bjh research paper

Mean platelet counts are relatively decreased with malaria but relatively increased with endemic Burkitt Lymphoma in Uganda, Tanzania, and Kenya

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Summary

Platelet counts are decreased in *Plasmodium falciparum* malaria, which is aetiologically linked with endemic Burkitt lymphoma (eBL). However, the pattern of platelet counts in eBL cases is unknown. We studied platelet counts in 582 eBL cases and 2 248 controls enrolled in a case-control study in Uganda, Tanzania and Kenya (2010–2016). Mean platelet counts in controls or eBL cases with or without malaria-infection in controls versus eBLcases were compared using Student's *t*-test. Odds ratios (ORs) and two-sided 95% confidence intervals (95% CIs) were estimated using multiple logistic regression, controlling for age, sex, haemoglobin and white blood cell counts. Platelets were decreased with malaria infection in the controls [263 vs. 339 × 10^9 platelets/l, $P < 0{\cdot}0001$; adjusted OR (aOR) = 3·42, 95% CI: 2·79–4·18] and eBL cases (314 vs. 367 × 10^9 platelets/l, *P*-value = 0·002; aOR = 2·36, 95% CI: 1·49–3·73). Unexpectedly, platelets were elevated in eBL cases *versus* controls in overall analyses (mean: 353 vs. 307 × 10^9 platelets/l, $P < 0{\cdot}0001$; aOR = 1·41; 95% CI: 1·12–1·77), and when restricted to malaria-positive (mean 314 vs. 263 × 10^9 platelets/l, $P < 0{\cdot}0001$; OR = 2·26; 95% CI: 1·56–3·27) or malaria-negative (mean 367 vs. 339 × 10^9 platelets/l, $P < 0{\cdot}001$; OR = 1·46; 95% CI: 1·17–1·83) subjects. Platelets were decreased with malaria infection in controls and eBL cases but elevated with eBL.

Keywords: Burkitt lymphoma, epidemiology, Epstein–Barr virus, non-Hodgkin lymphoma, Plasmodium falciparum malaria, platelet counts.

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Endemic Burkitt lymphoma (eBL) is an aggressive germinal centre B-cell lymphoma that is relatively common in countries with holoendemic *Plasmodium falciparum* (*Pf*) malaria transmission.[1] The aetiology of eBL is linked to *Pf* malaria,[2] childhood infection with Epstein–Barr virus,[3] and chromosomal abnormalities involving the translocation of the *MYC* oncogene on chromosome 8 into the vicinity of immunoglobulin gene enhancer elements on chromosomes 14, 22, or 2.[4,5] The role of malaria in eBL aetiology is supported by case-control studies showing that genetic variants, such as the sickle cell trait, that protect against malaria also protect against eBL[6] and sero-immunoepidemiological studies that demonstrate significantly altered anti-malaria[7] and anti-EBV antibody profiles.[8] Biomarkers for these infections may offer a rational way for discovery of eBL biomarkers.

Platelets have recently emerged as important players in the control of life-threatening infections,[9] including *Pf* malaria.[10] Consistent with this function, they are the second most abundant formed element of blood after red blood cells (RBCs) and are maintained in a narrow physiological range (150–450 $\times$ 10^9 platelets/l).[11,12] Platelets have been shown to kill *Pf* parasites by releasing platelet factor 4 (PF4)[13] which interacts with other molecules and is translocated into infected RBCs where it kills parasites.[14] Platelets also carry proteins in their surface membrane that facilitate binding and internalization of malaria parasites.[15] These proteins include CD36, which is a major receptor for *Pf* malaria parasites,[16] and is also involved in PF4-mediated parasite killing. These mechanisms are responsible for the removal of 5–60% of the malaria parasite biomass.[13]

Asymptomatic or clinical malaria is associated with decreased platelet counts and the reduction is greatest in children with the most severe form of malaria.[17–21] However, whether malaria infection is associated with decreased platelet count in children with eBL is presently unknown. Asare *et al.*[22] recently reported normal to increased circulating platelet counts but with apparently decreased platelet membrane glycoprotein expression and platelet function in children with eBL in Ghana, compared to controls who were studied by flow cytometric studies. However, their small sample size (16 cases and 15 controls) and lack of malaria data precludes firm conclusions. Based on studies showing that platelets inhibit malaria parasite growth,[13,14] we hypothesized that malaria infection would be associated with a reduction in platelet counts in children with eBL. Based on the assumption that eBL is causally related to malaria[6,7] we hypothesized that compared to healthy controls, eBL would be associated with decreased platelets.

