1 **TITLE PAGE**

2 **Loss of Humoral and Cellular Immunity to Invasive Nontyphoidal**

3 ***Salmonella* During Current or Convalescent *Plasmodium falciparum***

4 **Infection in Malawian Children**

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ABSTRACT

Invasive nontyphoidal *Salmonella* (iNTS) infections are commonly associated with *Plasmodium falciparum* infections, but the immunologic basis for this linkage is poorly understood. We hypothesized that *P. falciparum* infection compromises the hosts' humoral and cellular immunity to NTS which increases their susceptibility to iNTS infection. We prospectively recruited children aged between 6 and 60 months at a Community Health Centre in Blantyre, Malawi and allocated them to the following groups; febrile with uncomplicated malaria, febrile malaria-negative, non-febrile malaria-negative. *S.* Typhimurium (STm)-specific; serum bactericidal activity (SBA) and blood bactericidal activity (WBBA), complement C3 deposition and neutrophil respiratory burst activity (NRBA) were measured. SBA to STm was reduced in febrile *P. falciparum* infected (Median -0.201og10, IQR [-1.85, 0.32]) compared to non-febrile malaria-negative (Median -1.42log10, IQR [-2.0, -0.47], p=0.052). In relation to SBA, C3 deposition on STm was significantly reduced in febrile *P. falciparum* infected (Median 7.5%, IQR [4.1, 15.0]) compared to non-febrile malaria-negative (Median 29%, IQR [11.8, 48.0], p=0.048). WBBA to STm was significantly reduced in febrile *P. falciparum* infected (Median 0.25log10, IQR [-0.73, 1.13], p=0.0001) compared to non-febrile malaria-negative (Median -1.0log10, IQR [-1.68, -0.16]). In relation to WBBA, STm-specific NRBA was reduced in febrile *P. falciparum* infected (Median 8.8% IQR [3.7, 20], p=0.0001) compared to non-febrile malaria-negative (Median 40.5% IQR [33, 65.8]). *P. falciparum* infection impairs humoral and cellular immunity to STm in children during malaria episodes, which may explain the increased risk of iNTS observed in children from malaria endemic settings. The mechanisms underlying humoral immunity impairment are incompletely understood and should be explored further.

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INTRODUCTION

Invasive infections with nontyphoidal *Salmonella* (NTS) serovars, principally Typhimurium and Enteritidis are estimated to cause over 2.1 million illnesses and 416,000 deaths per year (1). In malaria endemic settings, invasive NTS are commonly associated with current or convalescent episode of malaria, particularly severe malarial anemia (2, 3). Other factors associated with increased susceptibility to iNTS in children are immature immunity and malnutrition, while HIV infection is the driving force for iNTS susceptibility in adults (4, 5). About 6.5% of invasive bacterial infections (IBIs) occurs in *P. falciparum* infected children (6, 7), however in view of low sensitivity of blood cultures, *P. falciparum* infection could account for more than 50% of IBI in children living in malaria-endemic settings (8). Often children are diagnosed and treated for malaria while IBIs is unattended leading to poor health outcomes.

The association between malaria and iNTS was first reported in 1920s (9). Biggs *et al* recently reported that iNTS and malaria co-infections were common in febrile pediatric in-patients from a high malaria transmission area compared to those from a low malaria transmission area in Tanzania (10). In contrast, *S.* Typhi bacteremia was uncommon in febrile pediatric in-patients from a high malaria transmission area (10). In addition, the association between iNTS and malaria is observed in seasonal peaks during the rainy season (4, 5, 11, 12). However, the immunologic basis for increased iNTS cases in malaria endemic setting is not fully understood.

The link between NTS and malaria in humans and mice are extensively covered in the reviews by Uche *et al* (13) and Takem *et al* (14) . Phagocytes (including neutrophils and monocytes) are key players in controlling rapid replicating NTS within the gut mucosa hence preventing the spread of NTS to systemic organs (15). Studies in both human and mice have shown that *P. falciparum* derived products such as hemozoin, heme and heme oxygenase-1 mediate the reduction in phagocytosis and oxidative burst activities (16-18). Some have shown that during acute malaria, the pro-inflammatory cytokine IL-12 is reduced while anti-inflammatory cytokine IL-10 is increased (19-22). The anti-inflammatory environment, coupled with reduced phagocytosis and oxidative burst activities during malaria, are thought to create a favorable environment for NTS replication within the gut mucosa and blood stream compartments. However, the role of humoral immunity to NTS during *P. falciparum* infection has not been explored extensively, although its role in non-malarial children has been studied before (23-25).

Immunoglobulin G (IgG) antibodies to NTS targeting lipopolysaccharide (LPS) are thought to confer some protection against NTS bacteremia in African children (23, 25, 26). Opsonizing anti-NTS-LPS IgG antibodies mediate NTS killing in a cell-free manner through the complement cascade membrane attack complex (MAC) and also facilitate killing by phagocytes which involves phagocytosis and respiratory burst mediated killing (24). We envisaged that exploring the role of humoral immunity to iNTS during malaria will broaden our understanding of iNTS and malaria association that has previously focused on cellular immunity. Therefore, we examined cell-free bactericidal activities and cellular bactericidal activities against NTS in a cohort of uncomplicated *P. falciparum* infected children. We show that during malaria, *P. falciparum* infection impairs serum bactericidal immunity to STm via altered complement C3 deposition on STm in addition to impairment of phagocytes

respiratory burst which has been known before, providing comprehensive explanation for increased susceptibility to iNTS during malaria in children.

