5°C or higher, or a history of fever. Participants were recruited after obtaining informed consent from adults or assent from parents or legal guardians for minors. All patients were clinically suspected of having malaria and/or typhoid fever and were randomly selected. Following laboratory diagnosis, participants were classified into four groups: febrile patients negative for both malaria and typhoid fever, malaria-positive patients, typhoid fever-positive patients, and co-infected patients. Based on screening tests (including HIV testing) and exclusion criteria such as blood transfusion within the previous 48 hours, history of chronic disease, within the previous 48 hours, pregnancy or lactation, and use of immunosuppressive agents, a final selection of

participants was made. Additionally, asymptomatic controls aged above 2 years, with no history of fever within the previous 7 days and negative for malaria, typhoid fever, and HIV screening tests, were recruited from the community to serve as healthy controls. For biomarker analysis, participants were divided into five groups: febrile controls (n = 15), asymptomatic controls (n = 10), malaria-positive patients (n = 18), typhoid fever-positive patients (n = 15), and co-infected patients (n = 23) (Fig. 2).

2.3. Sample and data collection

Data on socio-demographic characteristics, clinical signs and symptoms, comorbidities, initial diagnosis, prescribed medications, and past medical history were collected by the principal investigator and trained technicians through face-to-face interviews using a structured case report form. A total of 5 mL of venous blood was collected from each participant for malaria diagnosis using rapid diagnostic tests (RDTs) and thick blood film microscopy, as well as for plasma extraction for the assessment of oxidative stress markers and for typhoid fever diagnosis using the Widal slide and tube tests and a rapid IgM/IgG typhoid test. In addition, stool samples were collected in sterile containers for the detection of *Salmonella* O and H antigens. Plasma was obtained by centrifugation of blood samples at 3000 rpm for 5 minutes and was stored in cryotubes at – 40°C until further analysis. All subsequent laboratory assays were conducted at the Molecular Diagnostic Research Laboratory (MDR-Lab), where the assay of oxidative stress markers and cytokines was performed.

2.4 Laboratory diagnosis

2.4.1 Diagnosis of malaria

Malaria diagnosis was performed using a Rapid Diagnostic test for screening and a Thick Blood Film for parasite confirmation and count. The SD Bioline Malaria Pf/pan (catalogue number # 05FK40) was used according to the manufacturer's instructions. A thick blood film was done to confirm the rapid diagnostic test, as described by [34] and parasite count was done as reported elsewhere [12]. Patients who tested positive by rapid diagnostic test (RDT) and were confirmed by thick blood film microscopy, or those positive by thick blood film microscopy alone, were considered finally malaria-positive, as reported by [4].

2.4.2 Diagnosis of Typhoid Fever

Typhoid fever diagnosis was based on serological and antigen detection techniques, including the qualitative and quantitative Widal test (cut-off titre $\geq 1/80$; Catalogue number# B03R-0017-P-0040), the Typhidot IgM/IgG rapid test from the AllTest device kit (Catalogue number# IT7-402), and a rapid antigen-based test for *Salmonella* O and H antigens in stool samples (Catalogue number# ISTY-602).

The Widal test was performed according to the manufacturer's instructions. Acute serum was used with the test containing the somatic (O) and flagella (H) antigens of *S. typhi* and *S. Paratyphi* A, B and C. All positive results obtained through a slide test were confirmed and quantified by the 1/80 tube agglutination method. The Typhidot rapid test used in this study is a lateral flow immunochromatographic assay designed to detect IgM and IgG antibodies against the O and H antigens of *Salmonella typhi* and

Salmonella paratyphi in patient serum. The test was performed and interpreted according to the manufacturer's guidelines, as previously described by [35]. In addition, the rapid antigen test for *Salmonella* O and H antigens was used to supplement and confirm typhoid fever diagnosis. The procedure and interpretation of this test have been described elsewhere [38]. A diagnostic algorithm combining the results of these three tests was used to classify patients as positive or negative for typhoid fever, according to the method described by [4].

2.5. Molecular Markers analysis

2.5.1. Stress marker cortisol and oxidative stress parameters (Malondialdehyde, Catalase, Superoxide Dismutase)

Plasma cortisol concentration was determined using the ARIGO Laboratory kit (catalogue number# ARG81392) and Plasma Malondialdehyde (MDA) concentration using the Elab-science kit (Catalogue number# E-EL-0060) according to the manufacturer's instructions.

The activities of superoxide dismutase (SOD) and catalase (CAT) were measured using commercially available kits: the EnzyChrom™ Catalase Assay Kit (Catalogue No. ECAT-100) and the EnzyChrom™ Superoxide Dismutase Assay Kit (Catalogue No. ESOD-100) (BioAssay Systems, USA). All procedures were performed according to the manufacturer's instructions. Catalase activity was determined based on the degradation of hydrogen peroxide (H_2O_2) by catalase, using a redox dye. The change in color intensity measured at 570 nm was directly proportional to the catalase activity in the sample. Briefly, 10 μ L of patient plasma was mixed with 90 μ L of H_2O_2 substrate and incubated for 30 minutes. A standard curve was prepared using H_2O_2 standards. Subsequently, 100 μ L of detection reagent (comprising assay buffer, dye reagent, and horseradish peroxidase enzyme) was added to each test tube, followed by incubation for 10 minutes. Optical density (OD) was measured at 570 nm using a spectrophotometer (Thermo Scientific Genesys 150).

SOD activity was determined using a colorimetric assay provided by the kit. In this method, superoxide radicals (O_2^-) are generated through a xanthine oxidase-catalysed reaction. These radicals react with WST-1 dye to produce a colored product. SOD in the sample scavenges the superoxide radicals, thereby reducing the formation of the colored product. Thus, the colour intensity, measured at 450 nm, is inversely proportional to SOD activity. Before analysis, samples were diluted as required. Briefly, 20 μ L of diluted sample was added to a well of a clear, flat-bottom 96-well plate, followed by 160 μ L of working reagent (composed of assay buffer, xanthine, and dye conjugate). Then, 20 μ L of diluted xanthine oxidase enzyme was added to each well. Absorbance was measured immediately at 450 nm using a microplate reader, followed by incubation for 60 minutes and a second absorbance reading. SOD activity was calculated using the formula provided by the manufacturer.

2.5.2. Pro-inflammatory (IFN- γ , TNF- α) and anti-inflammatory (IL-10, IL-4) cytokine profiles

The assay of inflammatory cytokines was performed at the Molecular Diagnostic Research Laboratory using commercially available enzyme-linked immunosorbent assay (ELISA) kits and plasma samples. Interleukin-10 (IL-10; Catalogue No. 02012), interleukin-4 (IL-4; Catalogue No. 02008), and interferon-gamma (IFN- γ ; Catalogue No. 02002) were measured using STERMCCELL ELISA kits, while Tumor necrosis factor-alpha (TNF- α ; Catalogue No. ELH-TNF α) was quantified using a RayBiotech ELISA kit. All assays were conducted according to the manufacturer's instructions.

The quantification of cytokines was based on the sandwich ELISA principle. Briefly, standard cytokine solutions were prepared by serial dilution in assay buffer to obtain a range of concentrations specific to each biomarker. The standards were dispensed into designated wells of a microplate, followed by the addition of test plasma samples to the appropriate wells along with the sample diluent. A blank well containing only chromogen solutions A and B and stop solution, without plasma or reagents, was included. The plate was incubated at 37°C for 30 minutes, after which horseradish peroxidase (HRP)-conjugated reagent was added to all wells except the blank. Following incubation, the plate was washed four times with wash buffer to remove unbound components. Chromogen solutions A and B were then added, and the plate was incubated at 37°C for 15 minutes to allow color development. The reaction was stopped by adding the stop solution to each well. Optical density (OD) was measured at 450 nm within 15 minutes using a microplate reader (ICEN, Model IN-B149, China). The OD value of the blank well was set to zero. A standard curve was generated by plotting OD values against known cytokine concentrations, and this curve was used to determine the concentrations of cytokines in the test samples.