Here, we report platelet count results in eBL cases and controls enrolled in the EpideMiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) study in Uganda, Tanzania, and Kenya during 2010–2016.[23]

Patients and methods

Study population and data

Briefly, children aged 0–15 years diagnosed with eBL (61·4% histologically or cytologically confirmed) were enrolled as cases.[23] Eligibility was restricted to usual residents (≥4 months prior to enrollment) of six selected malaria holoendemic regions in northern Uganda, northeastern Tanzania and western Kenya.[23] Controls were selected from healthy general population children residing in the same regions as the eBL cases. The controls were enrolled contemporaneously from 300 random villages (100 per country) in the study areas.[23] The controls were age- and sex-frequency matched to the distribution of historical eBL cases from the study region.[23] Village level microgeographical data, including proximity to surface water and population density, were captured to adjust for local malaria transmission.[23] Individual level data, including age, sex, and a history of malaria treatment as an in- or outpatient were captured using structured interviewer-administered questionnaires. Venous blood samples (clinical: 4 ml and research: 10 ml) were collected in EDTA tubes. The clinical blood samples were immediately tested for a complete blood count using commercial haematology analyzers, including study-provisioned QBC Star

(QBC Diagnostics Inc., Philipsburg, PA, USA). Other automated haematology analyzers available at the local hospitals were used (Sysmex 500i, Sysmex Corp., Kobe, Japan; Medonic M16, Boule Diagnostics AB Domnarvsgatan, Spanga, Sweden; Huma count 5L, HUMAN Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany). Malaria infection was diagnosed based on thick film microscopy or commercial malaria antigen rapid diagnostic tests (malaria-RDTs).[24]

Ethical approval

The study was approved by the Uganda Virus Research Institute's Research and Ethics Committee, Uganda National Council for Science and Technology (H816), Tanzania National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/1023), Moi University/Moi Teaching and Referral Hospital Institutional Research and Ethics Committee (000536), and National Cancer Institute's Special Studies Institutional Review Board (10-C-N133). Guardians of the children gave written informed consent. Children aged ≥7 years old gave assent.

Statistical analysis

Analyses were restricted to subjects with platelet count data (83·5% of eBL cases and 76·6% of controls,[23] Figure S1), after confirming that the age, sex, village characteristics and malaria history of eBL cases and controls with platelet counts were not different from those excluded because of missing platelet counts. Primary analyses focused on platelet counts as the independent variable and malaria or eBL as the outcome variables. These analyses were done on the combined dataset; however, country-specific sensitivity analyses were conducted to verify the consistency of the findings (results not shown).

Means and standard deviations (SDs) and medians and interquartile ranges (IQRs) of platelet counts, haemoglobin and white blood cell (WBC) counts in controls were calculated in malaria-positive and -negative eBL cases and controls. The mean and median values for platelet counts, haemoglobin and WBCs were similar; thus, means are used to describe the distributions in this paper. Because platelet counts vary by age, ethnicity and other factors, mean values in the malaria-uninfected controls were used to estimate the normal values expected in healthy children in each study country (Table SI). We used Student's *t*-test to compare the means of platelet counts, haemoglobin level and WBC counts in malaria parasitaemia/antigenaemia-positive and -negative controls and eBL cases. Because an inverse relationship between platelet count and mean platelet volume holds true in different human populations[25] and different animal species,[26] we calculated the correlation coefficient between platelet counts and mean platelet volume[11] as a quality control check of the validity of platelet count results, using data from Uganda and Kenya (mean platelet volume was not available in Tanzania).

We generated binary categorical variables for platelet counts, haemoglobin, and WBC count defined as having a value equal to or greater than the mean *versus* not, in malaria-uninfected controls in their age group and country. Odds ratios (ORs) and two-sided 95% confidence intervals (95% CIs) of associations between eBL or malaria infection and platelet counts (as a binary variable) were calculated using multiple logistic regression. The association between eBL and platelet counts (as a binary variable) was assessed stratified by malaria parasitaemia/antigenaemia-positive or -negative status. Associations were mutually adjusted for sex, age group, village characteristics, malaria parasitaemia/antigenaemia, history of malaria treatment, haemoglobin and WBC counts as confounders.[24] The measures of malaria above imperfectly correlate with lifetime malaria exposure, which is the actual risk factor for eBL.[27,28] Hence adjusting for these measures may bias the results towards the null and lead to conservative estimates.[27,28] Haemoglobin and WBC counts were considered as confounders and used to control for disease-related effects on the bone marrow and splenic function.[29,30] Finally, we searched the published literature for papers that reported individual pretreatment platelet count results in BL cases to gain insights about the distribution of platelet counts in BL cases worldwide.

