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Is asymptomatic malaria really asymptomatic? Hematological, vascular and inflammatory effects of asymptomatic malaria parasitemia

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Summary Asymptomatic malaria infections are highly prevalent in malaria endemic regions and most of these infections remain undiagnosed and untreated. Whereas conventional malaria symptoms are by definition absent, little is known on the more subtle health consequences of these infections. The aim of our study was to analyze the hematologic, vascular and inflammatory effects of patent and subpatent asymptomatic malaria parasitemia in children and adults on the Indonesian island Sumba. Both children and adults with parasitemia had increased high-sensitive C-reactive protein levels compared to aparasitemic individuals. In addition, children, but not adults with parasitemia also had lower platelet counts and Hb levels and higher levels of von Willebrand factor and platelet factor-4, markers of endothelial and platelet activation, respectively. These findings suggest that asymptomatic malaria infections have subtle health consequences, especially in children, and should be regarded as potentially harmful.

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Introduction

Despite considerable achievements in the recent decade, the burden of malaria has remained high with an estimated number of 584,000 deaths in 2013 of which the majority of cases occurred in children under the age of five years.[1] While *Plasmodium falciparum* is responsible for the vast majority of malaria-related morbidity and mortality, increasing evidence suggests that the clinical burden of *Plasmodium vivax* malaria has been underestimated or might be increasing in areas were *P. falciparum* prevalence is on the decline.[2–4]

In malaria endemic areas, a large proportion of malaria infections are asymptomatic. Most of these asymptomatic infections remain undiagnosed and untreated. A proportion of these individuals also carry gametocytes and may therefore play an important role in malaria transmission.[5–8] Whether asymptomatic infections are also responsible for adverse health effects is less clear. Previous studies have found associations between asymptomatic infections and anemia, thrombocytopenia and inhibited cognitive function, while the association with nutritional status yielded conflicting results.[9–13] Moreover, differentiating between asymptomatic parasitemia as causal factor or as a marker of a recent symptomatic infections is not straightforward.[14] The majority of studies relied on microscopy for detection of parasitemia, which is known to underestimate the prevalence of infection because of the abundance of infections with parasite densities below the microscopic threshold for detection (subpatent parasitemia).[15]

Endothelial cell activation with release of the platelet binding protein von Willebrand factor (vWF) is a well-known phenomenon during febrile *P. falciparum* and *P. vivax* infections.[16–18] In patients with severe malaria, this may be associated with a secondary deficiency in the vWF-inactivating enzyme ADAMTS-13.[19–21] Release of vWF has been suggested to play an etiologic role in malaria-related thrombocytopenia and to mediate sequestration of parasitized erythrocytes.[22] Sequestration is a complex process in which members of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family on infected erythrocytes interacts with CD36 and a variety of other endothelial receptors.[23] Sequestration is an important mechanism for avoiding parasite clearance in the spleen that may be facilitated by activation of endothelium and vWF release.

Most of the studies that involve asymptomatic malaria infections include children, while studies including adults and/or non-African populations are sparse. Enrollment of adults is especially important as an increasing body of evidence supports a role of chronic infectious diseases in the pathogenesis of cardiovascular diseases.[24,25] Raised levels of C-reactive protein (CRP), vWF and vWF propeptide, platelet factor-4 (PF4) and d-dimer, which are established markers of inflammation, endothelial activation/dysfunction, platelet activation and fibrin turnover, respectively, have been associated with cardiovascular events and other health problems.[26–32] In addition, preliminary data have also suggested an association between higher osteoprotegerin (OPG) levels and lower beta2-glycoprotein-1 levels with cardiovascular diseases.[33,34] Marked increases in these markers are found in patients with symptomatic malaria infections, but it is unclear whether these are also increased in asymptomatic parasitemia. The aim of our present study was therefore to determine whether asymptomatic microscopic as well as submicroscopic parasitemia in children and adults are associated with increased inflammation and platelet- and endothelial-cell activation. A cohort of asymptomatic children and adults from three villages in the same district in Southwest Sumba Island, each with different malaria transmission rates, was established and participants were surveyed twice with a five-month interval.

Methods

Study site

This study was performed in the Southwest Sumba district of the Indonesian island Sumba, which is located in East Nusa Tenggara Province. Southwest Sumba district has a tropical climate with a dry season from May to November and a wet season from December to April. Both *P. falciparum* and *P. vivax* are endemic in West Sumba with substantial heterogeneity in malaria prevalence across the district. A recent survey showed an overall malaria prevalence in West Sumba district of 6.8% in the wet season and 5.0% in the dry with prevalence ranging from 0 to 34% between sub-villages.[35]