RESULTS

Transient loss of serum bactericidal immunity to *S.* Typhimurium during current or convalescent *P. falciparum* infection

We have previously shown that acquisition of serum bactericidal activity (SBA) to STm correlates with the decline in iNTS infections in childhood in individuals not infected with *P. falciparum* (23, 25). Therefore firstly, we examined the SBA to determine whether SBA to STm is reduced in *P. falciparum* infected children. We found that SBA to STm was reduced but did not reach statistical significance difference in children with acute malaria (Median -0.201og10, IQR [-1.85, 0.32]) compared to non-febrile malaria-negative children (Median -1.42log10, IQR [-2.0, -0.47], p=0.052) (Figure 1A). SBA to STm was significantly reduced in children with acute malaria (Median -0.201og10, IQR [-1.85, 0.32], p=0.0007) and at day 14 in convalescence (Median -0.49log10, IQR [-2.0, 0.49] p=0.0054) compared to febrile malaria-negative children (Median -1.85log10, IQR [-2.85,-1.24]) (Figure 1A). SBA to STm at 30 day in convalescence (Median -1.851og10, IQR [-2.24, 0.06]) was similar to febrile malaria-negative children (Median -1.85log10, IQR [-2.85,-1.24], p=0.43) and non-febrile malaria-negative children (Median -1.42log10, IQR [-2.0, -0.47], p=0.39) (Figure 1A). Furthermore, in a subset of children we found that 6/23 (26%) had robust SBA to STm (ability to kill STm by at least ≥ -1.0 log10 change in STm cfu/ml) at acute malaria phase compared to 16/23 (69.5%) at day 30 in convalescence (Figure 1B). We also found that out of 16 children that lacked robust SBA to STm at acute malaria phase, 10/16 (62.5%) attained robust SBA to STm at day 30 in convalescence.

We have previously shown that acquisition of SBA to STm correlates with age in healthy children (25). We found that acquisition of robust SBA to STm correlated with age development in febrile non malaria children and in non-febrile malaria negative children SBA (spearman's r =-0.43, p=0.0037 and r=-0.38, p=0.0086 respectively) (Figure 2B and 2A). Interestingly, we observed that during acute *P. falciparum* infection, at day 14 and 30 in convalescence, SBA to STm did not kill STm by at least ≥ -1 log10 change in STm cfu/ml in some older children (≥24 months considered serum immune to STm (23)) and SBA to STm poorly correlated with age (acute malaria spearman's r=0.23, p=0.11, day 14 r=0.15, p=0.37 and day 30 spearman's r=-0.16, p=0.39 respectively) (Figure 2C-2E).

Since SBA to STm is mainly mediated by anti-STm IgG antibodies targeting LPS (23, 25). Un-expectedly, we found that SBA to STm in non-febrile malaria negative children poorly correlated with anti-STm-LPS IgG antibody titres (spearman's r=0.038, p=0.81) while in febrile non-malaria children SBA correlated with anti-STm-LPS IgG antibody titres (spearman's r= -0.34, p=0.03) (Figure 3A and 3B). Interestingly, we observed that during acute malaria, SBA to STm poorly correlated with anti-STm-LPS IgG antibody titres (spearman's r=0.19, p=0.20) while the correlation of SBA to anti-STm-LPS IgG antibody titres was superior at day 14 and day 30 in convalescence, however this was only statistically significant at day 14 (spearman's r= -0.37, p=0.04 and r= -0.29, p=0.15 respectively) (Figure 3C-3E). These findings suggest that *P. falciparum* infection induced the transient loss of serum bactericidal activity to STm in *P. falciparum* infected children which is independent of age and acquired antibody immunity.

To explore this further, we randomly selected serum samples (n=10) of children (>24 months old) to examine levels of complement C3 and C5b-9 deposition during malaria (Figure 4).

Interestingly, we found that C3 deposition on STm was significantly lower in febrile *P. falciparum* infected children (Median 7.5%, IQR [4.1, 15.0]) compared to febrile malaria-negative children (Median 60%, IQR [21.5, 71.5], p=0.003) and non-febrile malaria-negative children (Median 29%, IQR [11.8, 48.0], p=0.048) (Figure 4C and 4E). C3 deposition was also lower in febrile *P. falciparum* infected children (Median 7.5%, IQR [4.1, 15.0]) compared to day 30 in convalescence (Median 19%, IQR [12.1, 58.8], p=0.027) and was similar at day 14 in convalescence (Median 19.5%, IQR [10.7, 28.7], p=0.113) (Figure 4C-4D).

C5b-9 deposition on STm was significantly lower in febrile *P. falciparum* infected children (Median 21%, IQR [9.6, 31.0]) compared to febrile malaria-negative children (Median 34%, IQR [29, 74.5], p=0.012) but was not significantly different from that seen in non-febrile malaria negative children (Median 28%, IQR [14.35, 40.8], p=0.57). There was no significant differences in C5b-9 deposition on STm in febrile *P. falciparum* infected children (Median 21%, IQR [9.6, 31.0]) compared to day14 (Median 24%, IQR [24.8, 46.5], p=0.084) and day 30 in convalescence (Median 38%, IQR [29, 64], p=0.084) (Figure 4E-4F). Taken together, this suggests a transient increase in consumption of C3 complement component during acute malaria which rebound to levels comparable to non-malaria children by day 14 in malaria convalescence.

Serum is a pre-requisite for blood cells killing of *S.* Typhimurium

S. Typhimurium is a facultative intracellular organism that requires the action of both cellular and humoral immunity to be effectively controlled. We therefore examined if whole blood killing of STm was also reduced during *P. falciparum* infection. We found that whole blood

bactericidal activity (WBBA) to STm was reduced during malaria at acute stage (Median 0.25log10, IQR [-0.73, 1.13], p=0.0001), day 14 (Median -0.511og10, IQR [-1.53, 0.57], p=0.110) and day 30 in convalescence (Median -0.191og10, IQR [-0.96,0.64], p=0.009) and in febrile malaria negative children (Median 0.18log10, IQR [-0.66, 0.87], p=0.004) compared to non-febrile malaria-negative children (Median -1.0log10, IQR [-1.68, -0.16]) (Figure 5A).

Humoral immunity enhances intracellular killing of STm (24). We observed that washed blood cells bactericidal activity (WBCBA) to STm during malaria, in febrile malaria-negative children and non-febrile malaria-negative children was abrogated in all washed blood cells conditions examined (Figure 5A-B). To determine if serum mediated immunity is required for efficient washed blood cells killing of STm. We examined the assay after STm was opsonised with autologous serum, and we found that killing of STm was partially restored (Figure 5A-5C). We show that WBBA to STm is reduced during malaria and in febrile illness in children and that serum opsonisation is be essential for cellular killing.

