Thrombocytopenia as Additional Marker of Severity in African Children with Plasmodium Falciparum Malaria

Gérardin P[1,2*], Ka AS[1] and Imbert P[1,3]

[1]Department of Pediatrics, Hôpital Principal, Dakar, Senegal
[2]INSERM CIC1410 Clinical Epidemiology, CHU Reunion, Groupe Hospitalier Sud Réunion, France
[3]Department of Infectious Diseases and Tropical Medicine, Hôpital d'Instruction des Armées Bégin, Saint-Mandé, France

[*]**Corresponding author:** Gerardin P, Department of Pediatrics, Hôpital Principal, Dakar, Senegal and INSERM CIC1410 Clinical Epidemiology, CHU Reunion, Groupe Hospitalier Sud Réunion, France, Fax +262.262.35.97.11, Tel: +262.262.35.90.00 (+ 5-87-53), E-mail: patrick.gerardin@chu-reunion.fr

Abstract

Background: Thrombocytopenia (platelets counts $< 100,000/mm^3$) is predictive of death in African children with falciparum malaria exposed to low seasonal transmission. However, the impact of this indicator on malaria severity was not fully addressed.

Method: We compared platelet counts according to multiple indicators of malaria severity including 1990 and 2000 World Health Organization (WHO) criteria, 24-hour Pediatric Risk of Mortality (PRISM) score, 24-hour Paediatric Logistic Organ dysfunction (PELOD) score, and Major Therapeutic Procedures (MTPs), defined as requirement for mechanical ventilation, hemodynamic support, blood transfusion, hemodialysis, or use of sedatives drugs. We next assessed the discriminatory performance of thrombocytopenia, alone or together with a combination of WHO 2000 criteria in predicting these outcomes.

Result: Children fulfilling both WHO definitions of severe malaria had lower mean platelet counts than mild cases in accordance with both ($129,000$ versus $173,000/mm^3$; $P<0.001$). Thrombocytopenic children had higher mean $PRISM_{h24}$ and $PELOD_{h24}$ admission scores than non-thrombocytopenic counterparts (5.8 versus 4.6; $P<0.01$; 6.2 versus 4.6; $P<0.001$, respectively). They did not need more MTPs (63.0% vs 52.6%, $P=0.07$). Among 288 children, the combination of impaired consciousness, respiratory distress, or thrombocytopenia, had the same discriminatory performance in predicting outcome than WHO 2000 criteria (sensitivity, 100%, specificity, 28%, positive predictive value, 80%, negative predictive value, 100%).

Conclusion: Thrombocytopenia is an indicator of life-threatening malaria and should therefore be proposed as additional marker of severity, especially in non-immune African children.

Keywords: Severe Malaria; Children; Africa; Thrombocytopenia; PRISM; PELOD

Introduction

Thrombocytopenia defined for European populations as a platelet count lower than $150,000/mm^3$ is a common finding among children with falciparum malaria [1-3]. The lower limit of the normal platelet count in African populations has been defined at $100,000/mm^3$ with a normal range of $100-300,000/mm^3$ Defined at a value less than $100,000/mm^3$, thrombocytopenia occurs relatively infrequently in cases of acute malaria but this cut-off was shown of great prognostic relevance in a cohort study of 288 Senegalese children exposed to low seasonal transmission [4-6]. Thus, in this previous study was demonstrated a strong independent association between thrombocytopenia in-hospital mortality (odd ratio 13.3) along with inverse correlations between platelet counts and age, or between platelets and parasitemia, as well as associations between thrombocytopenia and several organ dysfunctions (such as respiratory distress or cerebral malaria). In addition, the prognostic value of thrombocytopenia was also found in children requiring endotracheal intubation for life-threatening malaria in the same area [7]. However, these data lacked both internal and external validity. On the one hand, the prognostic value of each severe malaria criterion is inconsistent across the different levels of transmission and needed to be gauged with different tools assessing disease severity irrespective of the WHO definition, such as broadly used generic severity scores and organ dysfunction scores. On the other hand, the relationship between thrombocytopenia and severe malaria (SM) is still controversial in other settings [8-11]. Furthermore, HIV infection and quinine may confound this relationship, both being associated with SM (as risk factor or treatment) and classical causes of thrombocytopenia [12-14].

In the present study, we sought to add internal validity to our previous findings by studying the relationships between platelet counts and SM using two broadly used pediatric scoring systems and the WHO criteria for benchmarking the definition of SM.