Results

Characteristics of study subjects

We studied 582 eBL cases and 2 248 controls in Uganda, Tanzania, and Kenya with platelet count data (Table I). No significant differences were seen between eBL cases and controls with platelet counts *versus* those that were excluded because platelet counts were missing. The eBL cases studied were significantly younger than the controls, although the absolute difference was small [mean: 7·4 (SD 3.7) years vs. 7·8 (SD 3.4) years, $P = 0{\cdot}014$]. The eBL cases were more likely than the controls to be male (63·9% vs. 53·1% in controls; $P < 0{\cdot}0001$; Table I). As previously reported,[23] the eBL cases were more likely than the controls to reside in villages with a high mean population count or in villages near surface water. The eBL cases were more likely to have a lower prevalence of malaria parasitaemia/antigenaemia at enrollment and a lower frequency of history of malaria-related fevers reported in the past 12 months or history of inpatient malaria treatment in the past 12 months (Table I). However, consistent with a role of malaria, they were more likely to report a history of outpatient malaria in the past 12 months (Table I). Consistent with lymphoma diagnosis being associated with B symptoms, eBL cases were more likely than controls to report a fever at enrollment and also to report a non-malaria-related fever within the past six months.

Platelet counts decreased with malaria parasitaemia/antigenaemia in controls and eBL cases

We observed a wide range of variation in values of platelet count (19–999 $\times$ 10^9 platelets/l) and mean platelet volume

Table I. Distribution of demographical and malaria measures in controls and endemic Burkitt lymphoma cases in the EpideMiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) study.

Characteristics	Controls	Cases	*P*
All subjects	2 248 (100·0)	582 (100·0)	
Demographics			
Age, years (mean ± SD)	7·8 (3·4)	7·4 (3·7)	0·014
Age group, years			0·002
0–2	119 (5·3)	50 (8·6)	
3–5	504 (22·4)	155 (26·6)	
6–8	714 (31·8)	161 (27·7)	
9–11	548 (24·4)	123 (21·1)	
>12–15	363 (16·2)	93 (16·0)	
Sex			<0·0001
Male	1 193 (53·1)	370 (63·9)	
Missing/unknown	0	3	
Village characteristics			
Proximity to water			<0·0001
Far (>500 m)	1 075 (47·8)	99 (20·6)	
Missing/unknown	0	102	
Population density of children			0·017
Low	1 407 (62·6)	273 (56·8)	
Missing/unknown	0	101	
Malaria status/history of malaria treatment			
Malaria rapid diagnostic test			<0·0001
Positive	957 (42·6)	151 (26·2)	
Missing/unknown	1	5	
Inpatient malaria treatment			0·289
Yes, past 12 months	255 (11·4)	55 (9·7)	
Yes, >12 months	409 (18·3)	95 (16·7)	
Never	1 574 (70·3)	418 (73·6)	
Missing/unknown	10	14	
Outpatient malaria treatment			<0·0001
Yes, past 12 months	920 (41·1)	265 (46·7)	
Yes, >12 months	188 (8·4)	99 (17·4)	
Never	1 130 (50·5)	204 (35·9)	
Missing	10	14	
History of fevers and hospital admission			
Has fever at enrollment			<0·0001
Yes	147 (6·6)	348 (61·8)	
Missing/unknown	8	19	
Reported ≥ 1 fever in the last 12 months			<0·0001
Yes	1 570 (75·0)	135 (62·5)	
Missing/unknown	154	366	
Reported ≥ 1 fever due to malaria in the past 6 months			<0·0001
Yes	1 557 (69·6)	334 (59·8)	
Missing/unknown	10	23	
Reported ≥ 1 fever not due to malaria in the last 6 months			<0·0001
Yes	373 (16·7)	273 (49·4)	
Missing/unknown	16	29	
Reported ≥ 1 hospital admission			<0·0001
Yes	826 (37·0)	377 (66·4)	
Missing/unknown	13	14	

Column percentages provided for each characteristic. *P*-values are based on a chi-square test for differences in the distribution of characteristics between eBL cases and controls.

