Ex Vivo Microsphiltration Profile of Plasmodium Falciparum Infected Red Blood Cells in Patients with Malaria in Kéniéroba, Mali: Exploring the Spleen in Vivo Retention Function

Bourama KEITA (binkokeita@gmail.com)
USTTB FMOS: Universite des Sciences des Techniques et des Technologies de Bamako Faculte de Medecine et d'Odontostomatologie https://orcid.org/0000-0002-8960-696X
Seidina A.S. Diakité
MRTC: Malaria Research and Training Center
Agnes M. Guindo
MRTC: Malaria Research and Training Center
Drissa S. Konaté
MRTC: Malaria Research and Training Center
Karim Traoré
MRTC: Malaria Research and Training Center
Modibo Sangaré
MRTC: Malaria Research and Training Center
Sory I. Diawara
MRTC: Malaria Research and Training Center
Ibrahim Sanogo
MRTC: Malaria Research and Training Center
Bakaina Diarra
MRTC: Malaria Research and Training Center
Mahamadou Diakité
MRTC: Malaria Research and Training Center

Research

Keywords: Microsphiltration, Malaria, Plasmodium falciparum, splenic retention

Posted Date: June 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-557105/v1

Abstract

Malaria pathophysiology is not still fully understood. The main mechanisms of malaria involve the synergistic interactions between host and parasite. Although, the role of the spleen has been mentioned in various clinical forms of malaria, a supportive clinical evidence is still needed. We conducted a pilot study to determine the impact of the spleen functional state in different clinical forms of malaria.

Ex vivo microsphiltration was used to assess the splenic function in patients received during routine consultation with malaria at the Kéniéroba health center, a high malaria endemic area in Mali.

A total of 25 patients were enrolled for microsphiltration. Two patients (8%) had a no palpable spleen (Hackett stage 0), 22 patients (88%) had a palpable spleen with at deep inspiration (Hackett stage 1) and only one patient (4%) presented a palpable spleen (Hackett stage 2). Parasitaemia ranged from 5360 trophozoites/µl to 342720 trophozoites/µl with a mean parasitemia of 50774 trophozoites/µl ± 65540 trophozoites/µl; the mean hemoglobin rate was 11.2 ± 1.2 g/dl with the extremes of 8.7 g/dl and 13.4 g/dl. The retention rate of the infected red blood cell ranged from 11.11% to 94.44% with an average of 65.4% ± 23.7%. A higher ex vivo retention rate of infected red blood cells was observed in patients with Hackett stages greater than or equal to 1 (p= 0.03).

This pilot study proved the feasibility of the exploration of the spleen filtering function in malaria patients using the ex vivo microsphiltration.

Introduction

Malaria is the most important tropical parasitic disease with 228 million cases and 405,000 deaths in 2018 worldwide [1]. Africa bears a disproportionate share of the global malaria burden (93% morbidity and 94% mortability rates in 2018) [1]. Despite the ongoing basic and experimental research on malaria, the pathophysiology of the disease is not yet fully understood due to complex and multifactorial mechanisms. The main mechanisms involve synergistic host-parasite interactions [2].

The high frequency of splenomegaly in malaria endemic areas, the occurrence of splenic ruptures during or immediately, the more frequently marked severity of the first episodes in splenectomized patients are suggestive a central role of the spleen in the malaria pathogenesis [3]. Pathogenic stages of *P. falciparum* develop in red blood cells (RBCs) and modify their properties [4, 5]. Understandably, the spleen, responsible of controlling the RBCs deformability, influences the fate of infected RBCs. In fact, clinical manifestations o*f P. falciparum* infection result from the development of asexual stages within the RBCs [6]. Splenic microcirculatory beds filter altered RBCs, so the spleen can naturally eliminate subpopulations of infected or uninfected RBCs modified during *P. falciparum* malaria [7]. The spleen seems to be more protective against severe manifestations of malaria in naive patients [8]. It is involved in parasite clearance after certain antimalarial treatments, including artemisinin derivatives (artesunate and dihydroartemisinin) [9]. The loss of RBCs during malaria contributes to malaria anemia, a clinical form associated with subacute progression, frequent splenomegaly and chronic subclinical parasitaemia. This

loss is due the splenic clearance of either newly infected RBCs or uninfected RBCs (modified by parasites)[10]. However, this phenomenon seems to be associated with a reduced risk of serious complications associated with high parasitic loads, such as cerebral malaria [11]. These hypotheses remain speculative despite their relevance. Exploring the role of the spleen in the pathophysiology of malaria remains very complicated due to the in vivo experimental challenges.