Patients and Methods

The study took place in the pediatric department of the "Hôpital Principal", a tertiary care hospital in Dakar, Senegal, from October 1, 1997 to March 31, 1999. This setting is exposed to a low seasonal transmission, i.e., entomological inoculation rates < 1 infective bite per person per year [15]. Informed consent was obtained from first-degree relatives (parents or tutors with legal authority), according to the recommendations of the institutional board. The data collection, as detailed previously, was prospective [6]. All children (0-15 years old) with clinical signs of malaria, a Plasmodium falciparum positive blood film and a valid admission platelet count were enrolled in the study.

Platelets were counted from a whole blood sample by ST KS® Coultronics automaton. In case of severe thrombocytopenia (platelets < 20,000/mm³) or clinical hemorrhage, controls on citrate tube and blood coagulation tests (for prothrombin time, partial thromboplastin time, and fibrinogen) were performed on manual chronometric tests.

Malaria severity was assessed according to World Health Organization (WHO) definitions [16,17]. In accordance, children fulfilling both definitions were classified as 'severe', those matching only the 2000 WHO criteria were classified as 'moderate', those with no WHO criterion according to both definitions were classified as 'mild' [18].

The Pediatric Risk of Mortality (PRISM) is a widely recognized scoring system aimed at predicting death in children [19]. It is independent of platelet counts. It was prospectively assessed in this study and previously shown as discriminant as the 2000 WHO criteria (AUC 0.89 95% CI 0.82-0.95 versus 0.84 95% CI 0.81-0.90) [18,20].

The Pediatric Logistic Organ dysfunction (PELOD) is the first organ dysfunction score, developed and validated in children [21]. It is derived from the PRISM variables completed by data on liver enzymes, white blood cells count and mechanical ventilation. It integrates platelet counts without significant influence on its calculation. In this study, it was calculated retrospectively from the prospective dataset.

Major therapeutic procedures (MTPs), based on supplies available in Dakar, have been previously defined as mechanical ventilation, hemodynamic support, blood transfusion, hemodialysis, and use of sedatives drugs, e.g., phenobarbital, diazepam, clonazepam and thiopental [18]. MTPs had been used previously to evaluate malaria severity in Senegalese children [18]. More recently, they were used as surrogate marker of life-threatening malaria in a French national study covering more than 6,000 imported cases over a decade [22].

We successively explored the relationships between platelet counts and malaria severity, using WHO classification, the Pediatric Risk of Mortality (PRISM) score, the PELOD score, both assessed within 24 hours (h24) and MTPs

First, we compared in-hospital mortality according to malaria severity groups (mild versus moderate, moderate versus severe) and categories of admission platelet counts (thrombocytopenic, platelets < 100,000/mm³ versus non-thrombocytopenic, platelets ≥ 100,000/mm³) using Chi square tests. Second, we compared between the same groups, mean admission platelet counts, mean $PRISM_{h24}$ score and mean $PELOD_{h24}$ score, using two sample Mann-Whitney tests. Third, we compared the proportions of children requiring MTPs in the same manner using Chi square tests.

We next assessed the discriminatory performance of thrombocytopenia (platelet count < 100,000/mm³) in predicting death, alone or together with impaired consciousness (Blantyre coma score < 5) or respiratory distress (sustained lower chest wall recession or deep acidotic breathing), or with both conditions. Accordingly, we calculated the sensitivity, specificity, positive and negative predictive values of these combinations in predicting outcome and compared their performance to that of the WHO 2000 criteria [18].

These analyses were performed using Stata® (Stata Statistical Software: release 7; StataCorp. 2001). Odds ratios (OR) and 95% confidence intervals (CI) were given. A P value < 0.05 was considered statistically significant.

Results

During the study period, 319 consecutive children were admitted with falciparum malaria, of whom eight with mild malaria were excluded due to missing data, 23 of whom were excluded due to absence of platelet counts (two deaths, mean $PRISM_{h24}$ score: 5.7), leaving 288 children for inclusion (mean $PRISM_{h24}$ score: 5.2 ± 6.5).

Of 288 pediatric falciparum cases, 154 (53.5%) had been treated with an anti-malarial drug prior to admission, 114 (39.5%) of whom had received quinine injections. 215 children (74.6%) had SM according to the WHO 2000 criteria, and 73 (25.4%) had a mild form. Outcome was recovery without sequelae (n=249; 86.5%) or with neurologic sequelae upon discharge (n=13; 4.5%), or death (n=26; 9.0%).