2.6. Statistical analysis

Statistical analysis was performed using SPSS version 26.0, and graphs and figures were created using Graph-Pad Prism version 9. Categorical variables were present with frequencies and percentages, and the Chi-square test was used to compare group differences between categorical variables. For continuous variables, data were tested for normality using the Shapiro-Wilk test. Parametric data and nonparametric data were presented as means $\pm$ standard deviation and medians (25th-75th percentiles), respectively. Non-normal distributed data were log-transformed during analysis to follow a normal distribution. For the difference between 02 or more groups, the Student T-test, ANOVA (with Post-Hoc Tukey) and Kruskal-Wallis with Dunn's test were used where appropriate. Correlation analysis was done using Pearson's correlation test or Spearman's test as appropriate. To test if any of these markers measured could discriminate between malaria, typhoid fever and the co-infection during fever, a Receiver Operating Characteristic Curve (ROC) analysis was performed. Diagnostic performance was assessed with sensitivity, specificity, predictive values, and Likelihood ratios with precision at 95% confidence intervals. Principal component Analysis and cos² Circle (Variable factor Mapping) were done using the R statistical software (version 4.5.3) on log-transformed data. In this study, a *p*-value < 0.05 was considered significant at 95% CI.

3. Results

3.1 Socio-demographic characteristics

General socio-demographic characteristics of patients ($n = 71$) and Healthy control ($n = 10$) are presented in Table 1. Among febrile patients selected for the rest of the study, the ratio female/male was 1.47, and the median age was 26 (17–37), whereas among controls, the ratio female/male was 0.11 and the median age was 24 (22–32). Aside from fever, the most common symptoms were headache (63%, $n = 51$), abdominal pain (37%, $n = 30$), general body pain (25.9%, $n = 21$), vomiting (18.5%, $n = 15$) and sweating (7.4%, $n = 6$). There was no significant difference with age, and our study population ($p = 0.9$), but an association was found between gender and our study population ($p = 0.014$). An increase in body temperature was obtained between the co-infection and asymptomatic control group ($p = 0.009$).

Table 1
Socio-demographics Characteristics of the study population

Characteristics	HC (n = 10)	FC (n = 15)	M (+) (n = 18)	T (+) (n = 15)	M/T (n = 23)	p-value
Age (mean ± S.D)	26.7 (± 6.78)	28.4 (± 14.86)	25.56 (± 13.53)	29.9 (± 12.5)	28.83 (± 16.84)	0.9
Gender	9 (90)	10 (53.33)	11 (61.1)	5 (33.3)	8 (34.8)	0.014
Male, n (%)						
Female, n (%)	1 (10)	5 (46.67)	7 (38.9)	10 (66.7)	15 (65.2)	
Body temperature mean ± S.D	36.6 (± 0.9)	37.27 (± 0.96)	37.6 (± 1.21)	37.52 (± 0.9)	38.08 (± 1.29)	0.009
Average weight (mean ± S.D)	75.76 (± 7.64)	63.83 (± 23.9)	67.44 (± 14.1)	71.3 (± 19.22)	65.93 (± 23.63)	0.4
Clinical presentation on admission						
Fever, n (%)	/	15 (100)	18 (100)	15 (100)	23 (100)	0.033
Headache, n (%)	/	7 (46.7)	16 (88.9)	9 (60)	19 (82.6)	0.021
Abdominal Pain, n (%)	/	7 (46.7)	5 (27.8)	6 (40)	12 (52.2)	$p > 0.05$
Profuse Sweating, n (%)	/	0 (0.0)	4 (22.2)	0 (0.0)	2 (8.7)	0.007
Anorexia, n (%)	/	0 (0.0)	2 (11.1)	1 (6.7)	2 (8.7)	$p > 0.05$
General Body Pain, n (%)	/	4 (26.7)	2 (11.1)	6 (40)	9 (39.1)	$p > 0.05$
Jaundice, n (%)	/	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	$p > 0.05$
Shaking Chill, n (%)	/	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	$p > 0.05$
Vomiting/Nausea, n (%)	/	5 (33.3)	4 (22.2)	4 (26.7)	2 (8.7)	$p > 0.05$
						$p > 0.05$
						$p > 0.05$
Median Parasitemia (25 th - 75 th)	/		1800 (120-8638)	/	2400 (306-12000)	0.3

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Socio-demographic Characteristics of the study population

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Jaundice, n (%)	/	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	$p > 0.05$
Shaking Chill, n (%)	/	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	$p > 0.05$
Vomiting/Nausea, n (%)	/	5 (33.3)	4 (22.2)	4 (26.7)	2 (8.7)	$p > 0.05$
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						$p > 0.05$
Median Parasitemia (25th-75th)	/		1800	/	2400	0.3

HC: Healthy Control; FC: Febrile Control; M(+): Malaria only; T(+): Typhoid Fever only; M/T: Malaria and Typhoid Fever Co-infection. Parametric data presented as mean $\pm$ standard deviation, and non-parametric data presented as median (25th-75th). Non-parametric data were log-transformed to follow a normal distribution and used for statistical analysis. Categorical variables are presented as count (percentage, %). Differences in categorical variables were compared using Pearson's χ^2 test. A Student T test and ANOVA were used for difference between group with. Post-Hoc test (Tukey's Test). A $p < 0.05$ was considered significant.

Characteristics	HC (n = 10)	FC (n = 15)	M (+) (n = 18)	T (+) (n = 15)	M/T (n = 23)	p-value
			(120– 8638)		(306– 12000)	
HC: Healthy Control; FC: Febrile Control; M(+): Malaria only; T(+): Typhoid Fever only; M/T: Malaria and Typhoid Fever Co-infection. Parametric data presented as mean $\pm$ standard deviation, and non-parametric data presented as median (25th-75th). Non-parametric data were log-transformed to follow a normal distribution and used for statistical analysis. Categorical variables are presented as count (percentage, %). Differences in categorical variables were compared using Pearson's χ^2 test. A Student T test and ANOVA were used for difference between group with. Post-Hoc test (Tukey's Test). A $p < 0.05$ was considered significant.						

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3.2 Laboratory finding

3.2.1 significant increase in stress markers and reduction in antioxidants in patients compared to controls.

Cortisol level was twice as high in co-infection compared to mono-infections and asymptomatic control ($p < 0.0001$). For oxidative stress markers, a higher production of Malondialdehyde was obtained in co-infection compared to control ($p < 0.001$); the same was seen during Typhoid fever only ($p < 0.05$). A reduction of antioxidant markers SOD and CAT was seen during the co-infection ($p < 0.001$). Asymptomatic control harbouring the highest level of SOD and CAT activities (Fig. 3).

3.2.2 Increased pro-inflammatory and anti-inflammatory cytokine concentrations in patients compared to healthy controls.

Regarding pro-inflammatory cytokines IFN- γ and TNF- α , a significant production of IFN- γ was obtained during malaria ($p < 0.001$), typhoid fever ($p < 0.01$), febrile control ($p < 0.001$) and the co-infection ($p < 0.0001$) compared to healthy control (HC). TNF- α level was statistically different between the Malaria (+) and febrile control subjects ($p < 0.01$). A reduction of this pro-inflammatory cytokine was seen during malaria, compared to febrile control and asymptomatic control, but it was not statically significant ($p > 0.05$) (Fig. 4).

For anti-inflammatory cytokines IL-4 and IL-10, a significant diminution of IL-4 was obtained in the typhoid fever group compared to the healthy control ($p < 0.05$). An increase of IL-4 was seen during the co-infection compared to typhoid fever mono-infection ($p < 0.05$). For IL-10 concentration, a significant increase was seen in the febrile control group ($p < 0.05$), malaria mono-infection ($p < 0.0001$), typhoid fever mono-infection ($p < 0.0001$) and co-infection ($p < 0.01$) compared to the asymptomatic control group. Also, we noted that IL-10 level was most pronounced in malaria ($p < 0.0001$) and typhoid fever ($p < 0.01$) mono-infection compared to the co-infection group. Aside from these results, a higher concentration of IL-10 was obtained during malaria and typhoid fever mono-infection compared to febrile control (Fig. 4).

NB

TNF- α data were not available for 2 co-infected patients. IL-10 and IL-4 data were not available for 3 Malaria patients and 5 co-infected patients (due to technical difficulties occurring in the laboratory during the ELISA test).