Many techniques to explore the filtering function of the spleen have been described. Microsphiltration is an experimental technique, which mimics the mechanical retention of particles with little deformation in the human spleen [12, 13]. This technique can be used in vitro on suspensions of plasmodium culture but also ex vivo with parasites coming directly from patients with malaria. In addition, ex vivo microsphiltration of RBCs from patients with malaria could be used to explore the retention capacity of their spleen. Indeed, RBCs from patients with malaria have previously undergone splenic filtration in vivo and could therefore provide information on the splenic filtering functionality of the patient. Thus, the state of deformability of the RBCs, a determining factor in their splenic retention ex vivo, would reflect the ability of the spleen in vivo to filter out the less deformable RBCs.

In the present study, we plan to explore the role of the spleen in the occurrence of different clinical forms of malaria using *ex vivo* microsphiltration as tools to assess the splenic filtering function in malaria patients.

Methods

Study site and design

The study was conducted in Kéniéroba village at 55 km from Northwest of Bamako along the Niger River. Malaria transmission continue throughout the year but intense during rainy season from May to January. We conducted a cross-sectional survey to collect data in outpatients received during the routine consultation in local health center in 2018 transmission season. All confirmed *P. falciparum* malaria patient egal or more than 5000 trophozoite/µl of parasitemia with 8g/dl of hematocrit or more was enrolled when possible to perform the microsphiltration the same day.

Sampling

It was an exhaustive sampling about patients aged six months and more treated for thick smear confirmed malaria in health center during the routine consultation, who agreed to participate in the study and from whom the blood sample could be collected and brought to laboratory for the retention assay.

Microsphiltration

Splenic retention of *P. falciparum* infected-RBCs in malaria patients has been studied using previous described tip microsphiltration method [14].

Briefely, calibrated metal microbeads composed of 96.5% tin, 3.0% silver and 0.5% copper of different diameters were used to make layers in tips of very narrow spaces to imitate the inter-endothelial clefts of the micro-veins of the red pulp of the human spleen (sinus). An equal-weight mixture (1 g) of microsphere powder (5–15 µm in diameter and 15–25 µm in diameter) was suspended in 5 mL 1% PBS / AlbuMAX I solution (life technologies Cat#11020-021). A total of 800 µL of this suspension of microspheres were poured into an inverted anti-aerosol pipette tip of 1000 µL (Neptune, BarrierTips) and left to stand, leading to the formation of a layer of microspheres 5 mm thick at - above the aerosol filter. The microspheres were obtained from the Spherical Powder industry (24A, rue de la Résistance-BP 438, Annemasse 74108, France).

A total of 600 µL of RBCs suspension (patientsRBCs washed with RPMI and suspended at 1% hematocrit in PBS / AlbuMAX II 1%) were introduced instantly upstream of the layer of microspheres and entrained through the layer of microspheres with 6 ml of PBS / AlbuMAX II 1% using an electric pump (Syramed µsp6000, Arcomed'Ag). The filtration rate was 60 ml / H. A downstream sample (6.6 mL) and an aliquot of the upstream sample were collected to determine parasitemia. Each sample was filtered in duplicate. The mean parasitaemias (% of infected RBCs in the RBC suspension) in the upstream (PAm) and downstream (Pav) samples were determined. The RBC retention rate (RR) for each sample was calculated using the following formula: TR = [(PAm - PAv) / PAm] × 100 [14]. The cells were counted using an Accuri C6 flow cytometer (Becton - Dickinson) after labeling with Syto 61.

Statistical analysis

We used descriptive statistics to summarize data, quantitative variables are represented by means and standard deviation and categorical variables are characterized as frequencies. The t-test was used to assess statistical differences between means, plot-box was used to visualize data distribution by quartiles. Simple linear regression was applied to determine the correlation between rates of spleen retention and parasitemia, hemoglobin levels and age. For each variable, missing data was defined as no record of cases and unknown data, in this case the variable was not tested. A difference was considered significant at $P < 0.05$. All reported P values were bilateral. Statistical analyzes were performed on the complete data using statat version 14 software, the figures using prism version 8 software.