Children who had been treated with quinine had comparable platelet counts than untreated counterparts (geometric means, 141,000 versus 152,000/mm³).

Children classified severe by both WHO definitions had lower mean platelet counts than children classified mild by both WHO definitions (mean platelet count: 129,000 versus 173,000/mm³, P<0.001) (Table 1).

Severity categories		Mild	Moderate	Severe		
WHO classification & Platelet counts (×1000/ mm³)	2000	mild 1990 & mild 2000	mild 1990 & severe 2000¶	severe 1990 & severe 2000	< 100	≥ 100
Patients	215	73	70	145	138	150
Fatality Rates (%)	12.0	0	0	17.9	15.9	2.7
P values*			<.0001[b]		<.0001[c]	
Platelets (×1000/ mm³) Mean 95% CI	131 123-138	173 142-203	135 112-157	129 116-141	- -	- -
P values**		<.001[a]	N.S.[b]			
PRISM score Mean 95% CI	6.5 5.5-7.3	1.2 0.8-1.5	1.8 1.2-2.4	8.8 7.8-9.4	5.8 4.8-6.3	4.6 3.9-5.4
P values**		N.S.[a]	<.0001[b]		<.01[c]	
PELOD score Mean 95% CI	6.4 5.9-6.9	2.3 1.5-3.1	3.4 2.4-4.4	7.9 6.9-9.0	6.2 5.3-7.1	4.6 3.8-5.3
P values**		<.01[a]	<.0001[b]		<.001[c]	
M.T.P use %	156 73	10 14	29 41	127 88	87 63	79 53
P values*		<.001[a]	<.0001[b]		N.S[c]	

* Chi square or ** Mann-Whitney tests comparing proportions or geometric means, as appropriate: [a]mild cases according to both WHO definitions versus cases considered mild according to '1990 WHO' and severe according to '2000 WHO'; [b] cases considered mild according to '1990 WHO' and severe according to '2000 WHO' versus severe cases according to both WHO definitions; [c] thrombocytopenic versus non thrombocytopenic children. According to PELOD score, this group should therefore be considered as a moderate form of acute malaria
N.S.: non significant

Table 1: Malaria severity according to 1990 and 2000 WHO definitions evaluated using fatality rates, platelet count, Paediatric Risk of Mortality (PRISM) and Paediatric Logistic Organ Dysfunction (PELOD) scores, and Major Therapeutic Procedures (MTPs) as endpoints (n=288)

Thrombocytopenia (platelet count < 100,000/mm³) was not more likely in children classified severe with both definitions than in children classified mild with both definitions (52.4% versus 38.3%, P=0.05), irrespective of quinine use. Under these conditions of prevalence and recruitment of SM, it would have required 251 and 126 subjects in both groups to make this difference reach statistical significance with a power of 80% (β=0.20 and α=0.05, one-sided test). Ditto, thrombocytopenia could not discriminate between severe and moderate malaria, or between moderate and mild malaria (data not shown).

Several WHO 2000 criteria were associated with thrombocytopenia: impaired consciousness (OR, 2.0; 95% CI, 1.2-3.3, P<0.02), respiratory distress (OR, 1.7; 95% CI, 1.1-3.0, P<0.04), jaundice (OR, 4.1; 95% CI, 2.0-8.7, P<0.0001), hyperparasitemia (OR, 2.9; 95% CI, 1.3-6.5, P<0.01) and hemoglobinuria (OR, 5.1; 95% CI, 1.1-35.7, P<0.03). Thrombocytopenia did not increase the risk of metabolic acidosis (OR, 1.65; 95% CI, 0.8-3.4) or abnormal bleeding (OR, 5.6; 95% CI, 0.6-130.5).

Thrombocytopenic children had higher admission PRISM24 and PELOD24 scores than non-thrombocytopenic peers (mean scores, 5.8 vs 4.6; 6.2 vs 4.6; P<0.01 and P<0.001, respectively). They did not need more MTPs (63.0% vs 52.6%, P=0.07) (Table 1). Under this prevalence of malarial thrombocytopenia, it would have required 285 and 310 subjects in both groups to find thrombocytopenic children needed more MTPs than non-thombocytopenic children, with a power of 80% (β=0.20, α=0.05, one-sided test).