1. Increased in IFN- γ /IL-10 Ratio and reduction in TNF- α /IL-10 Ratio level in co-infected patients compared to healthy controls.

The ratio between IFN- γ /IL-10 and TNF- α /IL-10 was assessed, due to the implications of these 3 cytokines in various immune regulations and viability of data conserving their association in disease severity and fatal conditions [36–38].

For IFN- γ /IL-10 ratio, a significant increase was noted during malaria and typhoid fever co-infection compared to malaria mono-infection ($p < 0.05$) and Healthy control ($p < 0.0001$). An increase was also obtained in the febrile control group ($p < 0.001$) and typhoid fever mono-infection group ($p < 0.01$) compared to the control group (Fig. 5).

Regarding TNF- α /IL-10, a reduction of the ratio was obtained during malaria compared to Healthy control ($p < 0.05$) and febrile control ($p < 0.01$) (Fig. 5).

3.2.4 Complex interplay between stress markers and cytokines during malaria and/or Typhoid fever infections.

A complex relationship was obtained between cortisol, oxidative parameters and cytokines during malaria, Typhoid fever and the co-infection (Supplementary table 1). Cortisol was positively correlated with Log₁₀Parasitemia during the co-infection ($r = 0.74$, $p < 0.0001$) and slightly during malaria ($r = 0.56$, $p = 0.056$). A strong positive correlation between Body temperature and Cortisol was obtained in the febrile control group ($r = 0.67$, $p = 0.0067$), Malaria (+) ($r = 0.61$, $p = 0.007$), Typhoid fever (+) ($r = 0.63$, $p = 0.012$) and the co-infection ($r = 0.56$, $p = 0.005$). Between anti-oxidant markers, a negative correlation was seen between cortisol and SOD during Typhoid fever ($r = -0.7$, $p = 0.003$) and Malaria ($r = -0.5$, $p = 0.044$). The

same was seen between cortisol and CAT during Malaria ($r = -0.5$, $p = 0.033$) and the co-infection ($r = -0.6$, $p = 0.005$).

Regarding cytokines, a positive correlation between IFN- γ and cortisol was obtained in the febrile control group ($r = 0.56$, $p = 0.032$) and during the co-infection ($r = 0.47$, $p = 0.025$). The same was seen in other groups, although not significantly. Conversely, cortisol was negatively correlated with anti-inflammatory cytokines IL-4 during the co-infection ($r = -0.46$, $p = 0.049$) and with IL-10 during malaria ($r = -0.56$, $p = 0.033$) and during the co-infection ($r = -0.66$, $p = 0.003$) (supplementary Table 1). Cytokines IL-4 ($r = -0.5$, $p = 0.032$) and IL-10 ($r = -0.775$, $p = 0.0003$) were negatively correlated with Parasitemia during the co-infection.

3.2.5 Association between each marker and the different study cohort groups

To investigate the relationships among the eight biomarkers measured in this study, as well as their associations with the five study groups, principal component analysis (PCA) was performed. A PCA biplot and a $\cos^2$ (cosine squared) correlation circle were generated for the contributions of each biomarker and their relationships with the study groups.

The $\cos^2$ values showed how well each variable was represented in the first two PCA dimensions (Supplementary Table 2). IFN- γ was the best represented variable on Dim1 ($\cos^2 = 0.600$), followed by cortisol (0.396) and MDA (0.385). On Dim2, TNF- α showed the highest representation ($\cos^2 = 0.518$), followed by IL-4 (0.342) and IL-10 (0.318). CAT showed moderate representation across both dimensions, whereas SOD was poorly represented, with a total $\cos^2$ value of 0.046.

Figure 6A presents the variable factor map showing two principal biological axes. Dim1 (21.9%) was mainly associated with IFN- γ , cortisol, and MDA, while Dim2 (17.2%) was primarily associated with TNF- α , IL-4, and IL-10. CAT showed an opposite orientation to IFN- γ on Dim1, whereas SOD remained close to the origin, indicating weak representation in the two-dimensional space.

Figure 6B shows the PCA biplot illustrating group distribution according to biomarker profiles. Healthy controls clustered separately from infected groups along Dim1. Febrile controls occupied a central position, partially overlapping with malaria and typhoid fever groups. Malaria and typhoid fever patients showed similar clustering patterns, while co-infected patients were positioned furthest along Dim1, indicating stronger associations with IFN- γ , cortisol, and MDA.

3.2.6 Discriminative capacities of stress markers, cortisol, and inflammatory cytokines using a ROC curve

Biomarkers of oxidative stress and cytokines were evaluated, and their potential as diagnostic markers for malaria and typhoid fever was assessed using receiver operating characteristic (ROC) curve analysis. Using febrile controls as the reference group and malaria, typhoid fever, and co-infected patients as

disease groups, we determined the diagnostic accuracy of the biomarkers investigated in this study. An area under the curve (AUC) > 0.6 with a p-value < 0.05 was considered statistically significant. Among the oxidative stress markers measured, only cortisol demonstrated a significant diagnostic performance, with an AUC of 0.80 ($p = 0.0023$) in distinguishing co-infected patients from febrile controls (Fig. 7). Sensitivity, specificity, predictive values, and likelihood ratios are presented in Table 2. Regarding cytokines, IL-10, IL-4, and TNF- α showed potential as diagnostic markers for malaria and typhoid fever. IL-10 (AUC = 0.95, $p < 0.0001$) and TNF- α (AUC = 0.80, $p = 0.0038$) demonstrated strong diagnostic performance for malaria among febrile patients. For typhoid fever, IL-10 (AUC = 0.80, $p = 0.009$) and IL-4 (AUC = 0.82, $p = 0.0022$) were identified as potential markers. TNF- α (AUC = 0.73, $p = 0.029$) also showed moderate diagnostic value for identifying co-infection. Clinical cut-off values and their associated diagnostic performance are presented in Fig. 7 and Table 2.

Table 2

Diagnostic performance of Cortisol, IL-4, IL-10 and TNF- α during malaria, typhoid fever and typho-malaria co-infection.

Parameter	Cuff-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Lr (+)	Lr (-)	Accuracy
Febrile control as control and Malaria (+) as disease								
IL-10 (pg/mL)	> 338.6	93.3 (73.8–99.6)	86.7 (64.2–97.7)	87.5 (66.2–97.8)	92.9 (72.1–99.6)	7 (1.9–25.61)	0.77 (0.01–0.52)	90
TNF-α (pg/mL)	< 155.8	72.2 (49.5–89)	86.7 (64.2–97.7)	86.7 (64–97.7)	72.2 (50–89)	5.4 (1.45–20.3)	0.32 (0.15–0.69)	78.78
Febrile control as control and Typhoid Fever (+) as disease								
IL-10 (pg/mL)	> 325.9	80 (56–94.6)	66.7 (41.5–86.5)	70.6 (47.1–88.3)	76.9 (50.5–93.7)	2.4 (1.12–5.13)	0.33 (0.1–0.88)	73.33
IL-4 (pg/mL)	< 21.5	80 (56–94.6)	73.3 (48.5–90.8)	75 (51.1–91.5)	78.6 (53.4–94.2)	3 (1.25–7.2)	0.27 (0.09–0.79)	76.66
Febrile control as control and Co-infection as disease								
TNF-α (pg/mL)	< 175.1	75 (53.8–90.2)	66.6 (33–76.6)	65.2 (44.9–82.3)	66.7 (41.5–86.5)	1.7 (0.95–2.9)	0.45 (0.19–1.07)	65.79
Cortisol (ug/dl)	> 13.44	82.6 (64.1–94.2)	60 (35.1–81.7)	76 (35.1–81.7)	69.2 (42.3–89.3)	2.07 (1.08–4)	0.29 (0.11–0.77)	73.68

PPV: Positive Predictive Value, NPV: Negative Predictive Value; Lr: Likelihood ratio.