(6·1–18·2 fl) in all the controls, including 5·1% of controls with values <150 × 10^9 platelets/l and 10·1% with values >450 × 10^9 platelets/l. The platelet count and volume values were inversely correlated with each other in all controls ($r = -0{\cdot}021$, $P = 0{\cdot}0001$), as well as in controls who were either malaria-negative ($r = -0{\cdot}018$, $P = 0{\cdot}01$) or malaria-positive ($r = -0{\cdot}59$, $P = 0{\cdot}001$; Figure S2). The mean platelet count was significantly decreased in controls with malaria parasitaemia/antigenaemia *versus* those without (263 vs. 339 × 10^9 platelets/l, *P*-value < 0·0001, Fig 1A; aOR = 3·42, 95% CI: 2·79–4·18, Table II) and in the eBL cases with malaria parasitaemia/antigenaemia *versus* those without (314 vs. 367 × 10^9 platelets/l, *P*-value = 0·002, Fig 1B; aOR = 2·36, 95% CI: 1·49–3·73,Table III). The association of decreased platelet count with malaria parasitaemia/antigenaemia in the controls was observed in all the countries, but with slight variation in the magnitude of association (Uganda: aOR = 4·50,Tanzania: aOR = 3·43 and Kenya: aOR = 2·65; *P*-value for interaction = 0·030). This association was also observed in the eBL cases but with variation in the magnitude of association, albeit with overlapping confidence intervals, in the three countries [Uganda: aOR = 3·56 (95% CI: 1·85–6·84), Kenya: aOR = 3·09 (95% CI: 1·10–8·67) and Tanzania: aOR = 0·80 (95% CI: 0·23–2·73); *P*-value for interaction = 0·637].

Platelet counts significantly elevated in eBL cases compared to controls

As noted above, the platelet counts showed wide variability, but the distribution in malaria parasitaemia/antigenaemia-negative and -positive eBL cases was broader and skewed to the right compared with the distribution observed in the controls (Fig 2). Thus, there were more eBL cases than controls with thrombocytopenia (defined as <100 × 10^9 platelets/l: 6·9% vs. 0·8%) and thrombocytosis (defined as >650 × 10^9 platelets/l: 6·7% vs. 1·3%). Platelet counts were significantly elevated in eBL cases compared to controls (mean: 353 vs. 307 × 10^9 platelets/l, $P = 0{\cdot}001$; Fig 1C). Considering a platelet count above the upper normal range (>450 × 10^9 platelets/l) as a cutoff, eBL cases were more likely than the controls to have a value above this range both in the overall data (25·6% vs. 10·1%), and when the results were stratified by malaria parasitaemia/antigenaemia-positive (15·9% vs. 4·8%) and malaria parasitaemia/antigenaemia-negative status (28·9% vs. 14·0%). Consistent with the observation above that platelet counts were more dispersed in eBL cases than controls (Fig 2), the eBL cases were more likely than the controls to have a platelet count <150 × 10^9 platelets/l in the overall data (11·9% vs. 5·1%); however, this was pronounced in controls who were malaria parasitaemia/antigenaemia-negative (12·7% vs. 2·1%) compared to those who were malaria parasitaemia/antigenaemia-positive (9·3% vs. 9·1%; Fig 2).

When platelet count was considered as a binary variable of having a value equal to or greater than the mean *versus* not, eBL was associated with having an elevated platelet count (OR = 1·89, 95% CI 1·57–2·28; Table III). The association remained after mutually adjusting for sex, age, village characteristics, malaria parasitaemia/antigenaemia, history of malaria treatment, haemoglobin and WBC counts as confounders (aOR = 1·41,95% CI: 1·12–1·77) and in analyses stratified by malaria parasitaemia/antigenaemia-positive (OR = 2·26; 95% CI: 1·56–3·27) and malaria parasitaemia/antigenaemia-negative status (OR = 1·46; 95% CI: 1·17–1·83).

Haemoglobin significantly decreased with malaria parasitaemia/antigenaemia in the controls and eBL cases

Malaria parasitaemia/antigenaemia in the controls was associated with significantly decreased haemoglobin (mean: 119 vs. 126 g/l, $P < 0{\cdot}0001$), but not in the eBL cases (mean: 104 vs. 100 g/l, $P = 0{\cdot}09$). Thus, a statistically significant association was observed between malaria parasitaemia/antigenaemia and decreased haemoglobin only in the controls (aOR = 2·50,