The prevalence, overlap and mortality associated with the combination of impaired consciousness, respiratory distress syndrome, and thrombocytopenia, the three most common conditions in Dakar, a low seasonal transmission area, are shown in Figure 1 [6,14].

Of 215 severe cases, 28 children had no impaired consciousness, respiratory distress syndrome, or thrombocytopenia (overlap of the three indicators: 87.0%) whereas out of thrombocytopenic children (n=138), only 28 had no WHO criterion (overlap: 79.7%). For this purpose, the overlap of these three variables was predictive of more than half of the deaths observed (n=15/26), and associated with an in-hospital mortality of 38.5%. Of the 26 children who died, only one, who had impaired consciousness, did not present at least two of these indicators. The presence of two indicators predicted 10 deaths with an in-hospital mortality comprised between 8.3% and 15.4%, the worse prognosis being observed with the overlap of impaired consciousness and thrombocytopenia (in-hospital mortality: 15.4%). Among 288 children, the combination of impaired consciousness, respiratory

distress, or thrombocytopenia, had the same discriminatory performance in predicting outcome than the 15 WHO 2000 criteria joined together (sensitivity, 100%, specificity, 28%, positive predictive value, 80%, negative predictive value, 100%). In comparison, the WHO 1990 criteria were twice more specific and therefore performed better in predicting survival (sensitivity, 100%, specificity, 55%, positive predictive value, 69%, negative predictive value, 100%).
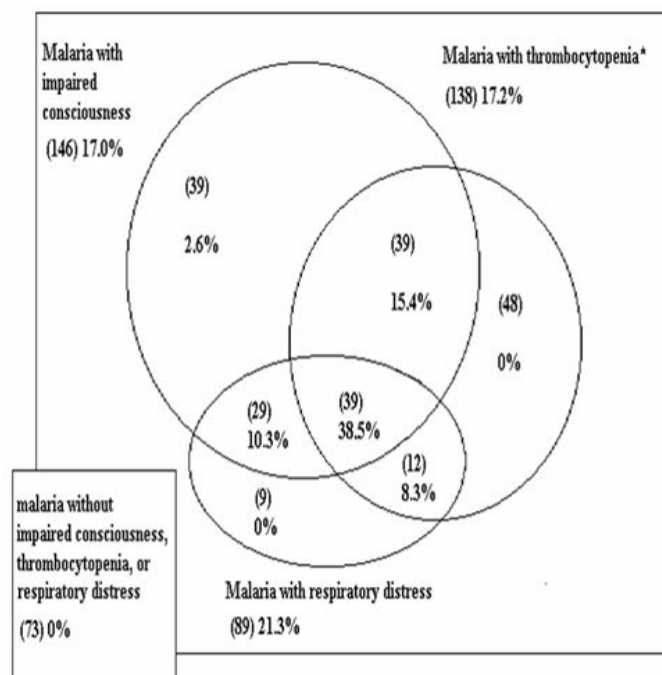
Total numbers are given in parentheses and related lethality is given as a percentage.

*This circle features malaria cases with WHO (2000) criteria excluding those with impaired consciousness or respiratory distress (n=48, 20 of whom are thrombocytopenic, overlap 41.7%, lethality 0%)

Figure 1: Numbers, overlap and mortality of the three commonest conditions affecting outcome of 215 severe malaria cases among 288 children hospitalized in Dakar, Senegal

Discussion

In this study, we addressed the question of malaria severity in children, using in turn 1990 or 2000 WHO criteria or a combination of both, to define severe malaria.

Over the last two decades, there have been many attempts to assess SM in children, most of these being performed in countries with moderate-to-high endemicity [23-25]. The conclusions of these studies question the validity of the WHO criteria for all settings and constitute major impetus for clinical redefinition of severe pediatric malaria.

For instances, in Kilifi, Kenya (0-69 infective bites per person per year), Marsh et al. showed that 1990 WHO criteria failed to identify high-risk children and could be advantageously replaced by simple bedside assessments of impaired consciousness and respiratory distress [23]. In Ifakara, Tanzania (> 300 infective bites per person per year), Schellenberg, *et al.* stressed the relevance of dehydration as well as of different respiratory and neurologic distress features [25].