4. Discussion

Over the past two decades, numerous biomarkers have been developed to detect oxidative stress, which induces damage to DNA, proteins, lipids, and carbohydrates. These biomarkers play an important role in disease prevention and therapeutic management [39]. However, studies investigating stress levels and disease severity in cases of malaria and typhoid fever co-infection remain limited. Furthermore, the immune response in such co-infections is not fully understood, particularly regarding the role of cortisol in modulating immune changes. Given their potential relevance, these biomarkers may serve as valuable

tools for early diagnosis, especially among febrile patients. Therefore, this study aimed to assess oxidative stress balance, cytokine profiles, and the diagnostic performance of these markers using ROC curve analysis to identify reliable biomarkers for diagnostic applications.

The increase in Cortisol level in co-infection compared to mono-infections and controls (Fig. 3) represent increase in severity and could be due to a greater HPA axis activation, as reported by previous authors [15, 17]. This higher production of cortisol may also indicate a dysregulation of the HPA axis, with an inhibition of the negative feedback induced by cortisol to regulate its production [40]. A strong positive correlation was seen between cortisol and parasitaemia malaria co-infection with typhoid fever ($r = 0.74, p < 0.0001$). This result may indicates infection severity. [12]. These positive correlations observed between cortisol and Body temperature (supplementary Table 1) could further confirm the implication of cortisol in the pathogenesis of malaria, typhoid fever and the co-infection, as fever is a common sign during malaria and typhoid fever, reflecting stress responses and HPA axis activation [12, 41]. Fever reflects the time of blood cell lysis. Schizogony during malaria and intra-intestinal invasion by typhoid fever, with blood loss, may have induced an elevation of temperature, generating then an intense stress to the body with the stimulation of the HPA axis and cortisol release.

Regarding oxidative stress markers, a significant increase in Malondialdehyde was noted in these infectious groups (Fig. 3). Previous studies report an increase in malondialdehyde levels during malaria infection [22, 42–44], with the implication of oxidative burst by the Immune system or the pathogens as a generator of Free oxygen species, leading to lipid peroxidation. Previous reports found an increase in MDA in co-infected mice compared to the vehicle [45]. The reduction of the activities of anti-oxidant markers SOD and CAT in co-infection compared to control (Fig. 3) confirms the generation of Reactive oxygen species (ROS) and the detoxification process, results obtained by previous authors [46–49]. Fever is associated with oxidative stress. A negative correlation was obtained between SOD and MDA ($r = -0.53, p = 0.0041$) in the febrile control group, mainly due to the generation of ROS during the fever production of antioxidants and repair systems. Cortisol was negatively correlated with antioxidant enzymes (SOD and CAT) during malaria and typhoid fever infections (supplementary Table 1). It is well known that cortisol is release though stimulation of the Hypothalamus by endogenous pyrogens such as TNF- α , IL-1 β or IL-6. This result suggests a link between HPA axis activation, immunity, and oxidative stress, potentially impacting antioxidant enzyme activities [20].

Elevation of the levels of pro-inflammatory cytokines at the early stage of an infection contributes to the control and clearance of the microorganism. At the late stage, immunoregulatory cytokines will be produced to regulate the inflammatory process and homeostasis [50]. Increased levels of IFN- γ in the febrile control ($p < 0.001$), malaria positive ($p < 0.001$), typhoid fever ($p < 0.01$), and the co-infection ($p < 0.0001$) compared to the healthy control (Fig. 4) suggest a heightened cellular immune response induced by IFN- γ , especially during the co-infection. Previous reports have found the role of this cytokine in the pathogenesis of malaria [38] and typhoid fever [27]. Correlation analysis revealed that IFN- γ was positively correlated with body temperature during malaria ($r = 0.49, p = 0.039$), typhoid fever ($r = 0.54, p = 0.039$), and the febrile control group ($r = 0.8, p < 0.0001$). This could suggest a production of IFN- γ in response to

homeostasis disturbance, infection and immune response activation. A diminution of TNF- α was seen during typhoid fever compared to febrile control ($p < 0.05$), but no significant difference was obtained between other groups (Fig. 4). The same was seen by Lyke et al., who obtained no significant difference between malaria patients and control subjects [51], but these results also contradict what was obtained by [17, 52], with a higher production of TNF- α during malaria and during the co-infection with Typhoid fever. The diminution of TNF- α in patients with typhoid fever compared to febrile control could be due to the causal role of TNF- α in severity, ROS production, leading to a fatal condition.

Antibodies and humoral immunity are essential during disease [53]. A significant increase in IL-4 was obtained in the co-infection group compared to the typhoid fever group ($p < 0.05$) (Fig. 4). Antibodies play a crucial role in combating *Salmonella* [54], explaining the differences in IL-4 levels. Also, IL-4 can induce the production of antibodies as IFN- γ . The higher level of IL-4 during the co-infection can be due to IFN- γ production. IL-4 was positively correlated with body temperature during malaria ($r = 0.52$, $p = 0.046$) and the co-infection ($r = 0.43$, $p = 0.04$), explaining the implication of this cytokine in infection and immune response. A significant production of IL-10 was obtained in febrile control ($p < 0.05$), malaria ($p < 0.0001$), typhoid fever ($p < 0.0001$) and the co-infection ($p < 0.01$) groups, confirming the results of Eric et al., [17]. This increase explains the regulation of inflammation, as an attempt to control parasite and bacteremia clearance and limit tissue damage, as reported by other authors [36]. IL-4 and IL-10 were positively correlated during typhoid fever ($r = 0.58$, $p = 0.025$), indicating the need for TH2 cytokines and humoral immunity with antibody production. The mean of IL-10 was significantly lower in co-infection (315 ± 24.3) compared to malaria mono-infection (402.62 ± 40.25 , $p < 0.0001$) and typhoid fever mono-infection (370.41 ± 65.23 , $p < 0.01$). Effectively, IL-10 is associated with increased odds of severe malaria [55]. This decrease in IL-10 level in co-infection compared to mono-infections may be due to increased production of IFN- γ . A reduction of IL-10 was also obtained in children with *Plasmodium* and *salmonella typhimurium*, from Kenya [56]. By correlation test, IL-10 was found to be negatively correlated with parasitemia during the co-infection ($r = -0.75$, $p = 0.0003$). This negative association may not solely be explained by reduced inflammatory response control, suggesting the presence of other immunoregulatory molecules influencing this phenomenon, such as cortisol [20]. IL-10 was negatively correlated to IFN- γ during malaria mono-infection ($r = -0.56$, $p = 0.029$), which confirms the imbalance between TH1 and TH2 cytokines, with the increase of IL-10 and reduction of TNF- α level during malaria mono-infection. Looking at the Ratio between cytokines, IFN- γ /IL-10 ratio was significantly higher in co-infection compared to control ($p < 0.001$), malaria mono-infection ($p < 0.05$) (Fig. 5). The same increase was noted in the febrile control group compared to the control ($p < 0.0001$). This confirms an increased production of pro-inflammatory cytokines such as IFN- γ and a cellular immunity-driven response. The increased ratio IFN- γ /IL-10 with a reduction of IL-10 level in co-infection compared to mono-infection could indicate a down-regulation of anti-inflammatory responses in co-infection. TNF- α /IL-10 ratio revealed a reduction during malaria compared to asymptomatic control ($p < 0.05$) and febrile control ($p < 0.01$) (Fig. 5). This reduction indicates an immunity drive to the TH2 profile in mono-infection, as reported by Davenport et al., who obtained a higher production of IL-10 during malaria infection compared to controls with a down-production of TNF- α , explaining it by an attempt to counteract the mechanism of IFN- γ -induces increase

NO, which normally induces the production of TNF- α , leading to the activation of macrophages. Through a feedback mechanism, TNF- α will repress it on synthesis [56].

Regarding the effects of cortisol on cytokines, a negative correlation between Cortisol and anti-inflammatory cytokines was obtained during Malaria with IL-10 ($r = -0.67, p = 0.006$), and the co-infection with IL-4 ($r = -0.46, p = 0.049$), IL-10 ($r = -0.66, p = 0.003$). A positive correlation between Cortisol and IFN- γ ($r = 0.47, p = 0.025$) was obtained during the co-infection (Supplementary Table 1). These findings contradict previous authors' reports on the link between Immunity changes and cortisol, with the negative action of cortisol on pro-inflammatory cytokines and the activation of anti-inflammatory mediators [19, 20]. Some authors have found that a reduction of cortisol receptor synthesis could induce the inhibition of cortisol action [40], leading to cortisol resistance, with the production of pro-inflammatory hormones and anti-microbial substances. There may be an interaction between the human stress response hormone and the cell-mediated inflammatory cytokine IFN- γ . Many studies have been conducted already, suggesting that Gcs induce pro-inflammatory responses, a process depending on time and dose [57, 58].