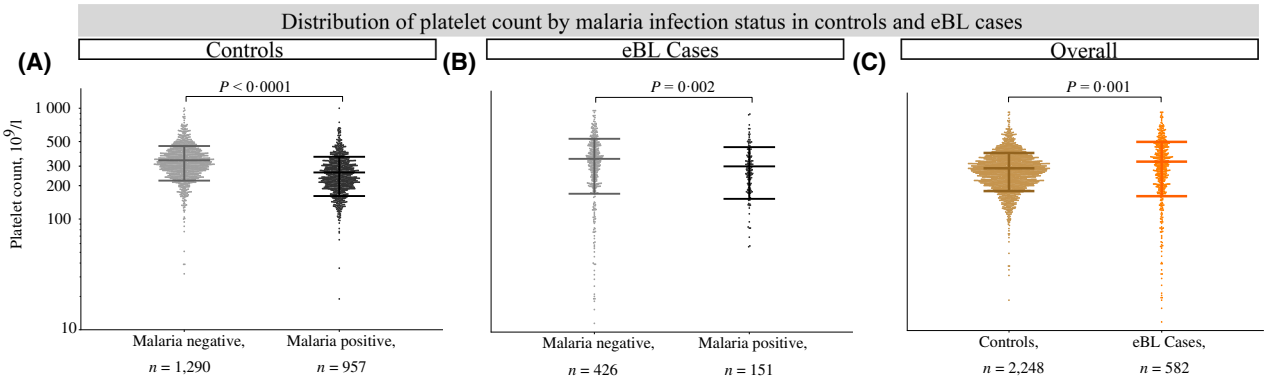

Fig 1. Distribution of platelet count by malaria infection status in the controls and eBL cases. [Colour figure can be viewed at wileyonlinelibrary.com]

Table II. Association between malaria parasitaemia/antigenaemia and platelet counts, haemoglobin and white cell counts.

	Controls				eBL cases			
	Malaria				Malaria			
Characteristics	− *n* (%)	+ *n* (%)	OR* (95% CI)	aOR† (95% CI)	− *n* (%)	+ *n* (%)	OR* (95% CI)	aOR† (95% CI)
Platelet count, 10^9/l								
≥Mean ‡	571 (44·3)	189 (19·8)	Ref	Ref	229 (53•8)	54 (35·8)	Ref	Ref
<Mean	719 (55·7)	768 (80·3)	3·23 (2·66–3·92)	3•42 (2•79–4•18)	197 (46•2)	97 (64·2)	2·09 (1·42–3·06)	2·36 (1•49–3•73)
P heterogeneity[4]			<0·0001	<0·0001			0·0002	0·0003
P-value for interaction§			0·014	0·030			0·864	0·637
Haemoglobin level, g/l								
≥Mean	677 (52·5)	307 (32·1)	Ref	Ref	65 (15•3)	20 (13·3)	Ref	Ref
<Mean	613 (47·5)	650 (67·9)	2·34 (1·96–2·78)	2•50 (2·08–3·01)	361 (84•7)	131 (86·8)	1·18 (0·6–9, 2·02)	1·06 (0•58–1•92)
P heterogeneity			<0·0001	<0·0001			0·549	0·860
P-value for interaction			0·626	0·797			0·087	0·098
White blood cell count, 10^9/l								
<Mean	772 (59·8)	550 (57·5)	Ref	Ref	194 (45•5)	79 (52·3)	Ref	Ref
≥Mean	518 (40·2)	407 (42·5)	1·10 (0·93–1·31)	1•28 (1•07–1•55)	232 (54•5)	72 (47·7)	0·76 (0·53–1·11)	1·07 (0•68–1•67)
P heterogeneity			0·258	0·008			0·152	0·781
P-value for interaction			0·130	0·044			0·955	0·932

OR, odds ratio; aOR, adjusted odds ratio.

*This column shows the odds ratios for associations based on data from all three study countries combined, adjusted for each country.

†Mutually adjusted odds ratios for association for all haematologic characteristics in the model, village characteristics (rural/urban and wet/dry), age, sex, malaria admission and treatment history

‡All binary categorical variables for platelet count, haemoglobin and white blood cell count were defined using the mean in malaria-negative controls in each age group and country. The reference group for platelet count is having a platelet count equal to or above the age group-specific mean in malaria parasitaemia/antigenaemia-negative controls.

§*P* heterogeneity values across study countries are provided in addition to *P*-values for interaction between study countries and the variables examined.

Table III. Association between eBL and platelet counts, haemoglobin and white cell counts.

Characteristics	Controls *n* (%)	Cases *n* (%)	OR* (95% CI)	aOR† (95% CI)
Platelet count, 10^9/l				
<Mean‡	1 488 (66·2)	296 (50·9)	Ref	Ref
≥Mean	760 (33·8)	286 (49·1)	1·89 (1·57–2·28)	1·41 (1·12–1·77)
P heterogeneity§			<0·0001	0·003
P-value for interaction§			0·605	0·443
Haemoglobin level, g/l				
≥Mean	984 (43·8)	6 (14·8)	Ref	Ref
<Mean	1 264 (56·2)	496 (85·2)	4·49 (3·52–5·73)	4·77 (3·63–6·25)
P heterogeneity			<0·0001	<0·0001
P for interaction			0·0002	0·001
White blood cell count, 10^9/l				
<Mean	1 322 (58·8)	276 (47·4)	Ref	Ref
≥Mean	926 (41·2)	306 (52·6)	1·58 (1·–32, 1·90)	1·44 (1·15–1·80)
P heteogeneity			<0·0001	0·001
P for interaction			0·012	0·010
Malaria rapid diagnostic test				
Negative	1 290 (57·4)	426 (73·8)	Ref	Ref
Positive	957 (42·6)	151 (26·2)	0·48 (0·39–0·59)	0·37 (0·29–0·48)
P heterogeneity			<0·0001	<0·0001
P for interaction			0·0001	0·017

OR, odds ratio; aOR, adjusted odds ratio.