Study design and participants

The study consisted of an observational prospective study enrolling randomly selected, asymptomatic adults and children from three areas. The following three areas were selected, based on the results from earlier malariological surveys[35]: a) Weepangali, a village with near absent malaria transmission; b) Onggol, a village with low malaria transmission and c) Noha, a village with high malaria transmission. Inclusion criteria for the study were: age 5 years or older, absence of a known chronic illness or use of chronic medication, no signs or symptoms of an acute illness in the past week and no plans for moving to another area in the coming six months. Before recruitment, meetings were held with the village heads and elders followed by a general village meeting at which the study was explained. Adults and children willing to participate were visited in their households in the days after the village meeting until the number of 150 participants per village (aiming for 2/3 adults and 1/3 children) was reached. After written informed consent was obtained, potential participants underwent clinical evaluation by an experienced infectious diseases specialist (J.B.), which included a medical history and physical examination. Participants fulfilling inclusion criteria were enrolled in the study. The number of participants who did not fulfill inclusion criteria was not recorded. Subjects with a tympanic membrane temperature of 38 °C or higher were excluded afterwards. Enrolled participants were sampled twice: in January 2010 and five months later in June 2010. Malaria slides were made for all participants and read on the same day. If positive, participants were treated the following day with a three-day age/weight

dependent curative course of artemether/lumefantrine according to Indonesian Ministry of Health guidelines.

Procedures and data collection

Clinical evaluation consisted of medical history and a physical examination. A standardized questionnaire was used which was completed with the help of a local translator. It contained questions regarding demographics, symptoms or signs suggestive of current illness, past medical history, including earlier presumed malaria episodes and their treatment, current and past drug use, and cardiovascular risk factors, including details on current or past tobacco use, alcohol intake and family history. Physical examination included measurements of weight, height, mid-upper arm circumference, tympanic membrane temperature, blood pressure and pulse, auscultation of heart and lungs, assessment of the presence of hepatosplenomegaly and skin examination.

Peripheral venous blood was collected during the initial visit in January and during the second visit in June. Blood was transferred to a K3-EDTA tube for a full blood count and CTAD vacutainer tube (Beckton Dickinson). The latter contains the anticoagulant sodium citrate and the platelet stabilizing substances theophylline, adenosine and dipyridamole. Peripheral venous blood was also used for preparation of a thick and thin malaria smear, a blood spot for PCR on filter paper (Whatman 3MM, Maidstone, UK) and measurement of glucose level using a portable glucose meter (GlucoTouch; LifeScan). The CTAD tubes were centrifuged at the spot at 2000 g for 10 min within 60 min of collection using a portable centrifuge (Portafuge, LW scientific) and platelet poor plasma was collected, transported on ice to a minus 20 °C freezer until further storage at minus 80 °C.

Laboratory analysis

Plasma markers

Plasma concentrations of vWF, vWF propeptide (vWF:pp), OPG, PF4, beta2-GPI, hs-CRP and thrombin–antithrombin (TAT) complexes were measured in the University Medical Center Utrecht using a semi-automated ELISA on a TECAN Freedom EVO robot (Tecan, Switzerland) as previously described with some minor modifications.[36] The following antibodies were used for the ELISA's: anti-human PF4 (MAB7951, AF795), anti-OPG (MAB8051, BAF805) and anti-C-reactive Protein (DY1707 Duoset) were purchased from R&D Systems (Abingdon, UK); rabbit anti-human VWF antibodies (A0082), peroxidase-conjugated rabbit anti-human VWF (P0226), rabbit anti-goat horseradish peroxidase (HRP) (P0449) and streptavidin-poly-HRP (P0397) were purchased from DAKO (Glostrup, Denmark); rabbit anti-VWF propeptide and rabbit anti-VWF propeptide/biotine were prepared as described by Borchiellini et al.[37] ADAMTS-13 activity was determined using the fluorescence resonance energy transfer (FRETS) assay for ADAMTS-13 activity (Peptides International, Inc., USA) whereby the ADAMTS-13 activity of normal pooled plasma (NPP) of healthy Dutch donors was set at 100%.

Malaria slide and PCR

Thick and thin blood smears were stained with Giemsa, and the number of parasites was quantified against 200 white blood cells. Parasite density was calculated using the patient's white blood cell count. Blood smears were read independently by a laboratory technician in the field and at the Eijkman Institute in Jakarta. A third reader reread the slides in case of disagreement. To determine molecular prevalence of parasites, DNA was extracted from filter papers using Saponin–Chelex extraction. A nested PCR reaction was performed after the data collection was completed at the Radboud university medical center in The Netherlands, amplifying the *P. falciparum* and *P. vivax* specific 18S ribosomal DNA fragment.[38,39] A nested PCR was performed, the nest 1 reaction contained *Plasmodium* genus specific primers. Two downstream species-specific PCR reactions were run to differentiate between *P. falciparum* and *P. vivax* infections.[39] Results were visualized on an ethidium bromide stained 2% agarose gel. Positive controls derived from cultured material and negative controls were included. The used DNA extraction method and PCR assay is able to pick up parasite densities below 5 parasites/μL blood.[40]

Ethics

This study received ethical clearance for the use of human subject from the Eijkman Institute Research Ethics Committee, Jakarta, Indonesia. Written informed consent was obtained from all participants or from parents or legal guardians for participants under the age of 17 years.