The PCA analysis further highlighted distinct immunometabolic patterns across the study groups. The strong association between cortisol, MDA, and IFN- γ along Dim1 suggests that oxidative stress, immune activation, and physiological stress responses are closely linked during infection, reflecting a predominantly Th1-driven inflammatory response against both *Plasmodium* parasites and *Salmonella Typhi* [17, 18, 42, 57]. In contrast, IL-10 showed an opposite orientation, supporting its anti-inflammatory and immunoregulatory role against pro-inflammatory mediators such as IFN- γ and cortisol [26, 59]. The negative contribution of CAT and SOD further indicates progressive exhaustion of antioxidant defences during sustained infection, consistent with increased ROS production and oxidative damage [3, 42, 46]. The PCA biplot also demonstrated clear separation of the study groups according to their biomarker profiles. Healthy controls clustered from inflammatory and oxidative stress markers, reflecting normal physiological conditions. Febrile controls occupied an intermediate position, suggesting a non-specific inflammatory response. Malaria and typhoid fever groups showed partially overlapping distributions, likely due to shared inflammatory and immunosuppressive mechanisms characterised by elevated IL-10 levels [27, 28, 36, 55]. In contrast, co-infected patients clustered furthest along Dim1 and were strongly associated with IFN- γ , cortisol, and MDA, indicating amplified oxidative stress and immune activation resulting from the combined burden of malaria and typhoid fever infections, as previously reported [45, 60].

ROC curve analysis was performed to evaluate the diagnostic performance of the studied biomarkers in discriminating malaria, typhoid fever, and co-infection among febrile patients. Among the stress markers, only cortisol showed significant diagnostic performance, with excellent discriminative capacity between co-infected and febrile control patients ($AUC = 0.80; p = 0.0023$), whereas the other stress markers showed poor diagnostic accuracy ($AUC = 0.5-0.6$). Elevated cortisol levels in co-infected patients may reflect increased disease severity and activation of the hypothalamic-pituitary-adrenal axis induced by the dual pathogen burden. Thus, cortisol levels above 13.44 may represent a potential biomarker for malaria-typhoid co-infection. Among cytokines, IL-10 showed strong diagnostic performance for malaria

(AUC = 0.95) and moderate performance for typhoid fever (AUC = 0.78), while IL-4 was a good marker for typhoid fever (AUC = 0.82). TNF- α also showed good discriminative ability for malaria (AUC = 0.80) and moderate performance for co-infection (AUC = 0.73). Overall, increased IL-10 levels associated with reduced TNF- α or IL-4 levels appeared to be associated with malaria and typhoid fever phenotypes among febrile patients. These findings suggest that cytokine profiling may improve discrimination between mono-infection and co-infection and help reduce misdiagnosis in febrile patients.

Febrile control group in the study has a potential influence on the diagnostic performance of evaluated biomarker using the ROC curve analysis. Febrile patients have no evidence of malaria and typhoid fever, but others infectious or inflammatory conditions could have influenced the AUC values and reduce the variability to discriminate between group. Conversely, if an undiagnosed condition produces biomarker responses that differ substantially from those associated with the investigated infections, the apparent discriminatory performance could potentially be overestimated. Therefore, the reported AUC values were interpreted in the context of a clinically febrile comparison group rather than a healthy control population.

Conclusion

Malaria and typhoid fever co-infection is associated with significant alterations in oxidative stress, physiological stress response, and cytokine balance. Co-infected patients showed increased cortisol and malondialdehyde levels, reduced antioxidant enzyme activities, and a predominance of Th1-type immune responses. PCA analysis further highlighted the close association of co-infection with IFN- γ , cortisol, and oxidative stress markers. The findings suggest that cortisol and selected cytokines, particularly TNF- α , may have potential as complementary biomarkers for distinguishing co-infected patients from other febrile conditions. However, further validation studies with larger populations are required before clinical application. Future studies using experimental models may also help clarify the mechanisms underlying glucocorticoid-mediated immune modulation during malaria and typhoid fever co-infection.

Limitations and Perspectives

One major limitation of this study was the relatively small sample size, which may have reduced the robustness of the statistical analyses. In addition, typhoid fever diagnosis relied solely on serological methods, which may not accurately reflect the true infection status of patients. Furthermore, the unequal gender distribution among participants may have introduced gender-related bias. Finally, the etiology of fever among febrile controls was not clearly defined, as patients with different febrile conditions were grouped.

Future research should focus on several key areas. First, larger multicenter cohort studies are needed to validate the diagnostic performance of cortisol, cytokines, and oxidative stress markers in malaria and typhoid fever co-infection, with a more characterisation of the febrile control group, to determine others microbiological causes of fever and validated the predictive AUC values. Second, improved diagnostic approaches incorporating culture-based and molecular techniques should be used to confirm typhoid fever infection status. Third, additional host biomarkers and gene expression profiles should be

investigated to better understand immune activation pathways and the molecular mechanisms underlying disease severity during co-infection. Fourth, longitudinal studies should assess the influence of nutritional status, gender, and other host-related factors on oxidative stress and immune responses. Finally, experimental studies using animal models may help clarify the immunoregulatory role of cortisol and its interactions with cytokine responses during malaria and typhoid fever co-infection.

Abbreviations

CAT Catalase

CIOMS Council for International Organisations of Medical Sciences

EBH Etoug Ebe Baptist Hospital

ELISA Enzyme-Linked Immunosorbent Assay

FC Febrile Control

HC Healthy Control

HPA Hypothalamic-Pituitary-Adrenal Axis

HRP horseradish peroxidase

IL Interleukin

IFN Interferon

IQR Interquartile Range

M (+) Malaria Mono-infection

M/T Malaria and Typhoid Fever co-infection

MDA Malondialdehyde

MDR-Lab Molecular Diagnostic Research Laboratory

NO Nitric Oxide

NOS Nitric Oxide Synthase

OD Optimal Density

PCA Principal Component Analysis

RDT Rapid Diagnostic Test

RNS Reactive Nitrogen Species

ROC Receiver Operating Characteristic

ROS Reactive Oxygen Species

SOD Superoxide Dismutase

T (+) Typhoid Fever Mono-infection

TGF Transforming Growth Factor-beta

Th1 T helper 1

Th2 T helper 2

TNF Tumour Necrosis Factor

Declarations

Ethics approval and consent to participate

The ethical clearance was obtained from the Centre Regional Ethics Committee for Human Health Research CRERSH-Ce (0226-CRERSHC/2022) and the Institutional Review Board of the Cameroon Baptist Convention (IRB2023-26), with administrative authorisation to do research from the Ministry of Public Health (1392-7/AAR/MINSANTE/SG/DRSPC). The research was carried out in compliance with current national and international regulations, as the Cameroon law on research (law n° 2022-008 of the 27th April 2022), including the CIOMS International Ethical Guidelines for Biomedical Research Involving Human (Council for International Organizations of Medical Sciences, CIOMS, 2016), the Declaration of Helsinki (2013). Informed consent was obtained from each participant before the beginning of the study

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Author Contribution

P.M.N. and W.F.M conceptualise the study, acquired funding and supervise the work. A.M.T and M.B.E were in charge of obtaining ethical approval, conducting the investigation, collecting samples in the field and performing laboratory analysis. A.T supervises the work at the Laboratory. A.M.T, S.D.K and K.M.N performed statistical analysis. A.M.T, M.B.E, and P.M.N wrote the original manuscript. N.L.G, K.M.N, S.D.K, and M.B.E critically review the manuscript for scientific content, language and clarity before submission.

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Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable requests.