*This column shows the odds ratios for associations based on data from all the three study countries combined, adjusted for each country.

†Mutually adjusted odds ratios for all haematologic characteristics in the model, malaria infection, village characteristics (rural/urban and wet/dry), age, sex, malaria admission and treatment history.

‡All binary categorical variables for platelet count, haemoglobin and white blood cell count were defined using the mean in malaria-negative controls in each age group and country. The reference group for platelet count is having a platelet count less than the agegroup-specific mean in malaria parasitaemia/antigenaemia-negative controls.

§*P* heterogeneity values across study countries are provided in addition to *P*-values for interaction between study countries and the variables examined.

95% CI: 2·08–3·01; Table II) but not in the cases. However, when eBL cases were compared to the controls, eBL cases were more likely to have decreased haemoglobin and this association remained statistically significant after mutually adjusting for sex, age, village characteristics, malaria parasitaemia/antigenaemia, history of malaria treatment, WBC counts and platelet count (aOR = 4·77, 95% CI: 1·12–1·77; Table III).

Leukocytosis associated with malaria parasitaemia/antigenaemia in controls but not in eBL cases

A small association was demonstrated between malaria parasitaemia/antigenaemia and leukocytosis in the controls (aOR = 1·28; CI: 1·07–1·55; Table II), but not among the eBL cases (aOR = 1·07,CI: 0·68–1·67). However, when eBL cases were compared with the controls, we observed a significant association between eBL and leukocytosis in analysis adjusting sex, age, village characteristics, malaria parasitaemia/antigenaemia, history of malaria treatment, haemoglobin and platelet count (aOR = 1·44; CI: 1·15–1·80; Table III).

Elevated platelet count in a disproportionate fraction of previously reported Burkitt lymphoma cases

We identified many Burkitt lymphoma (BL) cases and reports of case series in the literature; 101 of these reports included individual-level baseline platelet count data for 143 cases (Table IV, Table SIII). BL case series in the literature exhibit a wide range of platelet counts, including many of them with thrombocytopenia. However, similar to our results, a platelet count >450 × 10^9 platelets/l was observed in 14·7% of the BL cases identified in the literature, including 9·8% of the cases reported in the United States, 21% of the cases reported in Asia and 26% of the cases reported in Europe. A platelet count >450 × 10^9 platelets/l is expected in <1% of healthy adults in some populations.[31] Few cases were reported in Africa, the Middle East, Latin America and Oceania to support meaningful observations about those regions.

Discussion

Our study yielded two novel findings. The first was an observation that platelet counts were significantly reduced in eBL

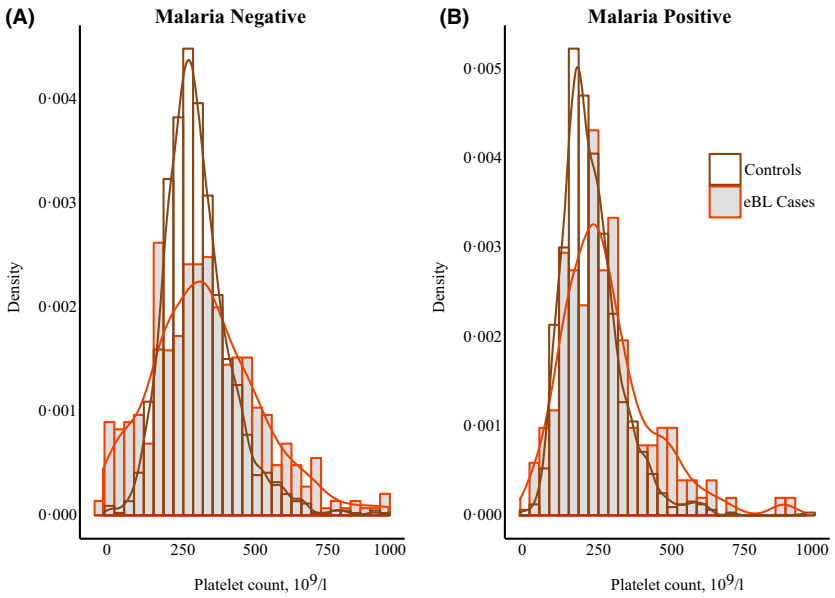

Fig 2. Density distribution of platelet counts in the controls and eBL cases shown seperately for malaria parasitemia/antigenemia negative (A) and malaria parasitemia/antigenemia positive (B) subjects. [Colour figure can be viewed at wileyonlinelibrary.com]