Reduced *S.* Typhimurium-specific neutrophil respiratory burst in malaria and febrile non-malaria children

To identify the specific bactericidal function that was altered in children with malaria and febrile illness, we examined neutrophils respiratory burst activity (NRBA) as it is a key mechanism for intracellular pathogen killing. We found that NRBA was significantly reduced in children during acute malaria (Median 8.8%, IQR [3.7, 20], p=0.0001) and also in febrile malaria-negative children (Median 9.4%, IQR [4.4, 19.5], p=0.0001) compared to non-febrile malaria-negative children (Median 40.5%, IQR [33, 65.8]) (Figure 6A and 6B). We observed that in *P. falciparum* infected children, there was a modest trend for improved respiratory

burst at day 14 (Median 17%, IQR [5.1, 31.5], p=0.135) and day 30 (Median 17%, IQR [6.1, 32], p=0.042) in convalescence compared to the acute malaria phase (Median 8.8%, IQR [3.7, 20]) (Figure 6B), but that even at 1 month there remained a significant defect. This shows that both malarial and non-malarial febrile children have impaired NRBA to STm. The pattern over time was similar to that seen for whole-blood killing, in keeping with oxidative burst being a dominant mechanism for the observed whole blood bacterial killing.

DISCUSSION

This study extends our understanding of how *P. falciparum* infection compromises phagocyte-dependent immunity to NTS in children (16-18), which provides additional explanation to the observed increased children's susceptibility to iNTS in malaria endemic settings. Our study describes the transient loss of serum bactericidal immunity to iNTS during current or convalescent *P. falciparum* infection in children. This loss of serum bactericidal immunity was specific to children who were febrile from malaria compared to other causes of fever. *P. falciparum* infection appears to compromise serum bactericidal immunity to iNTS in older children, and does not correlate with pre-existing IgG antibodies to STm LPS. This may likely be caused by the increased consumption of C3 complement component during *P. falciparum* infection.

In this study, we have demonstrated the transient loss of bactericidal humoral immunity to STm in children with current or convalescent *P. falciparum* infection. The loss in bactericidal SBA to STm was independent of age and anti-STm LPS IgG antibody titres during acute malaria and day 14 convalescent malaria, and robust SBA to STm was restored after 30 days convalescence. This was explored further by examining complement components deposition on STm. Consistent with previous findings (27), we found that deposition of mainly C3

complement component was transiently reduced during the acute phase of *P. falciparum* infection and rebound at days 14 and 30 in malaria convalescence to levels comparable to non-malaria controls. This is in keeping with lack of robust SBA to STm during acute malaria in some children with high anti-STm LPS-IgG antibody titres. Increased consumption of complement components, particularly C3, during acute malaria as observed in the current study and other studies (27), which are crucial for antibody dependent-complement killing of gram negative bacteria (23, 28), may favour the proliferation of STm. We observed that at day 14 in malaria convalescent children, SBA to STm remained poor despite complement proteins C3 on STm rebounding to normal levels, suggesting that other factors may be involved in compromising serum bactericidal immunity during malaria and this needs to be investigated further. *P. falciparum* has also developed complement killing escape strategies, it is possible that serum killing is abrogated during malaria via *P. falciparum* recruitment of factor H protein which prevents complement cascade activation via the C3b (29-31), ultimately blocking complement mediated NTS lysis. Furthermore, *P. falciparum* infection may compromise humoral immunity to NTS via reduction of antibody opsonisation capacity as observed in some studies (32), as well as defective complement cascade activation. This observation suggests that in children from settings where exposure to NTS is frequent, and malaria is highly endemic, humoral bactericidal immunity may be lost during repeated malaria episodes, increasing overall susceptibility to iNTS by favouring NTS proliferation and systemic infection.

We have showed that WBBA to STm is reduced in both *P. falciparum* infected and febrile malaria-negative children. Consistent with previous observations (24), our findings indicate that serum immunity plays a crucial role in both cell-free and intracellular killing of NTS. These findings provide support of antibody-based NTS vaccine development strategies, as

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they are likely to elicit both extracellular and intracellular protection against iNTS. Neutrophil respiratory burst constitutes a key mechanism of intracellular effector function for *Salmonella* killing (33). Consistent with results of previous studies (16-18, 21), we have shown that NRBA to STm is reduced in both *P. falciparum* infected and febrile malaria-negative children compared to non-febrile malaria-negative children. It has long been known that *P. falciparum* infection derived products including heme, heme oxygenase and hemozoin compromises neutrophils and monocytes effector functions in both humans and mice (16-18, 21, 34). In contrast to transient loss of serum killing to STm, we observed that both NRBA and WBBA were reduced for a period longer than 30 days in malaria convalescent children. This is in keeping with previous observation (18). Surprisingly, we found that NRBA was also reduced in febrile malaria-negative children compared to non-febrile malaria negative children. How non-malarial febrile illness compromises neutrophil respiratory burst is not clear. In this study, we did not confirm the aetiology of non-malarial febrile illnesses. Identifying the causes of these febrile illnesses may provide insights into the mechanisms behind impaired neutrophils respiratory burst. We recommend further investigations into the contribution of reduced C3 levels during acute phase of malaria to poor NRBA and WBBA to STm which was not explored in our current study. These findings suggest that the loss of intracellular killing of NTS in *P. falciparum* infected and non-malarial febrile children is likely due to impaired neutrophil respiratory burst activity.

Conclusion

We have demonstrated that *P. falciparum* infection transiently compromises the humoral immunity to NTS in children extending our knowledge that *P. falciparum* infection compromises cellular immunity to NTS. This study broadens our understanding of the

immunologic basis of increased susceptibility to iNTS during current or convalescent malaria, and the epidemiological association of malaria and iNTS in malaria endemic regions. The global immune defects induced by *P. falciparum* infection may render children from malaria endemic regions at risk of not only iNTS but also other enteric gram negative bacteria(35). Our study further highlights the need to improve management of concurrent malaria and IBI infections, particularly by developing rapid diagnostic test for IBIs, ideally to be run in parallel with malaria rapid diagnostic test. This could significantly improve the identification of malaria and IBIs, promote rational prescribing of antimicrobial agents and improve health outcomes.