Results

A total of 237 malaria episodes were identified, of which 10.5% (25/237) patients were included for microsphiltration. The sex ratio was 1.08. Only 4% (1/25) presented severe malaria with prostration and vomiting. Two patients (8%) of patients had a no palpable spleen (hackette stage 0), 22 patients (88%) had a palpable deep inspiration spleen (stage 1 of Hackett) and only one patient (4%) had a palpable spleen (Hackett stage 2) (Table 1). Patients were 9.68 ± 3.881 years old on average with the extremes of 4 and 18 years old. Parasitemia ranged from 5,360 to 342,720 trophozoites/µl with 50,774.40 ± 65,540,854 trophozoites/µl on average, the average hemoglobin level was 11.23 ± 1.20 g/dl with the extremes of 8.70 and 13.40 g/dl. Retention rates ranged from 11.11–94.44% with an average of 65.40 ± 23.77% (Table 2). We did not find significant difference between the average retention rates according to the clinical

phenotype of Malaria **(p = 0.2) (**Fig. 1A**)**. No correlation was observed between retention rates and parasitemia **(p = 0.23) (**Fig. 2A**)** or hemoglobin levels **(p = 0.21) (**Fig. 2C**)**. However, we observed a statistically significant difference between the average rate of splenic retention of RBCs infected and the Hackett stages **(P = 0.001) (**Fig. 1B**)**.

Table 1
Clinical characteristics of the study participants

Statut microfiltrated	**Frequency**	**Percentage**
Non-microsfiltrated	224	89.5
Microsfiltrated	25	10.5
Total	**239**	**100.0**
Hackett stages		
Stade 0	2	8.0
Stade 1	22	88.0
Stade 2	1	4.0
Total	**25**	**100.0**
Clinical phenotype		
Non-complicated malaria	24	96.0
Severe malaria	1	4.0
Total	**25**	**100.0**
Malaria severity criteria		
Hyperparasitemia	0	0
Prostration et hyperpasitemia	0	0.0
Vomiting and Prostration	1	100.0
Convulsions	0	0.0
Total	**1**	**100.0**
Statut anemia		
Anemia	11	44.0
Non-anemia	14	56.0
Total	**25**	**100.0**

Table 2
biological characteristics of study participants

Characteristics	Average	Standar deviation	Minimum	Maximum
Parasitemia	50774.40	65540.85	5360	342720
Hemoglobin levels	11.22	1.20	8.7	13.4
Retention rate	65.40	23.77	11.11	94.44

Discussion

Spleen role in clinical malaria symptoms occurrence has long time been discussed [3]. However, direct evidence for these claims has still not been reported due to difficulties in in vivo exploration of the splenic function. In this study, we proposed to explore the spleen function remotely in patients with malaria using ex vivo microsphiltration of RBCs infected. W carried out a cross-sectional from May to December 2018 in Kéniéroba village. In this pilot study, clinical and biological data as well as *ex vivo* microphiltration retention rates were collected from 25 malaria patients

Microsphiltration is an experimental exploratory technique of RBCs deformability. It mimics the splenic retention of less deformable RBCs. This technique has been validated on suspensions of parasitized RBCs at different stages in the development of *P. falciparum* in vitro [13]. In this study, we used the ex vivo microsphiltration performed on RBCs directly from patients to explore the filtering functional status of the spleen and to determine its impact on the clinical and biological parameters of malaria. We hypothesized that the ex vivo deformability of RBCs after the splenic filtration in vivo is indicative of the status of the patients spleens filtering function. Thus, high ex vivo microsphiltration retention rates of *P.falciparum*-infected RBCs would be associated with a dysfunction of the splenic filtering function in patients and vice versa.

The mean splenic retention rate of RBCs infected was 65.40 ± 23.77%, a much higher rate as compared to reports by Diakite et al. 2016 in which the mean retention rate was 54. 5 ± 4,7% in Hb AA subjects and 44.5 ± 3.3% in Hb AS subjects [15]. This high rate could be explained by the filtration conditions, in particular the time elapsed between the sampling and the microsphiltration.

Although anemia is associated with high retention of RBCs infected *in vivo* [11, 16], which may result into low ex vivo retention rates, we did not find a statistically significant correlation between the *ex vivo* retention rates of parasitized RBCs. and the hemoglobin level ($p = 0.21$). Our hypothesis assumed that low ex vivo retention rates would reflect high splenic retention in vivo that would be associated with a low arasitemia.

We found no correlation between ages ($p = 0.7$). Age could also influence the filtering function of the spleen in patients living in malaria endemic areas. In fact, in malaria endemic areas, patients are more likely to develop splenic insufficiency due to multiple challenges the spleen face during successive

infections [3, 17]. Analysis of parasitemia as a function of *ex vivo* retention rate did not reveal any correlation between these two parameters (p = 0.23). high parasite density was associated with serious manifestations of the disease such as cerebral malaria. This clinical phenotype is in favors of low splenic retention in *vivo* [7]. No correlation between the parasitemia and the *ex* vivo retention rates was found. Ended due to technical challenges we only worked on parasitemia more than 5000 parasites per microliter.