cases with malaria parasitaemia/antigenaemia. This observation was similar to the results in the controls and what has been reported in children without eBL but infected with malaria.[17,18,20,21] The decrease in platelet counts with malaria is attributed to platelet degranulation, which results in the release of PF4 to kill malaria parasites.[13] Our finding that platelet counts are decreased in eBL cases with malaria suggests that platelet anti-malaria response may be preserved in eBL cases. The second observation was that platelet counts were elevated in eBL cases compared to controls. This finding was unexpected, but it may be valid because it remained significant in analyses mutually adjusting for sex, age, village characteristics, malaria parasitaemia/antigenaemia, history of malaria treatment, haemoglobin and WBC counts and country.

Interestingly, we identified multiple reports in the literature of elevated platelet counts in eBL and sporadic BL cases. A baseline mean platelet count of 333 × 10^9 platelets/l was reported in 172 eBL cases studied in Uganda,[32] and one-quarter of those patients had counts >450 × 10^9 platelets/l. A study of 1005 eBL patients in Kenya did not report a mean platelet count,[33] but 10% of their patients had platelet counts >450 × 10^9 platelets/l. The mean platelet count was 542 × 10^9 platelets/l in 48 HIV-negative childhood BL cases in South Africa,[34] 360 × 10^9 platelets/l in 40 BL cases in another study in South Africa[35] and 271 × 10^9 platelets/l in 16 eBL cases in Ghana.[22] The observed mean platelet counts are substantially higher than the 230–237 × 10^9 platelets/l measured in apparently healthy children in Uganda[36] or 226 × 10^9 platelets/l measured in apparently healthy adults in Kenya[37] or elsewhere.[31] These results mirror the general pattern of elevated platelet count (14·7% of 143 BL cases with individual platelet counts) in BL cases in the worldwide literature.

Our findings that platelet counts and haemoglobin were both decreased with malaria parasitaemia/antigenaemia in controls and in eBL cases and in eBL cases as compared to the controls is consistent with the idea that relative thrombocytopenia is a disease effect due to malaria in the controls and eBL cases who are malaria positive or due to both malaria and eBL in the eBL cases, regardless of their malaria status. Both chronic malaria and eBL may suppress the bone marrow and are associated with splenic enlargement,[29,30] which can decrease platelet counts and haemoglobin. However, our data also suggest that BL is associated with disproportionately elevated platelet counts, thus, the reasons noted above would not explain the elevated platelet count pattern observed in eBL cases with or without malaria, or in BL cases from outside the malaria belt. It is theoretically possible that BL, regardless of malaria status, perhaps through infiltration or paracrine effects, modulates megakaryocyte physiology and increases the release of platelets into peripheral circulation. Such prematurely released platelets may have functional deficits, as suggested by Asare *et al.*[22] who observed that platelets in lymphoma cases had decreased platelet membrane glycoprotein expressions. However, our finding of the expected reductions in platelet counts in eBL cases with malaria parasitaemia/antigenaemia, similar to those in the controls with malaria parasitaemia/antigenaemia, suggests that platelets are functionally active against malaria, as reflected by their numbers in eBL cases and in children without eBL.[10]

While the explanation for disproportionately elevated platelet counts with BL is unclear to us, we suggest several possibilities.

Table IV. Summary of platelet counts at diagnosis for eBL cases with available platelet count data reported in the literature.

Platelet count, 10^9/l	Total, $n = 143$ (%)	Africa, $n = 5$ (%)	Asia, $n = 24$ (%)	Europe, $n = 39$ (%)	Middle East, $n = 7$ (%)	North America, $n = 61$ (%)	South America/ Caribbean, $n = 6$ (%)	Oceania, $n = 1$ (%)
<50	36 (25·2)	1 (20·0)	2 (8·3)	10 (25·6)	2 (28·6)	17 (27·9)	3 (50·0)	1 (100·0)
50–99	20 (14·0)	–	3 (12·5)	7 (17·9)	–	9 (14·8)	1 (16·7)	–
100–149	11 (7·7)	1 (20·0)	1 (4·2)	4 (10·3)	–	4 (6·6)	1 (16·7)	–
150–199	13 (9·1)	–	5 (20·8)	–	2 (28·6)	5 (8·2)	1 (16·7)	–
200–299	24 (16·8)	1 (20·0)	5 (20·8)	4 (10·3)	2 (28·6)	12 (19·7)	–	–
300–399	16 (11·2)	2 (40·0)	3 (12·5)	3 (7·7)	1 (14·3)	7 (11·5)	–	–
400–450	2 (1·4)	–	–	1 (2·6)	–	1 (1·6)	–	–
>450	21 (14·7)	–	5 (20·8)	10 (25·6)	–	6 (9·8)	–	–