METHODS AND MATERIALS

Recruitment of Study Participants and Follow-up

We recruited 154 children aged 6 to 60 months at a Community Health Centre in Blantyre, Malawi from January 2016 to August 2016. Study participants comprised 59 febrile children presenting with uncomplicated malaria; 49 febrile malaria-negative children; and 46 non-febrile malaria-negative children (Table 1). *P. falciparum* infected children were followed up at day 14 (n=42) and day 30 (n=41) during convalescence. Uncomplicated malaria group was comprised of children with acute phase of *P. falciparum* infection and presented to hospital for medical care, they were febrile (> 37.8 ºC) at the time of recruitment, had positive malaria rapid diagnostic test and positive malaria blood film, Blantyre Coma Score of 5 (36), haemoglobin (Hb) >5g/dl and serum glucose ≥45 mg/dl. Children with a positive HIV antibody test, severe anaemia (Hb ≤5 g/dl), malnutrition (weight for height Z-score <-2) or other chronic illness were excluded from the study. A 3 ml venous blood sample was collected from each participant at recruitment and during follow-up. Participants presenting with uncomplicated malaria were treated according to Malawi Government guidelines, before

blood sample collection. Ethical approval for the study was obtained from College of Medicine Research Ethics Committee (Protocol number P.08/15/1785) and written informed consent was obtained from parents or guardians of participating children.

Quantification of STm-specific SBA

Serum bactericidal activity (SBA) assays were performed as previously described (23). Briefly, serum or phosphate buffer saline (PBS) was mixed with STm D23580 (37) adjusted to $1.0x10^6$cfu/ml and incubated at 37^0C for 180 minutes. Test samples were serially diluted and plated in triplicate on Luria Bertani agar. Colony count of STm was done after 24 hours of incubation. Log 10 change in STm cfu/ml from the baseline was reported.

Quantification of STm-specific whole blood and washed blood cells killing

Three conditions were prepared as previously described (24); for condition 1, whole blood was used in whole blood bactericidal assay (WBBA), for condition 2, whole blood was washed twice with RPMI 1640 at 1,000 rpm for 10 minutes before using in a washed blood cells bactericidal assay (WBCBA). For condition 3, STm adjusted at $1.0x10^7$ cfu/ml was first opsonised with 1:10 serum from each participating child for 20 minutes at room temperature (RT) before challenging washed blood cells in a washed blood cells and serum-opsonised assay (WBCSOA). All conditions were challenged with STm adjusted at a final concentration of $1.0x10^6$cfu/ml. Colony counts were performed as described for the SBA experiment above.

Quantification of anti-STm IgG antibody titre by ELISA

These experiments were performed as previously described (25). Briefly, ELISA plates (Nunc-Immuno) were coated overnight with 100µl of carbonate-bicarbonate buffer (Sigma

Aldrich) per well containing 7.5µg/ml STm-LPS antigen (ALEXIS Biochemicals). Plates were washed with PBS containing 0.05% Tween 20 and blocked with 200µl/well blocking buffer (PBS/1% BSA) for 1 hour at 37°C. Test serum at 1:20 in dilution buffer (PBS/0.05% Tween 20/1% BSA) was serially diluted 3-fold and incubated at 37°C for 1 hour. After washing, 100µl of 1:2000 secondary Goat Anti-human IgG-AP antibodies (Southern Biotech) were added and incubated for 1 hour at 37°C. Finally, after washing, 100µl of SIGMAFAST™ p-Nitrophenyl phosphate substrate was added to each plate and read after 30 minutes using a Bio Tek reader ELx800 (Bio Tek Instruments, USA) at 405nm.

Quantification of complement components binding on the surface of STm

These experiments were performed as previously described (23, 28), 5µl of STm at $2x10^8$ cfu/ml was gently mixed with 45µl of undiluted serum or PBS (control) at RT for 20 minutes. Samples were washed twice with 1ml PBS by spinning for 5 minutes at 3,300g. 2µl of anti-C3c FITC conjugated antibody (Abcam) was added to 50µl of pellet to measure C3 deposition. 1µl of anti-C5b-9 neo-epitope antibody (Abcam) and 2µl of rabbit-anti-mouse FITC conjugated antibody (Abcam) were added to 50µl of pellet to measure MAC. Samples were washed twice with 1ml PBS after 20 minutes incubation at RT and fixed with 200µl 1% formaldehyde PBS. Samples were acquired on CyAN ADP flow cytometer (Beckman Coulter) and analysed using Flow Jo version 7.6.5.

Quantification of Neutrophil Respiratory Burst

Phagoburts test kit (Glycotope Biotechnology) was modified to measure neutrophil respiratory burst as previously described (24). Whole blood (45µl) was incubated on ice for 10 minutes then stimulated with serum opsonised STm at $1.0x10^8$ cfu/ml or wash solution containing instalmed-salts (control). Samples were then incubated for 10 minutes at 37 °C to

allow phagocytosis followed by 10 minutes incubation at 37°C after adding dihydrorhodamine 123 to promote oxidation. The reaction was stopped by 1:10 lysing solution for 20 minutes at RT. Samples were acquired on CyAN ADP flow cytometer (Beckman Coulter) and analysed using Flow Jo version 7.6.5.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, USA). Log10 change in bactericidal activity to STm, percentage of neutrophil respiratory burst positive cells or complement deposition were examined for normality of distribution using D'Agostino and Pearson omnibus normality test. Nonparametric data was compared using Mann-Whitney U test or Wilcoxon signed ranked test for paired t test. Median and interquartile range (IQR) were reported, and *p* value of less than 0.05 was considered statistically significant. Spearman's correlation coefficient *r* was used to determine relationships between bactericidal activity and age and anti-STm LPS IgG antibody titres during malaria.

NOTES

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Contribution: Conceived and designed the experiments: TSN, WLM. Wrote the manuscript: TSN, WLM. Performed the experiments: TSN, JTN. Analyzed the data: TSN, JTN, DT, JS, QD, KCJ, HCM, RSH, MAG, and WLM. All authors contributed to and have approved the final manuscript.