In Dakar, Senegal (< 1 infective bite per person per 10 years) where children are considered as non-immune, we found both WHO definitions accurate to select high-risk patients (e.g., sensitivity, 100%, positive predictive values, 69 to 80%), but their lack of specificity (28 to 55%) questioned the need for reducing false positives, (i.e., children meeting criteria not associated with in-hospital mortality), to restrict quinine use and spare intensive cares. To investigate this issue, we evaluated 1990 and 2000 WHO definitions, using fatality rates, PRISM and PELOD scores, and MTPs. Noteworthy, only the 1990 WHO criteria were associated with mortality. Interestingly, the PELOD could better discriminate than the PRISM between mild and moderate forms, which is coherent with the known superiority of organ dysfunction scores over generic severity scores in malaria, as shown recently with the Lambarene Organ Dysfunction Score (LODS), compared with the Signs of inflammation in children that kill (SICK) and the Pediatric Early Death Index for Africa (PEDIA) [26]. As expected, both children classified 'severe' or 'moderate' required ICU resources and the need for MTPs increased within the severity range, classified into mild, moderate and severe categories.

In the present study, we also go deeper in the understanding of the relationship between low platelet counts (<150,000/mm^3) and SM found in our previous study [6]. Thus, according to WHO classifications, platelet counts were lower in severe than in mild malaria and thrombocytopenia (platelets <100,000/mm^3), though not significantly, tended to be more common in the former group. Importantly, platelet counts correlated with PRISM score, a generic severity score that do not integrate platelet count in its calculation, and with PELOD score, a score aimed at predicting multiple organ dysfunction (MOD) in children [20,21]. This latter

finding may be explained by the possible association between thrombocytopenia and several WHO criteria that may reflect an endothelial activation as observed in the MOD syndrome, e.g., impaired consciousness with activation of brain microcirculation, respiratory distress with pulmonary vascular dysfunction, jaundice with hepatic dysfunction, respectively. However, whether platelets are critical effectors of SM pathogenesis and thrombocytopenia a marker of endothelial activation or a mere indicator of SM, remain to be elucidated in our children [27-28]. Indeed, the role of platelets in malaria is likely dual. On the one hand, platelet may have a protective function in the early stages of erythrocytic infection by killing malarial parasites [29]. In addition, thrombocytopenia may mediate disease severity through reduced transforming growth factor β1 (TGFβ1) and regulation of pro-inflammatory and anti-inflammatory cytokines [30]. On the other hand, platelets undergo microvesiculation and interact at the brain-endothelial cell surface with parasitized red blood cells (PRBCs) to induce PRBC clumping, an adhesive phenotype associated with SM, and especially with cerebral malaria [28,31]. In this situation, thrombocytopenia was shown protective by reducing PRBC clumping, while HIV infection increased platelet accumulation in brain microvessels [32,33]. Surprisingly, thrombocytopenic children did not require more MTPs than non-thrombocytopenic children, which is not in keeping with the findings of the recent French National study on severe imported malaria [22].

Although, the purpose of this study was not to determine the mechanisms of malarial thrombocytopenia, it should be objected that some of the thrombocytopenia observed in Dakar could be induced by quinine and that this drug adverse event might have partly skewed the analysis. Indeed, thrombocytopenia is a classical complication of quinine that is sometimes associated with harmful effects [12]. However, children treated with quinine prior to hospitalization had comparable platelet levels than untreated counterparts. Besides, thrombocytopenia wasn't more frequent in treated children, irrespective of the category of malaria severity. Therefore, the general sense of our results is unlikely to be affected by quinine-induced thrombocytopenia.

In Kenya, the study of blood components in children with falciparum malaria failed to demonstrate any link between thrombocytopenia and SM or lethality [7]. However albeit fivefold more important, this study suffered from several weaknesses, both in design and analysis, i.e., no control of confounders such as iron deficiency (ruled out in our previous work), low in-hospital mortality hindering the detection of differences between survivors and non-survivors, pooled analysis of SM rather than per clinical forms [6].

Ditto the study of Moulin *et al.* suffered from the same limitation, i.e., only four deaths among 234 cases, and of the choice of SM, a surrogate variable, as outcome measure rather than death [9,34].

Interestingly, our precursor results are now in keeping with those of very large study conducted in Papua, Indonesia (> 200,000 patients), which showed that severe malarial thrombocytopenia (platelets < 50,000/mm^3) was associated with an independent risk of death, alone (adjusted OR 2.77; 95% CI 2.20 - 3.48) or together with severe malarial anemia (adjusted OR 13.76; 95% CI 10.22 - 18.54) [25].