Data analysis

All data were recorded on standardized case report forms, which were subsequently digitalized in a database. Laboratory parameters were expressed as geometric means with 95% confidence intervals (95% CI) unless stated otherwise. Differences in laboratory parameters at the first visit between participants from the three areas were analyzed using one way ANOVA with Tukey post tests on log transformed data. The effect of subpatent and patent parasitemia on laboratory values was examined using a linear mixed model, adjusting for age, village and the correlation between observations from the same individual. Analyses were performed within SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Because of the multiple statistical comparisons, a two-sided *P* value of less than 0.01 was considered to be statistically significant.

Results

Characteristics of study population and parasitological data

A total number of 450 participants were enrolled. Seventeen participants were excluded from the analysis because of fever (n = 5) or absent malaria slide (n = 12). The characteristics of the remaining 433 participants,

aggregated by village, are presented in Table 1 and parasitological data are presented in Table 2. The proportion of participants with a positive result for the blood slide and PCR in the three villages was 0% and 9.3% in Weepangali, 4.8% and 11% in Onggol and 36.8% and 48.9% in Noha. Parasite prevalence in Noha was higher in children compared to adults, both by microscopy and PCR. Children and adults from Noha had a worse nutritional status compared to participants from Weepangali with a trend for a lower weight-for-age Z-score (WAZ) and a significantly lower height-for-age Z-score (HAZ) and BMI, respectively. Adult participants from Noha also had the lowest mean systolic blood pressure and the lowest prevalence of hypertension.

During the first visit respectively 30/45 (67%) and 27/52 (52%) of the PCR positive children and adults were slide positive and received a curative course of artemether–lumefantrine. At the second survey at the end of the wet season, a similar proportion of children and adults were PCR positive, although there was a not significant trend for a lower proportion of adults having a positive blood slide (27% vs 15.3%; P = 0.07). The proportion of *P. vivax* infections was also higher during the second visit in both children and adults. Of the children and adults who received antimalarial treatment at visit 1, 21/26 (81%) and 7/21 (33%) had a positive PCR at visit 2, indicating a new or persistent infection.

Laboratory markers across the three villages

Table 1 and Figs. 1 and 2 show laboratory parameters in children and adults from the three villages. At visit 1 at the end of the dry season, children, and to a lesser degree adults from Noha had a significantly lower mean platelet count and a higher hs-CRP concentration than participants from the other two villages (Table 1 and Fig. 1). The geometric mean (95% CI) of hs-CRP concentrations in children and adults from Noha were 574 (360–913) ng/mL and 458 (32–652) ng/mL, respectively compared with 250 (181–346) ng/mL and 219 (182–264) ng/mL in Onggol and 181 (141–233) ng/mL and 260 (211–320) ng/mL in Weepangali. Children from Noha also had a lower Hb concentration (P < 0.005; Table 1) and a there was a trend for a higher mean plasma vWF, vWF propeptide and PF4 levels (Fig. 2), suggesting mild endothelial cell and platelet activation. At the second visit, platelet count or Hb levels had not increased in children from Noha despite treatment of all slide positive individuals five months earlier. Instead, plasma levels of vWF, vWF propeptide and PF4 in children and vWF popeptide and PF4 in adults from Noha had increased further and differences across the areas were more outspoken and statistically significant. The geometric means of these parameters in children from Noha and Weepangali at visit 2 were: vWF 13.9 (12.9–15.1) μg/mL

Table 1 Baseline characteristics.

	Weepangali		Onggol		Noha	
	≤14 yrs	>15 yrs	≤14 yrs	>15 yrs	≤14 yrs	>15 yrs
Number, n	53	97	48	98	48	89
Male, n (%)	29 (54.7)	41 (43.6)	27 (56.3)	45 (45.9)	24 (50.0)	49 (54.5)
Age, yrs, median and interquartile range	9.0 (7.0–12.0)	34.0 (24.0–49.0)	7.0 (5.3–11.0)	34.5 (22.5–47.3)	9.0 (7.0–13.0)	34.0 (23.5–47.0)
Smoking, n (%)	1 (1.2)	32 (33.0)	2 (4.2)	30 (30.6)	4 (7.3)	29 (32.6)
Alcohol intake, n (%)	0 (0)	23 (23.7)	1 (1.9)	14 (14.3)	0 (0)	14 (15.7)
Family history CVD, n (%)	3 (5.7)	11 (11.3)	0 (0)	3 (3.1)	0 (0)	11 (12.6)
Length, m	1.24 (0.16)	1.56 (0.08)	1.20 (0.19)	1.58 (0.08)	1.23 (0.16)	1.59 (0.09)
Weight, kg	22.7 (8.6)	47.8 (8.9)	21.2 (8.7)	43.9 (7.7)	21.6 (6.5)	43.4 (7.0)
Weight-for-age Z-score	−2.41 (1.21)		−2.34 (1.36)		−2.84 (1.71)	
Height-for-age Z-score	−1.69 (1.15)		−1.50 (1.48)		−2.12 (1.18)[b]	
BMI Z-score	−2.02 (1.75)		−1.85 (1.24)		−2.10 (1.63)	
BMI		19.4 (3.0)		17.5 (2.2)[a]		17.1 (1.7)[a]
Systolic blood pressure, mmHg	101 (12)	128 (21)	109 (15)	131 (19)	105 (12)	122 (17)[b]
Diastolic blood pressure, mmHg	63 (7)	76 (12)	70 (10)	79 (12)	68 (10)	76 (11)
Systolic blood pressure ≥140 mmHg, n (%)	0 (0)	21 (21.6)	1 (1.9)	30 (30.6)	0 (0)	12 (13.5)[b]
Temperature, °C	36.6 (0.3)	36.4 (0.4)	36.8 (0.5)	36.4 (0.3)	36.7 (0.4)	36.5 (0.5)
Hemoglobin, mg/L	12.9 (1.7)	12.3 (3.0)	12.8 (1.2)	12.7 (3.0)	11.4 (1.7)[a,b]	12.7 (2.0)
Mean cell volume, fL	67.3 (6.1)	67.8 (8.6)	67.7 (5.0)	69.6 (8.9)	67.7 (6.3)	71.8 (8.1)[a]
White blood cells, $\times 10^9$/L	9.3 (3.1)	7.7 (2.3)	12.9 (7.8)	8.1 (2.3)	9.4 (2.9)	7.8 (2.6)
Platelets, $\times 10^9$/L	327 (85)	290 (116)	349 (95)	259 (91)	232 (95)	227 (78)
Mean platelet volume, fL	7.1 (0.9)	7.3 (1.3)	7.6 (0.8)	8.0 (0.8)	8.0 (0.7)	8.3 (0.8)