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Figures

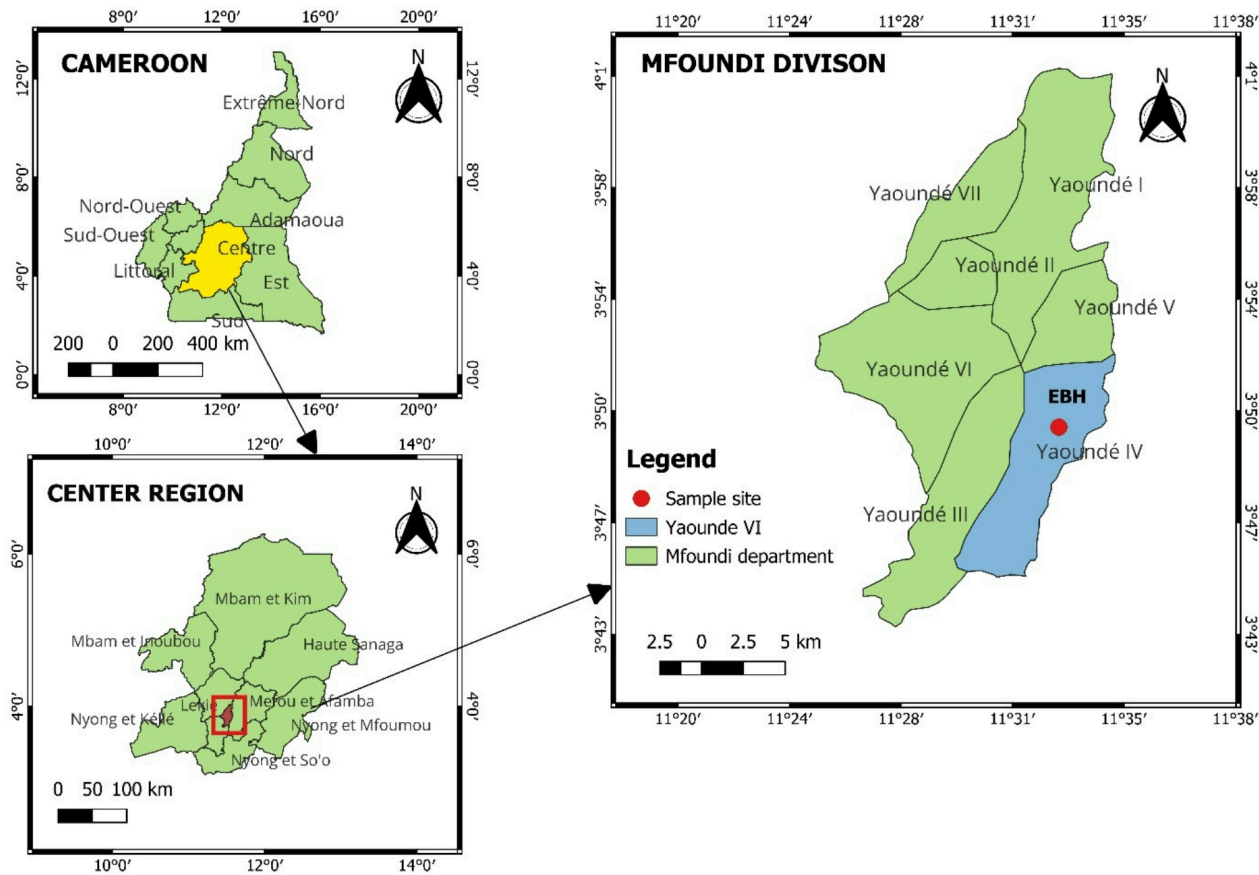


Figure 1

Study participant recruitment and classification. The Map shows the Centre region of Cameroon with the study site. Samples for this study were collected at the Etoug-Ebe Baptist Hospital (EBH), located in Yaoundé. The map was created using QGIS version 3.42.

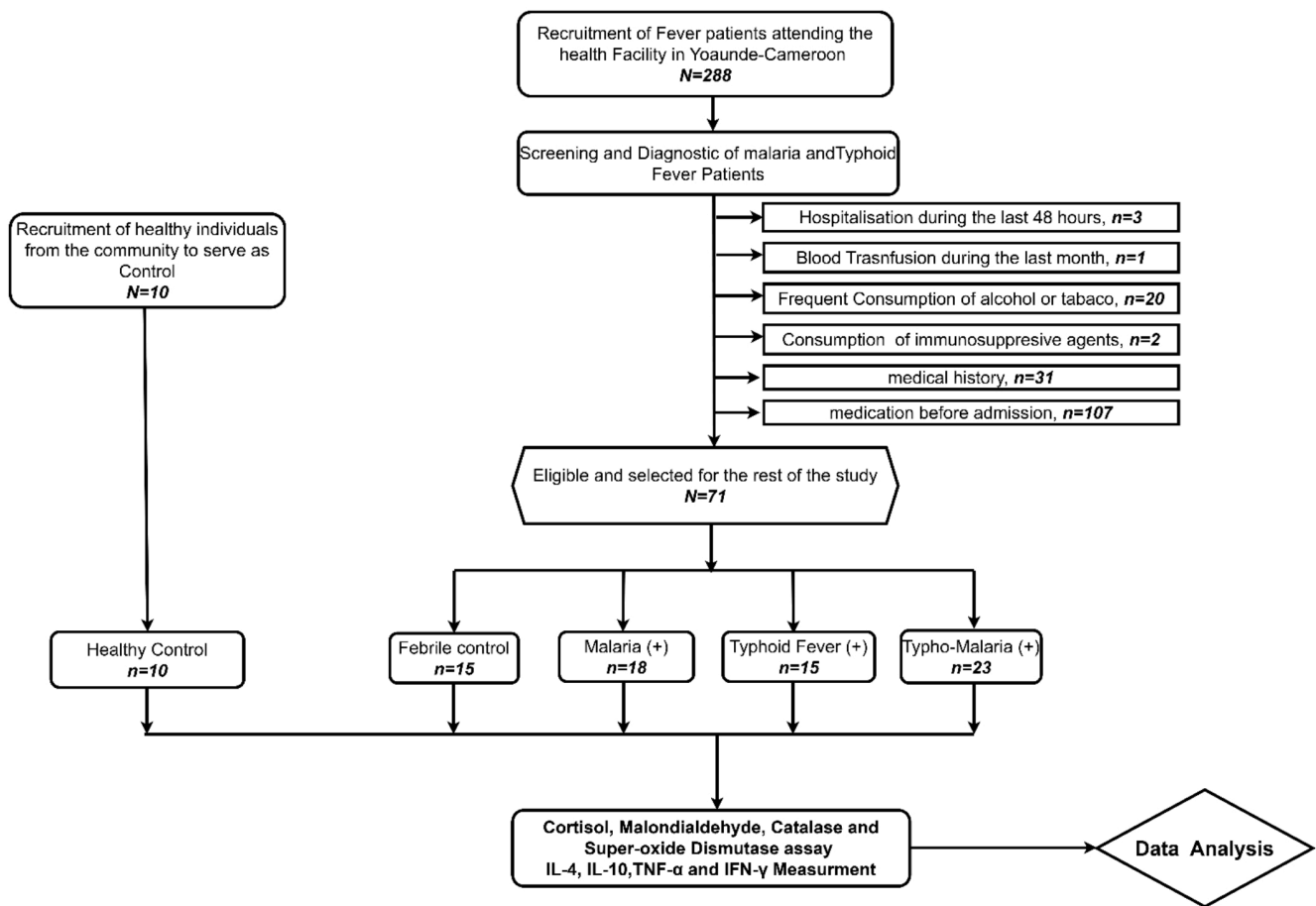


Figure 2

The Study flowchart indicating the strategy of selection and clustering of participants