First, platelets are known to be disproportionately elevated in several solid tumours, an observation which has led to the search for platelet-derived biomarkers as targets for early cancer detection or liquid biopsy.[38] Our results suggest that BL should be included and investigated as one of the malignancies with disproportionately elevated platelet counts. Second, platelets are biologically active in the germinal centre,[39] where BL originates.[40,41] Platelets play a role in B-cell maturation and B-cell isotype switching,[42] and are a rich source for transforming growth factor-β (TGF-β),[43] which is implicated in transforming cells through activation of reactive oxygen species.[44] Thus, platelets could enhance proliferation of initiated B cells and facilitate their escape of immune-mediated destruction and progression to BL. Finally, recent studies suggest that platelet count is a genetically controlled trait.[45,46] Thus, the platelet associations suggested by our study could be further clarified using genetic methods that focus on platelet-associated genetic variants.

We acknowledge that non-biological factors, such as machine measurement error,[47–49] could explain our results. However, our findings that platelet counts were decreased with malaria parasitaemia/antigenaemia in controls and eBL cases and the significant inverse correlation between platelet count and mean platelet volume,[11] both of which are expected patterns, undermine this explanation. Spurious elevation of platelet counts due to fragments of the cellular components of blood being counted as platelets is possible,[47,49] and has been shown to lead to overestimation of platelet count by 20–25%.[47,49] However, the likely causes of fragments that give rise to machine artefacts, such as leukemic manifestation[29,30] and severe malaria,[6,50] are rare in eBL.

The strengths of our study include using a large well-characterized dataset of eBL cases and controls from Uganda, Tanzania and Kenya and having detailed information about potential confounders.[27] The main limitation is the cross-sectional design,[51] which precludes temporal inferences. The discovery of genetic variants in genome-wide association studies (GWAS) associated with platelet counts[45,52,53] suggests an alternative way to explore platelet associations using Mendelian Randomization (MR), which will reduce concerns about reverse causation. MR takes advantage of the idea that an individual's genotype is randomized at conception,[54] and that genetic variants that are correlated with platelet count[55] are ascertained with great accuracy.[45,46] However, platelet GWAS have been conducted in European, Asian and African American ancestry populations,[45,52,53] who are ancestrally different from eBL belt populations.[56] Thus, it will be necessary to update platelet GWAS variants in East African eBL belt populations.

In conclusion, we show that platelet counts were decreased with malaria infection in controls and in eBL cases in Uganda, Tanzania, and Kenya. These findings suggest that platelet-mediated action against malaria is observed both in children with and without eBL. Secondly, we observed platelet counts were elevated in children with eBL regardless of malaria status, and that a disproportionate number of BL

cases reported in the literature have elevated platelet counts. These findings are novel and warrant further exploration.

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Author contributions

SMM conceived the idea, designed the study and supervised the work. MDO, PK, SJR, CNT, RTK, WNW, NM, EK, LWA, RJB, KB and JJG contributed to study design and supervised field work. TK, IO, IDL, HN, HD, MM, and PAW conducted fieldwork. SP conducted statistical analysis and interpreted data. SMM advised on statistical analysis. SP drafted the manuscript; JJG and SMM critically edited the paper. All authors contributed to the manuscript, read and approved the final manuscript.

Conflicts of interest

The authors declare to have no potential conflicts of interest regarding the present work.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Flow chart showing the selection of cases and controls in the EMBLEM study, by country, those excluded because they lacked platelet count data, the basis of diagnosis for the enrolled cases and the completeless of malaria data for both the cases and controls. Abbreviations: Y'=Yes, N'=No and 'n'=number of subjects with information.

Fig S2. Correlation between platelet count and mean platelet volume among all controls and stratified by malaria infection status.

Table SI. The distribution of mean platelet counts by age group among the matched population controls in the EMBLEM study stratified by malaria infection status.

Table SII. Summary of complete blood count data among eligible cases and controls in the EMBLEM study.

Table SIII. Results of literature search for Burkitt lymphoma cases with individual-level pretreatment platelet count data.

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