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544 **TABLES**

545

546 **Table 1: Study Participants' demographic and clinical features**

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	During malaria			Non malaria controls	
	Acute n= 59	**2 weeks** n=42	**1 month** n=41	**Febrile** n=49	**Non febrile** n=46
Female participants (%)	34 (58)	26 (62)	25 (61)	27(55)	15 (39)
Median age in months (range)	22.8 (6-59.8)	23.5 (6.6-60.2)	24.1 (7-61)	22.8 (6-59.3)	21.9 (6.7-50.7)
Median weight in kg (range)	10.2 (6.9-17)	10.5 (7 -16.1)	10.1 (7.1-16.3)	10.1 (6.9-15.4)	10.5 (7.1-15.5)
Median height in cm (range)	84 (66-113)	85 (75-113)	85 (75.6-113)	84.5 (74-104)	82.5 (64-106)
Median MUAC in cm (range)	14 (10.2-19)	13.8 (10-18.7)	13.95 (10.4-18.8)	13.4 (10-16.2)	14.2 (10.2-17.5)
Median Hb in g/dl (range)	9.5 (5.9-12.3)	9.7 (7.3-12.4)	10.3 (8.4-12.7)	10.8 (7.9-18.7)	11 (5.2-13.6)
Median absolute lymphocytes x10^3/µl (range)	3.4 (0.9-9.8)	5.3 (2.2-9.2)	5.3 (1.7-15.2)	4.2 (1.2-14.9)	5.4 (2.2-69.2)
Median absolute neutrophils x10^3/µl (range)	3.7 (0.93-11.4)	2.8 (1.2-3.9)	2.8 (0.5-5.9)	3.6 (0.2-19.9)	2.3 (0.18-22.2)
Splenomegaly (%)	20 (39)	1 (2.4)	0 (0)	4(8.2)	1(2.6)
Cough (%)	24(40.6)	9 (21.4)	9 (22)	29(59)	0(0)
Shortness of breath (%)	1(1.7)	0 (0)	0 (0)	13(26.5)	0(0)
Vomit (%)	21(35.6)	1(2.4)	2(4.9)	25(51)	0(0)
Diarrhoea (%)	14 (23.7)	0(0)	2(4.9)	14(28.6)	0(0)

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552 **LEGENDS**

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554 **Figure 1: Transient loss of serum bactericidal immunity to *S.* Typhimurium during**
555 **current and convalescent *P. falciparum* infection**

556 Serum bactericidal activity was reported as log10 change in STm cfu/ml from the baseline
557 and this was plotted as indicated during malaria and in controls. Red bars represent the
558 median and statistical differences were determined by Mann-Whitney U test (Fig 1A). Serum
559 bactericidal activity during malaria was linked (Fig 1B).

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562 **Figure 2: Relationship between serum bactericidal activity to *S.* Typhimurium and age**
563 **during malaria**

564 Serum bactericidal activity was reported as log10 change in STm cfu/ml from the baseline
565 and this was plotted against age in months as indicated in controls and during malaria.
566 Spearman's r correlation coefficient and *p* value was reported.

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569 **Figure 3: Relationship between serum bactericidal activity to *S.* Typhimurium and anti-**
570 **IgG antibody targeting *S.* Typhimurium LPS**

571 Serum bactericidal activity to STm was plotted anti-IgG antibodies targeting STm LPS in
572 controls and during malaria as indicated. Spearman's r correlation coefficient and *p* value was
573 reported.

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Figure 4: Reduced C3 deposition to *S.* Typhimurium during acute phase of *P. falciparum* infection in children

Serum (n=10) was randomly selected from >24 months children donors during malaria and controls. Serum bactericidal activity was reported as log10 change in STm cfu/ml from the baseline and this was plotted as indicated during malaria and in controls (Fig 4A). Serum bactericidal activity during malaria was linked (Fig 4B). Proportion of C3 and C5b-9 deposition on *S.* Typhimurium during malaria was linked (Fig 4D and Fig 4F). Red bars represent the median and statistical differences were determined by Wilcoxon signed rank test and Mann-Whitney U test.

Figure 5: Reduced blood cells killing of *S.* Typhimurium in malaria and non-malarial febrile children

Whole blood, washed blood or serum opsonised washed blood bactericidal activity was reported as log10 change in STm cfu/ml from the baseline and plotted as indicated during malaria and in controls. Red bars represent the median and statistical differences were determined by Mann-Whitney U test.

Figure 6: Reduced *S.* Typhimurium specific neutrophil respiratory burst activity in malaria and non-malarial febrile children

The representative gating strategy of neutrophils using forward scatter (FSC) and side scatter (SSC) expression followed by neutrophil respiratory burst activity plots in unstimulated or STm stimulated is shown (Fig 6A). Percentage of STm-specific neutrophils respiratory burst positive cells were plotted during malaria and in controls as indicated (Fig 6B). Red bars represent the median and statistical differences were determined by Mann-Whitney U test.