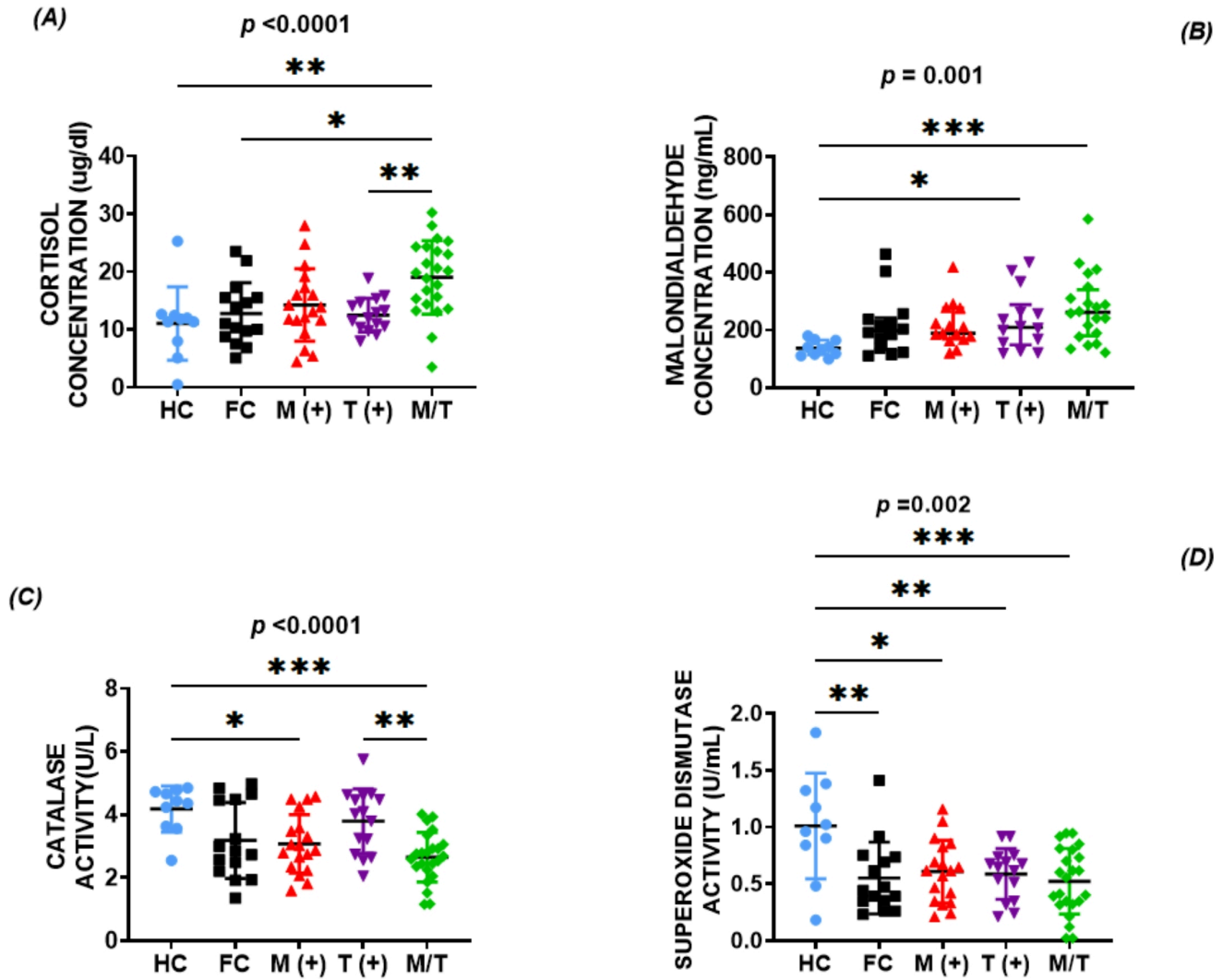


Figure 3

Stress markers stratified by infectious state. HC: Healthy Control ($n = 10$). FC: Febrile Control ($n = 15$), M (+): Malaria only ($n = 18$), T (+): Typhoid Fever only ($n = 15$), M/T: Co-infection ($n = 23$). Non-parametric data's as Malondialdehyde, were log-transformed to follow a normal distribution during analysis. ANOVA was used to determine differences between groups with post-Hoc tests (Tukey's Test). (A): Cortisol concentration, (B): Malondialdehyde concentration, (C): Catalase Activity; (D): Superoxide Dismutase activity. Lines between values for Malondialdehyde represent the median with IQR, and for cortisol, Catalase and Super-oxide Dismutase lines represent the mean with S.D. Asterisk denote signification: *: $p < 0.05$; **: $p < 0.01$ [0.001-0.01], ***: $p < 0.001$ [0.0001-0.001], ****: $p < 0.0001$.

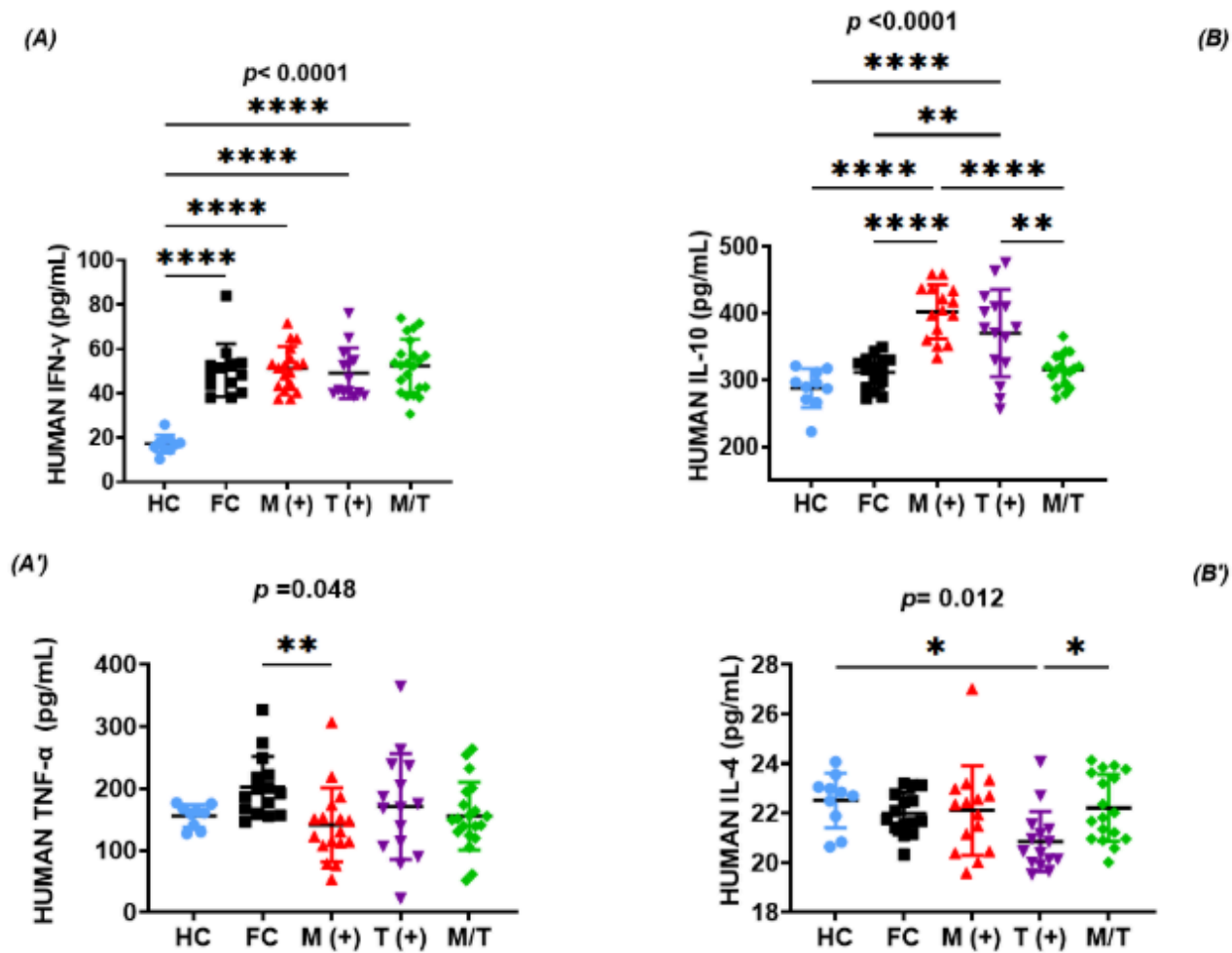


Figure 4

Pro and Anti-inflammatory cytokines stratified by infectious Status. HC: Healthy Control ($n = 10$). FC: Febrile Control ($n = 15$). M (+): Malaria only ($n = 15$). T (+): Typhoid Fever only ($n = 15$). M/T: Co-infected ($n = 18$). (A) and (A') represent Pro-inflammatory cytokines IFN- γ and TNF- α , respectively. (B) and (B') represent anti-inflammatory cytokines. IFN- γ and TNF- α were not normally distributed and were log-transformed to follow a normal distribution during analysis. The line between values for (A) and (A') figures represents the median with IQR. Lines between values for (B) and (B') figures represent the mean with S.D. Asterisk denote significance: *: $p < 0.05$; **: $p < 0.01$ [0.001-0.01], ***: $p < 0.001$ [0.0001-0.001], ****: $p < 0.0001$.