Data are provided as mean (standard deviation), unless indicated differently.
[a] $P < 0.05$ in comparison with Weepangali.
[b] $P < 0.05$ in comparison with Onggol.

Table 2 Microscopically detectable and submicroscopic parasitemia at first and second visit.

	Weepangali		Onggol		Noha	
	5–14 yrs	>15 yrs	5–14 yrs	>15 yrs	5–14 yrs	>15 yrs
January, number, n	53	97	48	98	48	89
Positive smear, n (%)		0	4 (8.3)	3 (3.1)	26 (54.2)	24 (27.0)
P. falciparum			3 (6.3)	1 (1.0)	18 (37.5)	19 (24.4)
P. vivax	0		1 (2.1)	2 (2.0)	7 (14.6)	2 (2.2)
Mixed			0	0	1 (2.1)	1 (1.1)
Unclassified			0	0	0	2 (2.2)
Positive PCR, n (%)	6 (11.3)	8 (8.2)	6 (12.5)	10 (10.1)	33 (68.8)	34 (38.2)
P. falciparum	6 (11.3)	8 (8.2)	5 (10.4)	7 (7.1)	24 (50.0)	28 (31.5)
P. vivax	0	0	1 (2.1)	2 (2.0)	4 (8.3)	3 (3.4)
Mixed	0	0	0	1 (1.0)	5 (10.4)	3 (3.4)
June, number, n	46	80	44	80	45	72
Positive smear, n (%)	0	0	4 (9.1)	2 (2.5)	22 (48.9)	11 (15.3)
P. falciparum			3 (6.8)	1 (1.3)	13 (28.9)	6 (8.3)
P. vivax			1 (2.3)	1 (1.3)	7 (15.6)	5 (2.2)
Mixed			0	0	2 (4.4)	0
Positive PCR, n (%)	9 (19.6)	12 (14.1)	6 (13.6)	7 (8.8)	29 (64.4)	25 (34.7)
P. falciparum	9 (19.6)	12 (14.1)	4 (9.1)	4 (5.0)	15 (33.3)	14 (19.4)
P. vivax	0	0	2 (4.6)	3 (3.8)	11 (24.4)	10 (13.9)
Mixed	0	0	0	0	3 (6.7)	1 (1.4)

and 10.5 (9.3–12.0) μg/mL ($P = 0.001$), vWF propeptide 6.8 (6.3–7.4) nM and 5.1 (4.4–5.8) nM ($P = 0.001$) and PF4 3.9 (3.2–4.9) pg/10^6 platelets and 1.9 (1.6–2.3) pg/10^6 platelets ($P < 0.001$). Geometric mean in adults from the same villages were: vWF propeptide 7.7 (7.2–8.2) nM and 6.6 (6.2–7.1) nM ($P = 0.012$) and PF4 4.2 (3.7–4.8) pg/10^6 platelets and 2.0 (1.7–2.3) pg/10^6 platelets ($P < 0.001$). There were no significant differences across the three areas in plasma levels of the other parameters, with the exception of a significantly lower beta2-GPI level in adults from Noha at the first visit ($P = 0.003$) (Supplemental Fig. 1). TAT levels were only detectable in a minority of the participants without a difference across the three villages (data not shown).