As expected, we observed high ex vivo retention of RBCs infected from patients with clinical Hacktt stages greater than or equal to 1 (p = 0.03). In fact, the Hackett clinical stage defines the degrees of splenomegaly that may be associated spleen function impairment.

Conclusion

This pilot study allowed to explore *ex vivo* filtering function of the spleen in patients with malaria. The retention of RBCs infected was observed in patients with clinical Hacktt stages greater. Methodological corrections, especially in terms of planning and sample size, will make it possible to generate data to draw reliable conclusions.

Abbreviations

MRTC	Malaria Research and Training Center
PBS	Phosphate Buffer Saline
Pf	Plasmodium falciparum
RBCs	Red Blood Cells
RPMI	Roswell Park Memorial Institute Medium
RR	Retention Rate
USTTB	University of Sciences, Technics and Technologies of Bamako
WACCBIP	West African Centre of Cell Biology of Infectious Pathogens

Declarations

Acknowledgements

We thank the parents, guardians and children who participated into this study, and the technical, clinical and nursing staf for assistance. We are grateful to many colleagues at MRTC for providing critical reviews of the manuscripts

which helped improve it. This publication uses data from the MalariaGEN SpotMalaria Project as described online https://www.malariagen.net/projects/ spotmalaria pending citeable publication; the

project is coordinated by the MalariaGEN Resource Centre with funding from Wellcome (206194, 090770).

Authors' contributions

Study setup : SASD. Sample collection : BK, MD. Data collection : BK, AMG, SASD. Data analysis : BK, SASD. Manuscript writing : SASD, BK. Manuscript review: SASD, BK. All authors read and approved the fnal manuscript.

Funding

This study is supported by a DELTAS Africa grant (DEL-15-007: Awandare). The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust (107755/Z/15/Z: Awandare) and West Africa

Ethics approval and consent to participate

Before starting this study, we obtained a community consent from traditional and customary chiefs prior to the study. The study was approved by the ethics committee of the faculty of medicine and Pharmacy of the University of Sciences, Technics and Technologies of Bamako

(USTTB), Mali. Written informed consent was obtained from a parent or guardian of each enrolled child.

Consent for publications

All authors read and approved the final manuscript. Competing interests, The authors declare that they have no competing interests.

Author details

1 Icer-Mali /FMOS-FAPH/ University of Sciences, Technics and Technologies of Bamako (USTTB), Bamako, Mali.

2 University Clinical Research Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali

References

1. OMS, *Rapport sur le paludisme dans le monde*. 2018.
2. Dalko, E., et al., *Multifaceted Role of Heme during Severe Plasmodium falciparum Infections in India.* Infect Immun, 2015. **83**(10): p. 3793-9.
3. Gomez-Perez, G.P., et al., *Plasmodium falciparum malaria and invasive bacterial co-infection in young African children: the dysfunctional spleen hypothesis.* Malar J, 2014. **13**: p. 335.
4. Cooke, B.M., N. Mohandas, and R.L. Coppel, *The malaria-infected red blood cell: structural and functional changes.* Adv Parasitol, 2001. **50**: p. 1-86.
5. Schwartz, R.S., et al., *Altered plasma membrane phospholipid organization in Plasmodium falciparum-infected human erythrocytes.* Blood, 1987. **69**(2): p. 401-7.
6. Scherf, A., et al., *Molecular mechanisms of Plasmodium falciparum placental adhesion.* Cell Microbiol, 2001. **3**(3): p. 125-31.
7. Huang, S., et al., *In vivo splenic clearance correlates with in vitro deformability of red blood cells from Plasmodium yoelii-infected mice.* Infect Immun, 2014. **82**(6): p. 2532-41.
8. Groom, A.C., E.E. Schmidt, and I.C. MacDonald, *Microcirculatory pathways and blood flow in spleen: new insights from washout kinetics, corrosion casts, and quantitative intravital videomicroscopy.* Scanning Microsc, 1991. **5**(1): p. 159-73; discussion 173-4.
9. Chotivanich, K., et al., *Central role of the spleen in malaria parasite clearance.* J Infect Dis, 2002. **185**(10): p. 1538-41.
10. Jakeman, G.N., et al., *Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes.* Parasitology, 1999. **119 (Pt 2)**: p. 127-33.
11. Buffet, P.A., et al., *The pathogenesis of Plasmodium falciparum malaria in humans: insights from splenic physiology.* Blood, 2011. **117**(2): p. 381-92.
12. Buffet, P.A., et al., *Ex vivo perfusion of human spleens maintains clearing and processing functions.* Blood, 2006. **107**(9): p. 3745-52.
13. Deplaine, G., et al., *The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro.* Blood, 2011. **117**(8): p. e88-95.
14. Lavazec, C., et al., *Microsphiltration: a microsphere matrix to explore erythrocyte deformability.* Methods Mol Biol, 2013. **923**: p. 291-7.
15. Diakite, S.A., et al., *Stage-dependent fate of Plasmodium falciparum-infected red blood cells in the spleen and sickle-cell trait-related protection against malaria.* Malar J, 2016. **15**(1): p. 482.
16. Buffet, P.A., et al., *Retention of erythrocytes in the spleen: a double-edged process in human malaria.* Curr Opin Hematol, 2009. **16**(3): p. 157-64.
17. Hommel, B., et al., *Hyposplenism revealed by Plasmodium malariae infection.* Malar J, 2013. **12**: p. 271.