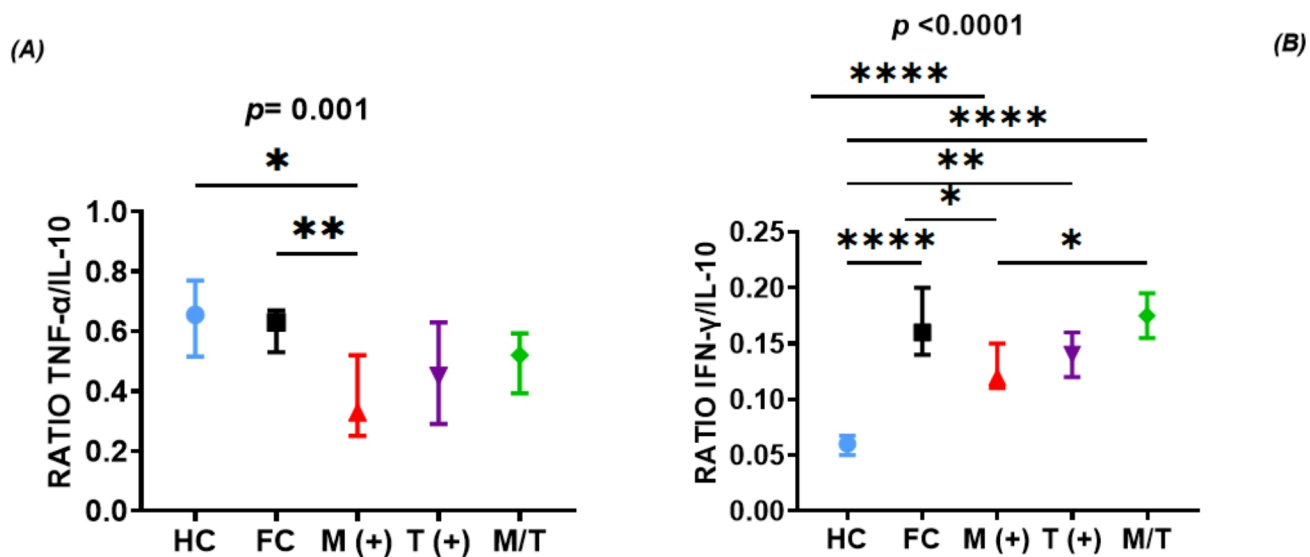


Figure 5

Ratio between Pro and anti-inflammatory cytokines between infectious groups. *HC*: Healthy Control ($n = 10$). *FC*: Febrile Control ($n = 15$), *M (+)*: Malaria only ($n = 18$), *T (+)*: Typhoid Fever only ($n=15$), *M/T*: Co-infected ($n = 23$). Ratio between TNF- α /IL-10 and IFN- γ /IL-10 was log transformed to follow a normal distribution and used for analysis. ANOVA was used to determine differences between groups with post-Hoc tests (Tukey's Test). Asterisk denote signification: *: $p<0.05$; **: $p< 0.01$ [0.001-0.01], ***: $p<0.001$ [0.0001-0.001], ****: $p<0.0001$.

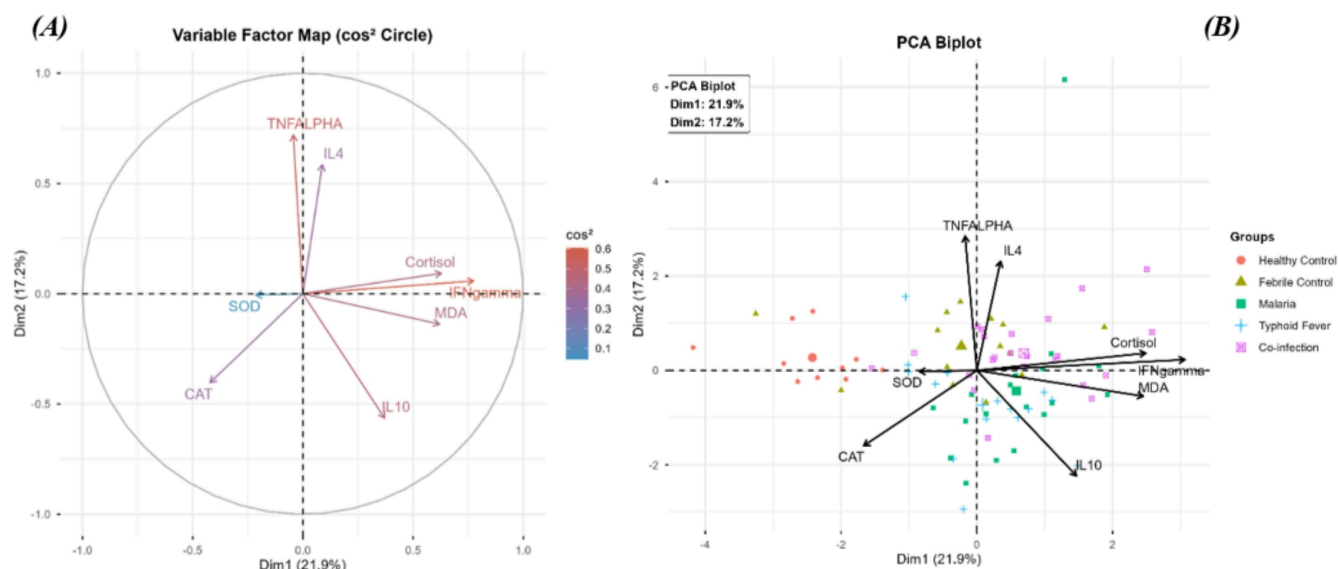


Figure 6

Principal Component analysis among our study cohorts (A) Cos² Circle and (B) PCA-Biplot.

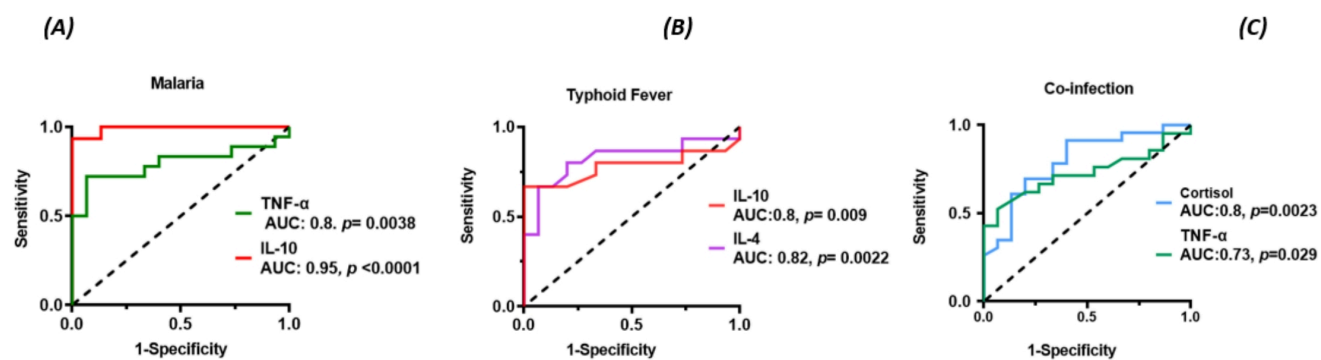


Figure 7

Graph showing ROC Curve for Stress and cytokine markers during (A) Malaria, (B) Typhoid fever and (C) the co-infection compared to Febrile control. Only significant markers are represented here ($p < 0.05$).

Supplementary Files

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