Effects of patent and subpatent parasitemia

To investigate the possible contribution of patent and subpatent parasitemia to the observed differences in laboratory parameters between the villages, participants of Onggol and Noha were grouped according to the results of the malaria slide and PCR. Laboratory data are presented in Figs. 3 and 4. Compared to non-infected children, platelet counts and Hb concentrations were significantly lower in children with subpatent parasitemia ($b = -54.6$, $se(b) = 22.50$, $P = 0.015$) and ($b = -1.53$, $se(b) = 0.32$, $p < 0.001$) and lowest in individuals with patent parasitemia ($b = -109.5$, $se(b) = 15.4$, $p < 0.001$) and ($b = -1.19$, $se(b) = 0.23$, $P < 0.001$), after adjustment

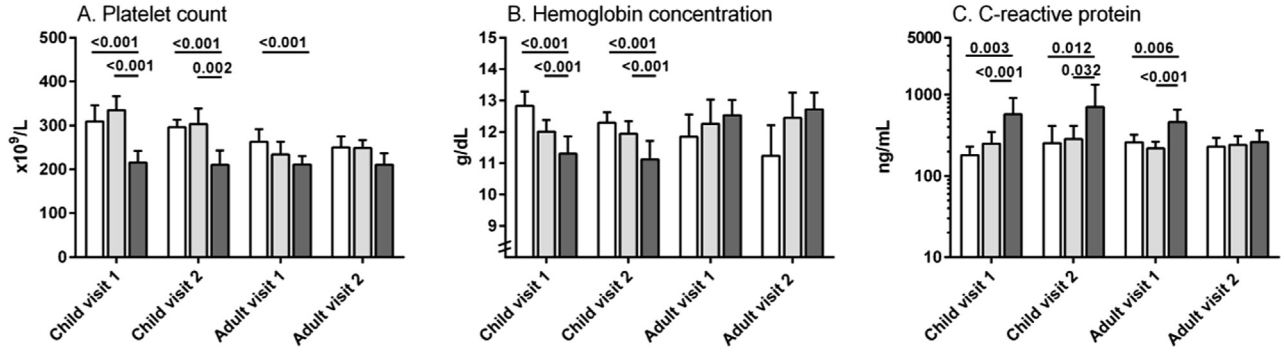

Figure 1 Platelet counts, hemoglobin and C-reactive protein levels in children and adults living in a village with near absent, low and medium/high malaria transmission. Data given are geometric mean with 95% confidence interval. The white bars are the village with near absent malaria transmission, the light gray bar the village with low malaria transmission and the dark gray bars the village with medium/high transmission. Visit 1 was in January at the beginning of the wet season and visit 2 in June, at the beginning of the dry season. CRP levels were log transformed for statistical analysis. Differences were assessed using one way ANOVA with Tukey post tests.

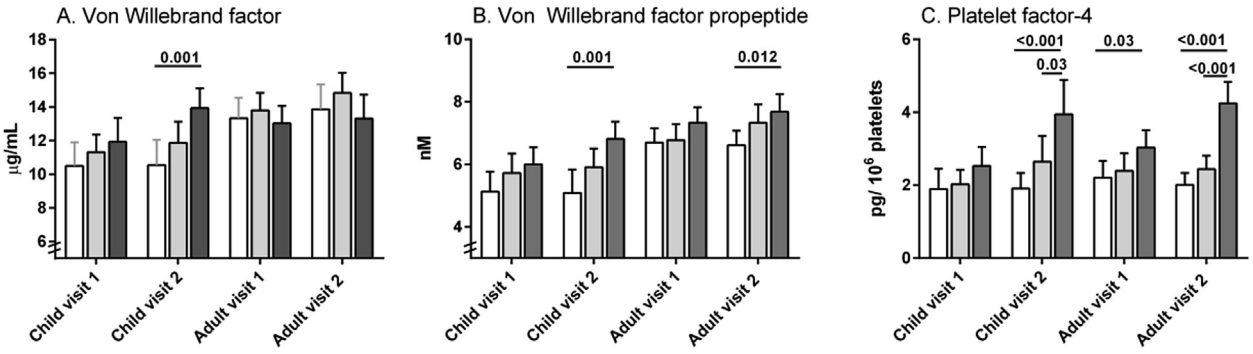

Figure 2 von Willebrand factor (vWF), vWF propeptide and platelet factor-4 levels in children and adults living in a village with near absent, low and medium/high malaria transmission. Data given are geometric mean with 95% confidence interval. The white bars are the village with near absent malaria transmission, the light gray bar the village with low malaria transmission and the dark gray bars the village with medium/high transmission. Visit 1 was in January at the beginning of the wet season and visit 2 in June, at the beginning of the dry season. Differences were analyzed on log transformed data using one way ANOVA with Tukey post tests.