HIV infection may constitute a significant bias in countries with 10 p. cents or more HIV prevalence [8]. Nonetheless, it was not the case for Senegal during the study period, since the HIV prevalence for children was estimated less than 0.1 p. cent in the 1990's and AIDS was diagnosed in less than 0.2 p. cent of our children [35,36].

Another important issue of this survey is the overlap between thrombocytopenia and the two other leading conditions associated with malaria (i.e., impaired consciousness and respiratory distress), which had the same sensitivity and negative predictive value in assessing the outcome than fifteen WHO (2000) criteria joined together. A great sensitivity and a high negative predictive value are required to ensure the triage of SM cases at the district level. Finally, in Dakar, the impact of assessing platelet counts in pediatric malaria led to simplify both diagnosis and prognosis of SM.

In conclusion, in the low transmission area of Dakar, Senegal, both 1990 and 2000 WHO definitions were suitable to detect high-risk children with SM. Furthermore, thrombocytopenia, defined as a platelet count lower than 100,000/mm^3, was a useful indicator of life-threatening malaria. Also, despite the absence of robust association between thrombocytopenia and SM (as defined by WHO criteria) owing to a lack of statistical power, the inverse correlation between admission platelet counts and two pediatric scoring systems of illness severity encourages us to consider thrombocytopenia as an additional marker of malaria severity in non-immune African children.

Acknowledgement

References

1. Dacie JV, Lewis SM (1975) Practical Haematology. 21-77.

2. Essien EM, Oruamabo RS (1976) Depression of platelet count during acute Plasmodium falciparum malaria infection. Nigerian J Paediatr 3: 69-70.

3. Piarroux R, Dumon H, Garnier JM, Lehman M Unal D, et al. (1993) Choice of therapy for imported cases of falciparum malaria in children: a retrospective study of 100 cases seen in Marseille, France. Trans R Soc Trop Med Hyg 87: 72-4.

4. Essien EM, Usanga EA, Ayeni O (1973) The normal platelet count and platelet factor-3 availability in some Nigerian population groups. Scand J Haematol 10: 378-83.

5. Essien EM. (1992) Platelets and platelet disorders in Africa. Baillière's Clin Haematol 5: 441-6.

6. Gérardin P, Rogier C, Ka AS, Jouvencel P, Brousse V, et al. (2002) Prognostic value of thrombocytopenia in African children with falciparum malaria. Am J Trop Med Hyg 66: 686-91.

7. Gérardin P, Rogier C, Ka AS, Jouvencel P, Diatta B, et al. (2007) Outcome of life-threatening malaria in African children requiring endotracheal intubation. Malar J 6: 51.

8. Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR (2002) Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. Br J Haematol. 119: 839-47.

9. Moerman F, Colebunders, B, D'Alessandro U (2003) Thrombocytopenia in African children can predict the severity of malaria caused by Plasmodium falciparum and the prognosis of the disease. Am J Trop Med Hyg 68: 379.

10. Moulin F, Lesage F, Legros AH, Maroga, C, Moussavou A, et al. Thrombocytopenia and Plasmodium falciparum malaria in children with different exposures. Arch Dis Child 88: 540-1.

11. Lampah DA, Yeo TW, Malloy M, Kenangalem E, Douglas NM, et al. (2015) Severe malarial thrombocytopenia: a risk factor for mortality in Papua Indonesia. J Infect Dis 211: 623-34.

12. van den Bemt PM, Meyboom RH, Egberts AC (2004) Drug-induced immune thrombocytopenia. Drug Safety 27: 1243-52.

13. Berkley JA, Bejon P, Mwangi T, Gwer S, Maitland K, et al. (2009) HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. Clin Infect Dis 49: 336-43.

14. Mwinga K, Vermund SH, Chen YQ, Mwatha A, Read JS, et al. (2009) Selected hematologic and biochemical measurements in African HIV-infected and uninfected pregnant women and their infants: the HIV Preventoin Trials Network 024 Protocol. BMC Pediatr 9: 49.

15. Trape JF, Lefebvre-Zante E, Legros F, Druilhe P, Rogier C, et al. (1993) Malaria morbidity among children exposed to low seasonal transmission in Dakar, Senegal and its implication for malaria control in tropical Africa. Am J Trop Med Hyg 48: 748-56.