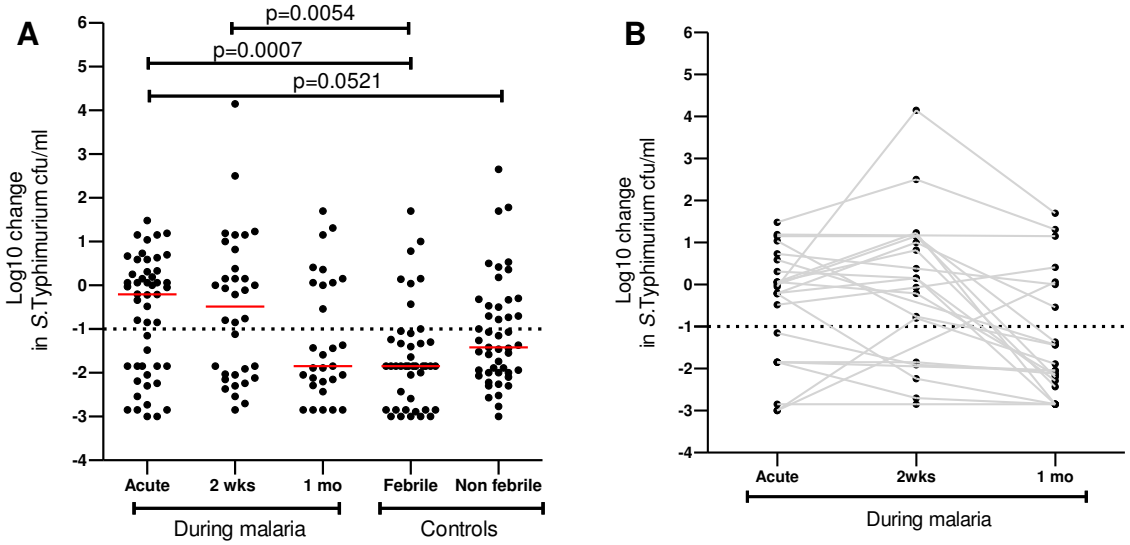


Figure 1

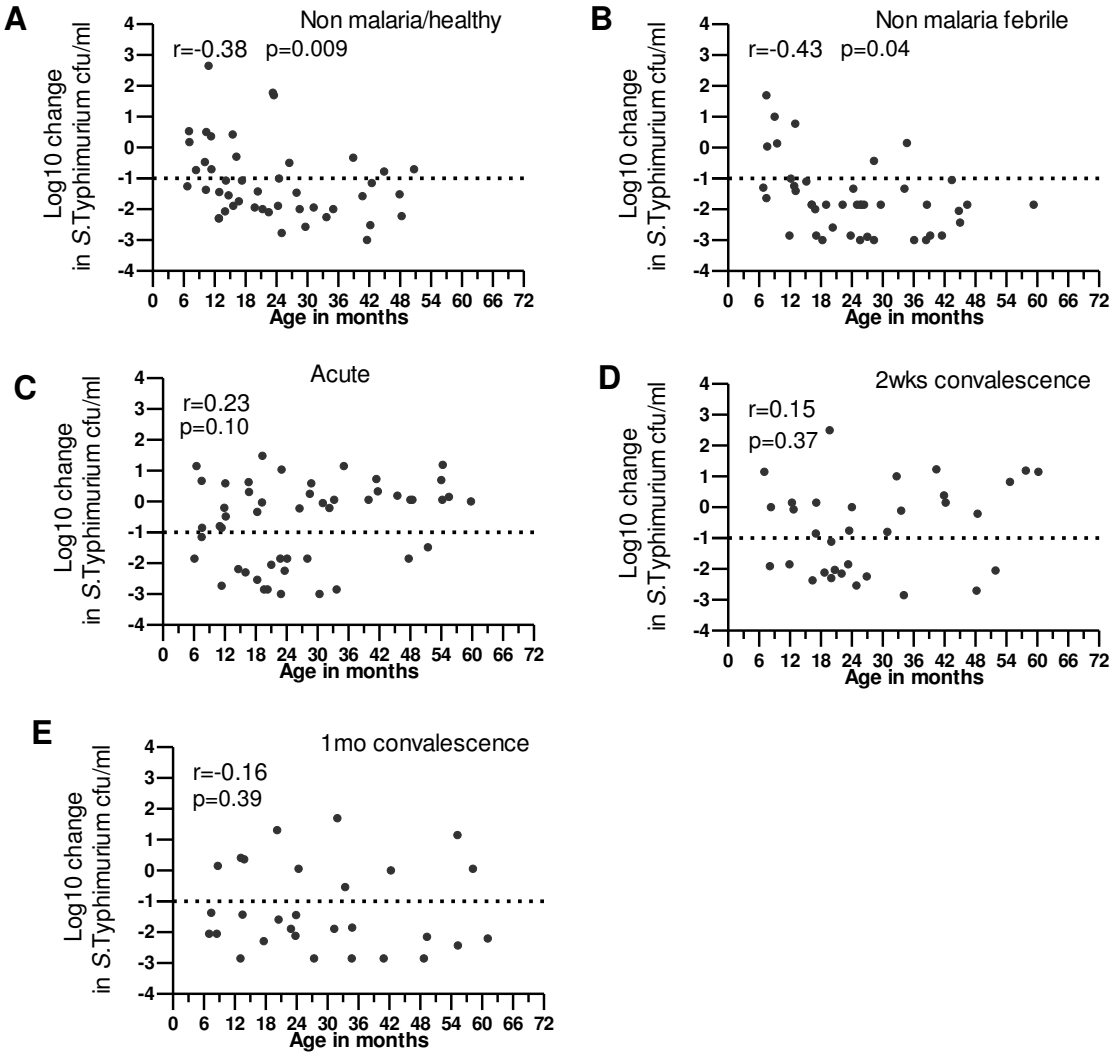
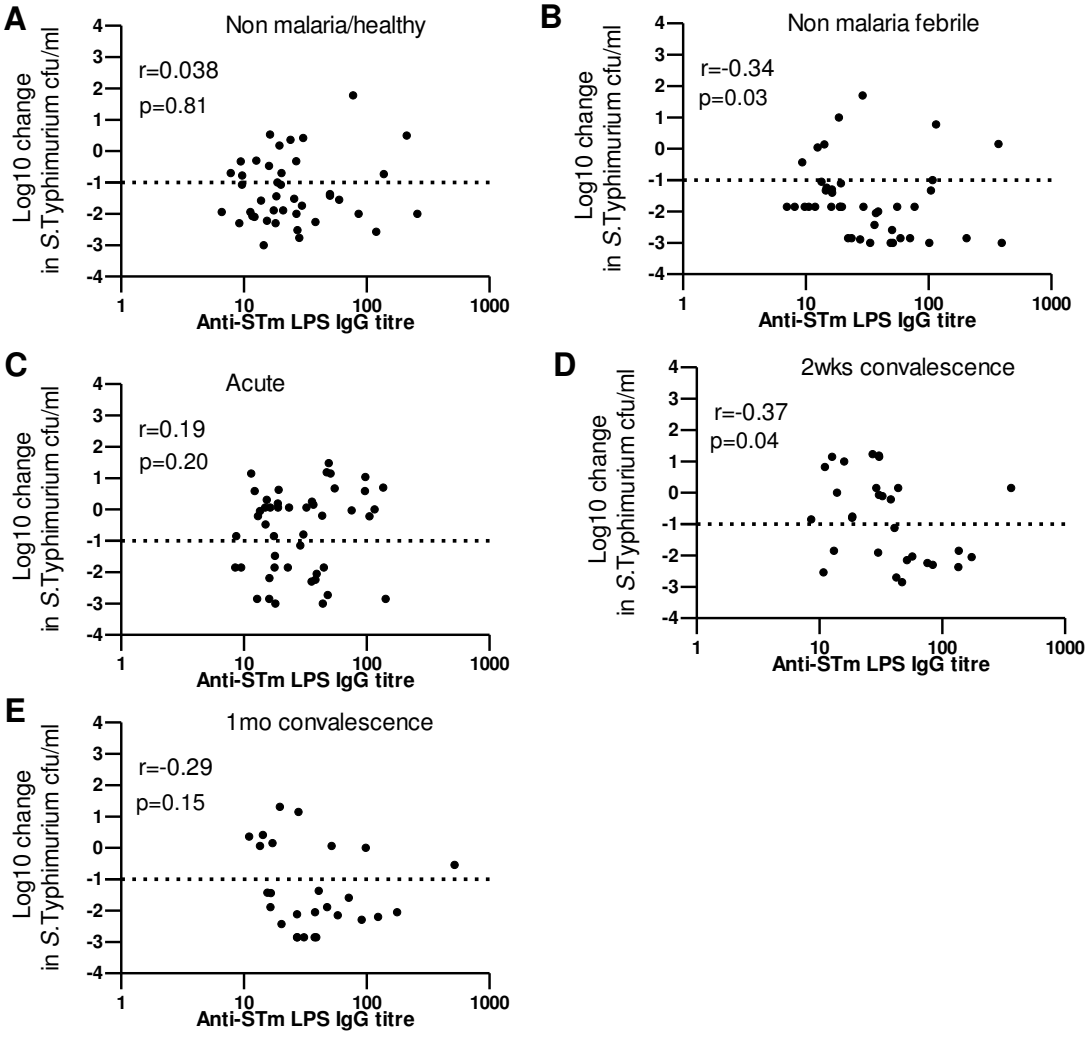