Figures

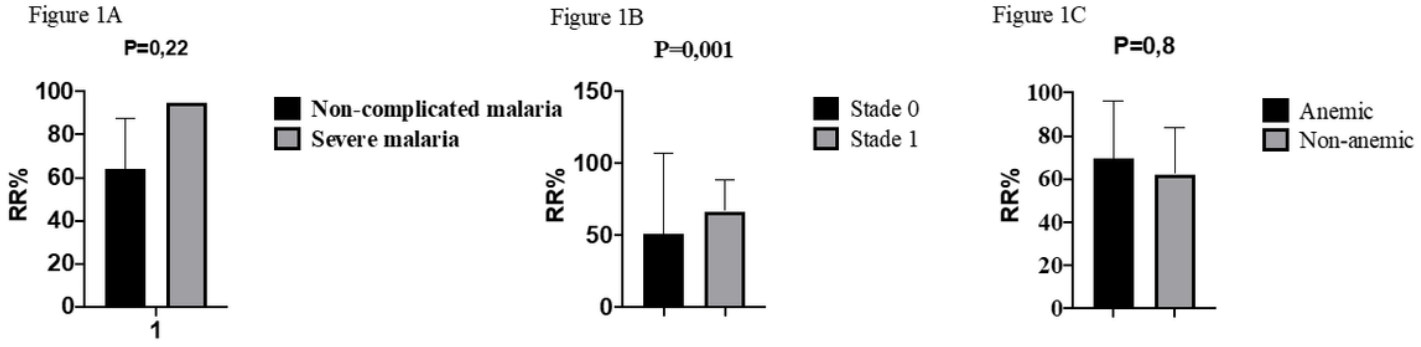

Figure 1

A: Mean spleen retention rates regarding the clinical phenotype B: Mean spleen retention rates regarding the clinical Hackett stage C: Mean spleen retention rates regarding the anemia status

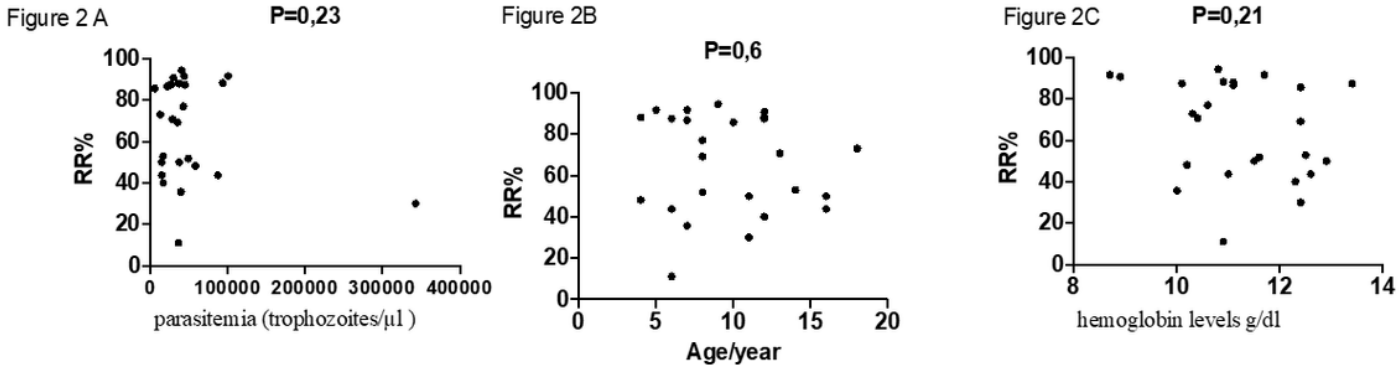

Figure 2

A: correlation between spleen retention rates and the parasitaemia B: correlation between spleen retention rates and the ages C: correlation between spleen retention rates and the hemoglobin levels