16. Warrell DA, Molyneux ME, Beales PF (1990) Severe and complicated malaria. 2nd edition. Trans Royal Soc Trop Med Hyg 84: 1-65.

17. World Health Organisation (2000) Severe falciparum malaria. Trans Royal Soc Trop Med Hyg 94: 1-90.

18. Imbert P, Gérardin P, Rogier C, Ka AS, Jouvencel P, et al. (2002) Severe falciparum malaria in children: a comparative study of 1990 and 2000 WHO criteria for clinical presentation, prognosis and intensive care in Dakar, Senegal. Trans R Soc Trop Med Hyg 96: 278-81.

19. Pollack MM, Ruttiman UE, Getson PR (1988) Pediatric risk of mortality (PRISM) score. Crit Care Med 16: 1110-6.

20. Gérardin P, Rogier C, Leteurtre S, Jouvencel P, Ka AS, et al. (2006) Evaluation of Pediatric Risk of Mortality (PRISM) scoring in African children with falciparum malaria. Pediatr Crit Care Med 7: 45-7.

21. Leteurtre S, Martinot A, Duhamel A, Proulx F, Grandbastien B, et al. (2003) Validation of the paediatric logistic organ dysfunction score: prospective, observational, multicentre study. Lancet 362: 192-7.

22. Mornand P, Verret C, Minodier P, Faye A, Theillier M, et al. (2017) the Pediatric Imported Malaria Study Group for the Centre National de Référence du Paludisme. PLoS One 12: e0180758.

23. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, et al. (1995) Indicators of life-threatening malaria in African children. New Engl J Med 332: 1399-404.

24. Waller D, Krishna S, Crawley J, Miller K, Nosten F, et al. (1995) Clinical features and outcome of severe malaria in Gambian children. Clin Infect Dis 21: 577-87.

25. Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, et al. (1999) African children with malaria in an area of intense Plasmodium falciparum transmission: features on admission to the hospital and risk factors for death. Am J Trop Med Hyg 61: 431-8.

26. Conroy AL, Hawkes M, Hayford K, Namasopo S, Opoka RO, et al. (2015) Prospective validation of pediatric disease severity scores to predict mortality in Ugandan children presenting with malaria and non-malaria febrile illness. Crit Care 19: 47.

27. Grau GE, Mackenzie CD, Carr RA, Redard M, Pizzolato G, et al. (2003) Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. J Infect Dis 187: 461-6.

28. Combes V, Taylor TE, Juhan-Vague I, Mège JL, Mwenechanya J, et al. (2004) Circulating endothelial microparticles in Malawian children with severe falciparum malaria complicated with coma. JAMA 291: 2542-4.

29. McMorran BJ, Marshall VM, de Graaf C, Drysdale KE, Shabbar M, et al. (2009) Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. Science 323: 797-800.

30. Hanisch BR, Bangirana P, Opoka RO, Park GS, John CC (2015) Thrombocytopenia may mediate disease severity in Plasmodium malaria through reduced transforming growth factor bete-1 regulation of proinflammatory and anti-inflammatory cytokines. Pediatr Infect Dis J; 34: 783-8.

31. Pain A, Ferguson SJ, Kai O, Urban BC, Lowe B, Marsh K, et al. (2001) Platelet-mediated clumping of Plasmodium falciparum-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. Proc Natl Acad Sci USA 98: 1805-13.

32. Wassmer SC, Taylor T, Maclennan CA, Kanjala M, Mukaka M, et al. (2008) Platelet-induced clumping of Plasmodium falciparum-infected erythrocytes from Malawian patients with cerebral malaria-possible modulation in vivo by thrombocytopenia J Infect Dis 197: 72-8.

33. Hochman SE, Madaline TF, Wassmer SC, Mbale E, Choi N, et al. (2015) Fatal pediatric cerebral malaria is associated with intravascular and platelets that are increased with HIV coinfection. mBio 6: e01390-15.

34. Rogier C, Gérardin P, Imbert P (2004) Thrombocytopenia is predictive of lethality in severe childhood falciparum malaria. Arch Dis Child 89: 795-7.

35. UNAIDS (2000) Report on the Global HIV/AIDS Epidemic. UNAIDS/00.13E, Geneva: World Health Organisation.

36. Imbert P, Rogier C, Gérardin P (2003) Letter in reply to Ladhani & Moerman. Am J Trop Med Hyg 68: 380-1.