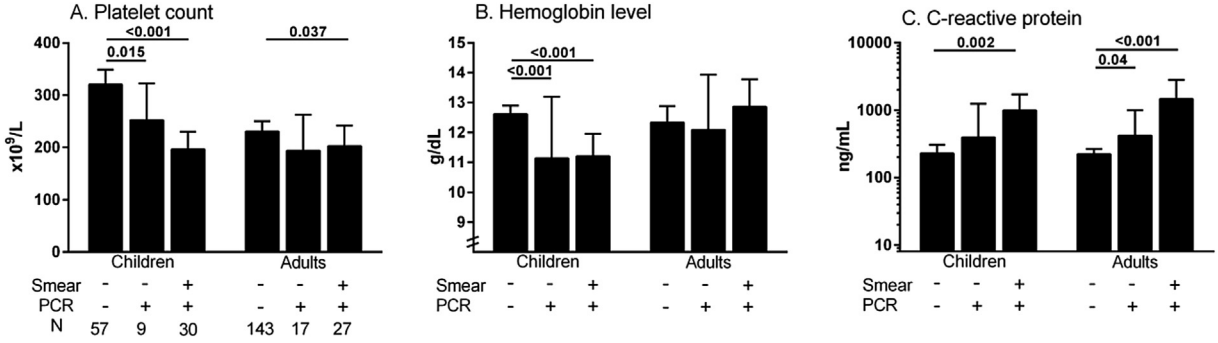

Figure 3 Platelet counts, hemoglobin and C-reactive protein levels in non-infected individuals and those with submicroscopic and microscopic parasitemia. Data presented are the geometric mean with 95% confidence interval of values obtained at the first survey in a low and middle/high malaria transmission village. Statistical difference was tested using a linear mixed model including data from both surveys, adjusting for age, village and the correlation between observations from the same individual. Values of hsCRP and PF4 were log transformed.

for age and the correlation between observations from the same individual (Fig. 3). Geometric means of platelet counts at visit 1 in non-infected children and those with subpatent and patent parasitemia were 321 (295—349) $\times$ 10^9/L, 252 (197—323) $\times$ 10^9/L and 196 (167—230) $\times$ 10^9/L. Hb concentrations in these children were 12.6 (12.3—12.9) g/dL, 11.1 (9.4—13.2) g/dL and 11.2 (10.5—12.0) g/dL, respectively. Adults with patent parasitemia also had a trend for a lower platelet count than non-infected adults (202 (169—242) x 10^9/L vs. 230

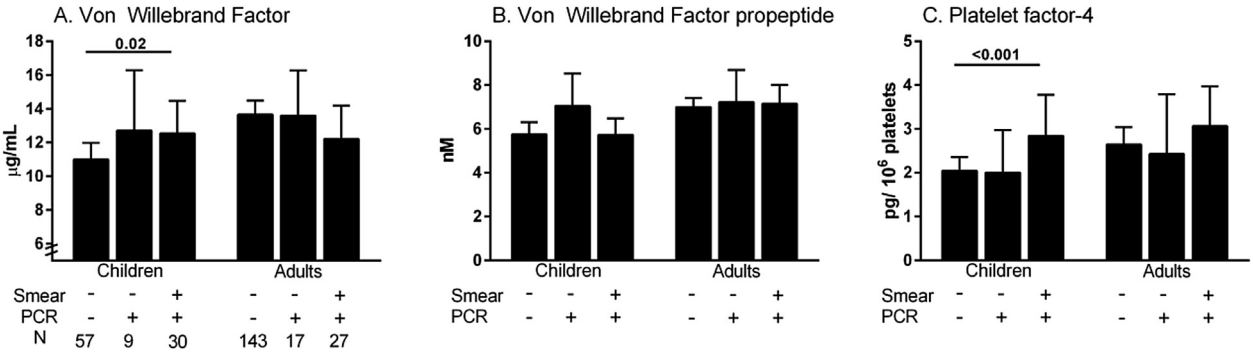

Figure 4 von Willebrand factor (vWF), vWF propeptide and platelet factor-4 levels in non-infected individuals and those with submicroscopic and microscopic parasitemia. Data presented are the geometric mean with 95% confidence interval of values obtained at the first survey in a low and middle/high malaria transmission village. Statistical difference was tested using a linear mixed model including data from both surveys, adjusting for age, village and the correlation between observations from the same individual. Values of PF4 were log transformed.

(212–251) × 10^9/L; P = 0.037), but there were no differences in Hb concentration. Compared to non-infected individuals, hs-CRP concentrations were higher in children and adults with patent parasitemia (log transformed values; b = 0.78, se(b) = 0.10, P < 0.001 and b = 0.73, se(b) = 0.09, P < 0.001, respectively). Corresponding geometric means of hs-CRP concentrations at visit 1 were 988 (567–1722) ng/mL and 1464 (767–2792) ng/mL in children and adults with patent parasitemia and 227 (269–306) ng/mL and 223 (187–265) ng/mL in aparasitemic children and adults. In addition, patent parasitemia in children, but not in adults, was associated with a significantly higher PF4 level per 10^6 platelets (log transformed value; b = 0.15, se(b) = 0.05, P = 0.002) and a trend for a higher vWF level (b = 1.74, se(b) = 0.75, P = 0.02) than in non-infected children (Fig. 4). Geometric means of PF4 and vWF were 2.8 (2.1–3.8) pg/10^6 platelets vs. 2.0 (1.8–2.4) pg/10^6 platelets and 12.5 (10.9–14.5) μg/mL and 11.0 (10.1–12.0) μg/mL, respectively. There were no significant differences in the other markers specified in the previous paragraph (data not shown). There was also no statistical difference in the nutritional scores HAZ and WAZ in children and BMI in adults between those with and without parasitemia (data not shown). Analyses were not adjusted for these nutritional scores, as they were not associated in univariate analysis with any of the studied parameters described above. Finally, we analyzed values of a selection of laboratory parameters taken in children from Onggol and Noha at the second visit in relation to the results of the malaria PCR at both visits (Fig. 5). Children with (sub)patent parasitemia at the first visit, but a negative PCR at the second, still had a trend for a lower platelet count and a higher plasma vWF level, whereas values of Hb and hsCRP were similar to values in non-infected children during visit 1. There were no significant differences in these laboratory values between individuals with *P. falciparum* and *P. vivax* (data not shown).