Figure 2



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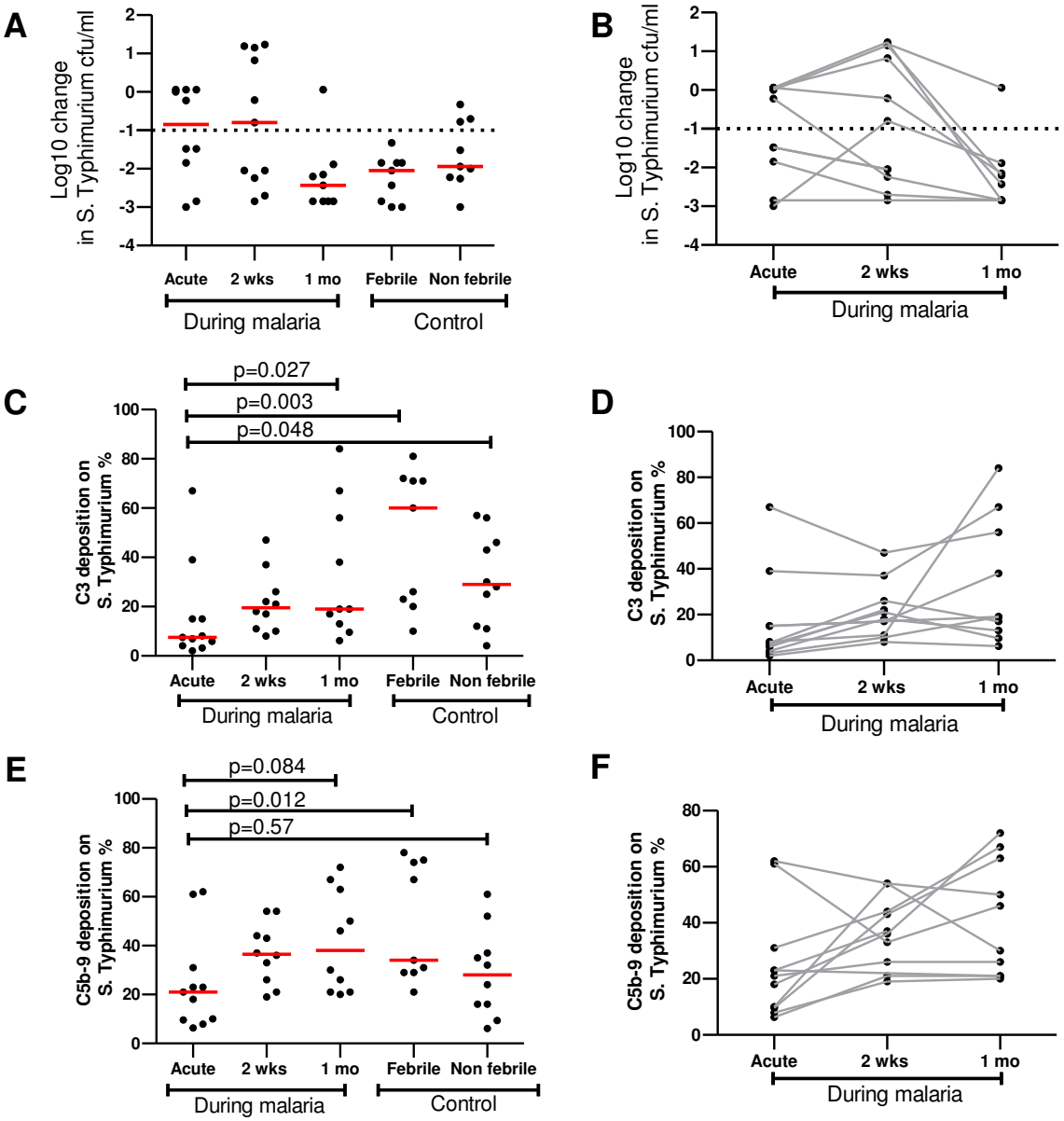