Discussion

In this study, we show that children who live in a high malaria transmission area and are infected with malaria parasites without symptoms suggestive of clinical malaria have low-grade inflammation (increased hsCRP levels), a lower Hb and platelet counts with, at the end of the wet season, evidence of platelet activation (increased PF4 concentrations) and mild endothelial activation (increased vWF and vWF propeptide concentrations). These effects of asymptomatic parasitemia were not mirrored in adults, with the exception of a

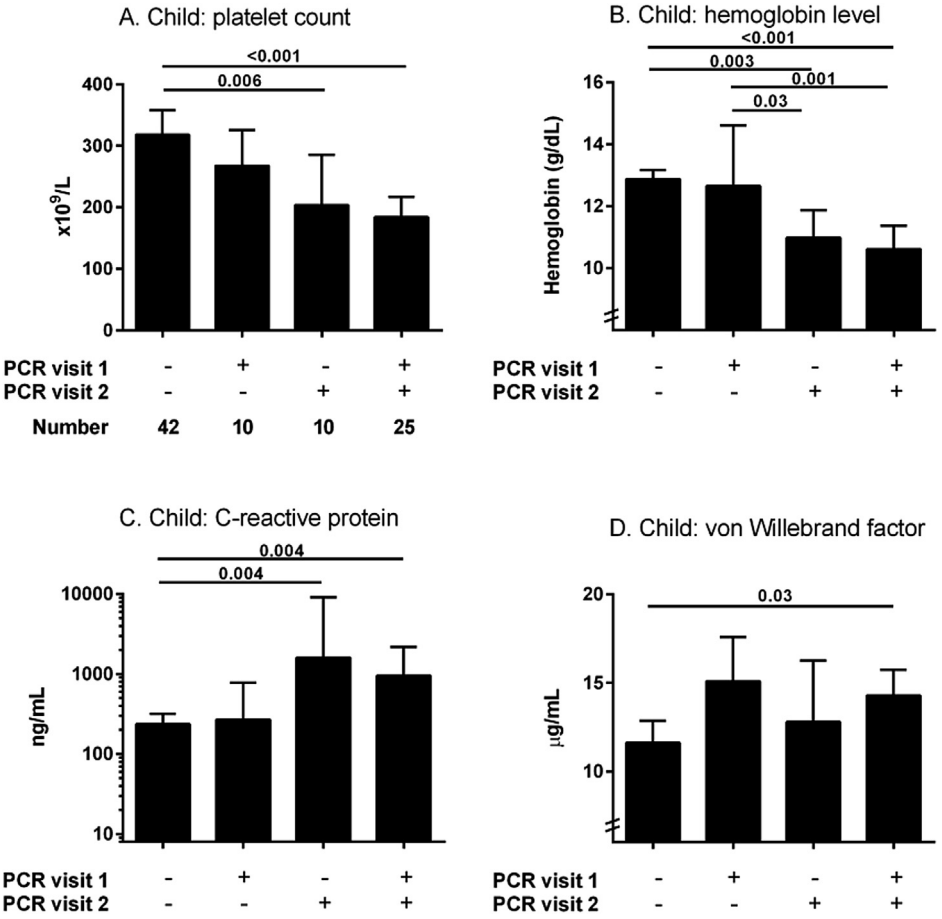

Figure 5 Value of selected laboratory parameters at second survey in children from low and middle/high malaria transmission area in relation to parasitemia status at first and second visit. Survey 1 was in January at the beginning of the wet season and survey 2 in June, at the beginning of the dry season. Data given are geometric mean with 95% confidence interval. Data were analyzed using one way ANOVA with Tukey post tests. CRP and vWF concentrations were log transformed.

higher hs-CRP concentration in adults with patent parasitemia. These findings indicate that the asymptomatic status of malaria, based on conventional malaria symptoms, ignores many subtle consequences of low-level malaria infections, and that all malaria infections, at least in children, should be regarded as potentially harmful.

To elucidate the role of asymptomatic parasitemia as a causal factor in these observations we explored a possible dose-response in parasite levels by comparing parasite-free individuals with individuals infected with submicroscopic concentrations and microscopically detectable parasites. We observed evidence for a dose-response in these associations in children for multiple parameters, including platelet counts and levels of Hb, hsCRP, PF4 and vWF.