Figure 4

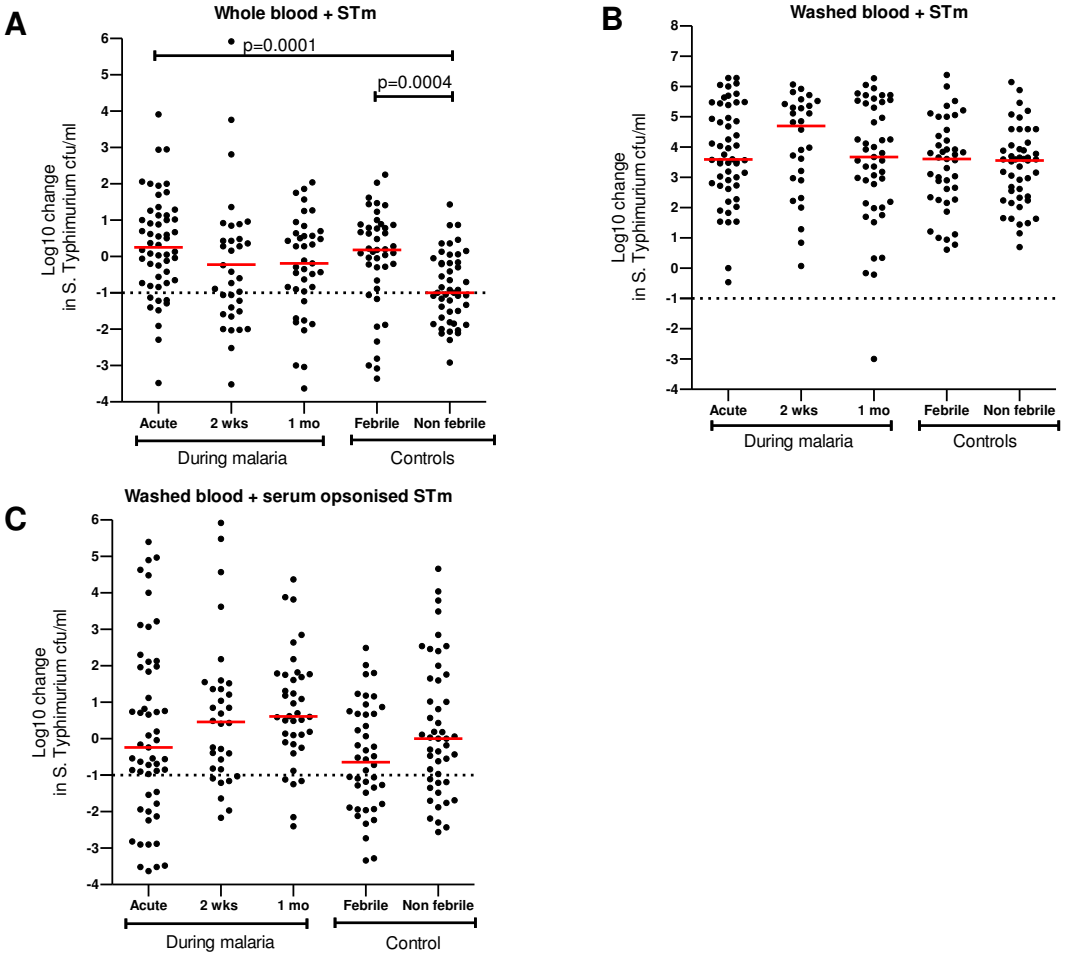

Figure 5

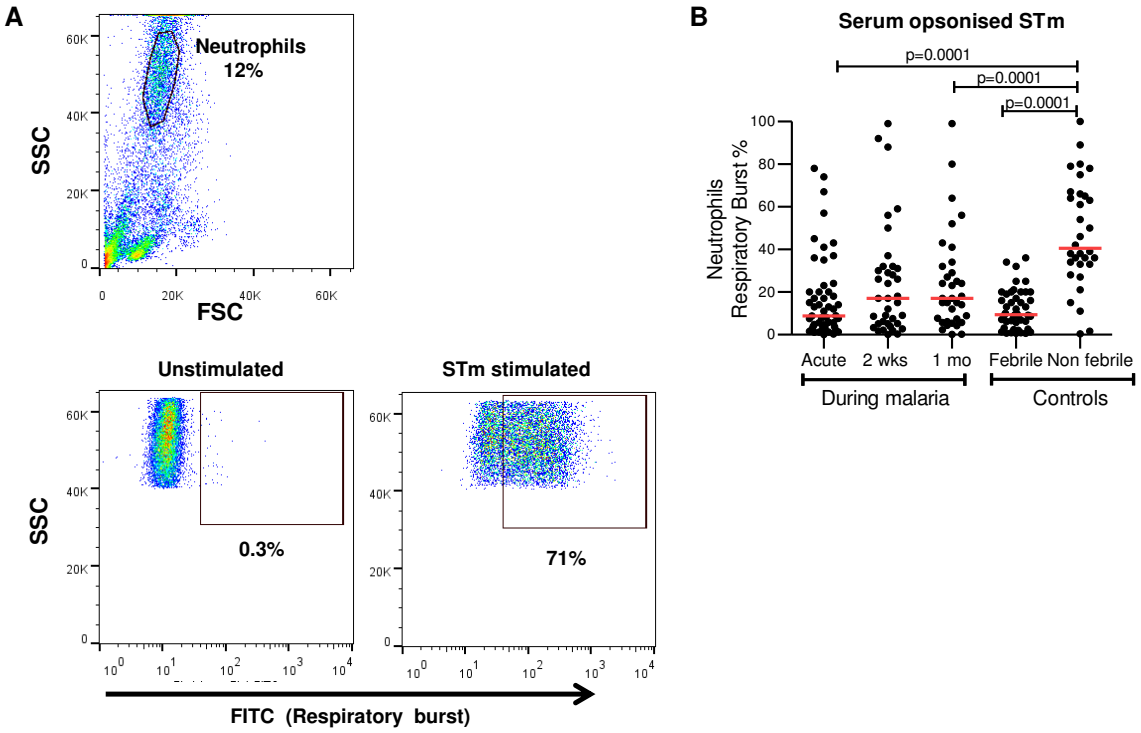

Figure 6