Our current data support recent findings by Park et al.,[41] who found patent, but not subpatent parasitemia in Ugandan children to be associated with higher levels of vWF, intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule (VCAM). Previous studies in Africa have also shown an increased CRP level in children and pregnant women with asymptomatic parasitemia.[42–45] Data on the acute phase response in populations outside Africa and in adults are sparse. Imrie et al.[46] did not find an elevated CRP level in the majority of children with microscopy positive asymptomatic malaria in Papua New Guinea. Our group had similar findings in asymptomatic microscopy positive school children from Sumba, although we did not use a high sensitive CRP assay in this study.[47] What our present study adds to these previous studies is the enrollment of both children and adults and the use of PCR for detection of subpatent parasitemia. The comparison between children and adults is especially interesting. Except for the elevated hsCRP levels, most of the other laboratory abnormalities associated with parasitemia were absent or less outspoken in adults than in children. Tolerance induced by repeated exposure is the most likely explanation for this observation. Our previous observations in healthy adult volunteers that platelet counts declined and vWF levels increased early during the course of a controlled human malaria infection, before parasites were microscopically detectable, supports this assumption.[17] It also suggests that the health consequences of asymptomatic parasitemia are more pronounced in children than in adults.

So far, the adverse consequences of asymptomatic parasitemia were predominantly thought to lie in its role in malaria transmission and the need to target asymptomatically infected patients to achieve malaria elimination.[5] Our suggestion that asymptomatic parasitemia itself has adverse consequences for health in children is based on the following assumptions. First, inflammation and platelet and endothelial activation are well known risk factors for the development of atherosclerosis and elevated hs-CRP levels are an independent risk factor for cardiovascular events.[26–28,31] Our results may therefore warrant further work looking at the association of raised concentrations of these parameters in childhood with cardiac endpoints in adulthood. Second, asymptomatic parasitemia was associated with lower Hb levels in children. Anemia is an important health problem in malaria endemic regions and responsible for significant morbidity and death, especially in children. Inflammation suppresses erythropoiesis, shortens erythrocyte survival and leads to disturbances in iron homeostasis. The latter is largely caused by increased production of the iron-regulatory hormone hepcidin. We previously showed in the same area that asymptomatic school children with smear positive parasitemia had higher levels of the iron-regulatory hormone hepcidin, leading to reduced iron availability for erythropoiesis.[47] Third, chronic inflammation is associated with malnutrition and cachexia in a wide variety of chronic diseases. Indeed, several but not all studies found presence of parasitemia to be associated with adverse nutritional parameters.[48,49] This may also explain the worse nutritional status in children from village 3 in our study, although we did not find an association of nutritional scores with parasitemia status or one of the parameters studied. Finally, a recent study in Ugandan children suggested asymptomatic parasitemia to result in cognitive impairments.[12]

Strengths of our study are that all participants were thoroughly examined by an infectious disease specialist to exclude symptomatic cases or concurrent non-malarial illnesses. In addition, as previously mentioned, most studies examining the effects of asymptomatic parasitemia were limited to children, whereas asymptomatic parasitemia is also common in adults. Finally, our study highlights the value of looking beyond fever and anemia to determine the symptomatic status of malaria infections. Limitations of our study were the low number of participants with submicroscopic parasitemia and *P. vivax* parasitemia, which hampered analysis of these subjects as a separate group. Secondly, potential selection bias was obscured by the fact that the number of participants who refused to participate or did not fulfill inclusion criteria was not recorded. Thirdly, whilst attractive, dose-response relationships have to be interpreted with caution. Current parasitemia may also be an indicator for longer term heterogeneity in malaria exposure and one may therefore hypothesize that the association between parasite status and platelet counts, Hb and the other parameters is the result of differences in previous exposure to microscopically detectable or possibly even symptomatic malaria.[14] We therefore restricted our analysis to the second time-point and adjusted estimates for parasite exposure in the previous exposure, which did not alter any of the described associations (data not shown). Finally, cardiovascular risk has been associated with a variety of other markers of inflammation, which have not been measured in this study. These include interleukin (IL)-6, IL-18, myeloperoxidase, soluble intercellular adhesion molecule-1, P-selectin and coagulation parameters other than TAT, such as d-dimer and fibrinogen.

In conclusion, our observations suggest that low-grade inflammation and platelet activation occur in children and adults with asymptomatic parasitemia, while mild endothelial cell activation and lower Hb levels are limited to children. In view of these possible adverse health consequences of asymptomatic parasitemia, efforts aimed at reducing asymptomatic parasitemia both at an individual and population level may be warranted. This is challenging, as persons with asymptomatic parasitemia will not seek clinical care due to absence of clinical illness. Therefore, other approaches may be required that actively aim to detect and treat all malaria infections in the community, regardless of concurrent symptoms suggestive of acute malaria attacks.

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Conflict of interest

The authors declare to have no commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2015.08.005.

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