Journal of african clinical cases and reviews / Journal africain des cas cliniques et revues

www.jaccrafrica.com ISSN 1859-5138 Open access

Original article

Post-transfusion malaria: incidence and factors associated at the mother and child center in Yaoundé

Paludisme post transfusionnel : incidence et facteurs associes au centre mère et enfant de Yaoundé

ANA Yanda[1,2], IAM Manga[1], JE Ngoué*[3], A Boula[1,2], M Oloume[5], EW Kamgueng[2], TN Jocelyn[2], KSD Hélène[5], MN Isabelle[3], DHM Awa[2,3]

Résumé

Introduction : Le plasmodium a été retrouvé dans le sang de donneurs bien qu'asymptomatiques, ce qui constitue un risque pour les receveurs de développer un paludisme post transfusionnel. Notre travail visait à évaluer l'incidence du paludisme post transfusionnel chez les enfants et les facteurs associés.

Méthodologie : Nous avons mené une étude transversale analytique impliquant 104 enfants chez qui le paludisme a été exclu avant la transfusion sanguine au Centre Mère et Enfant de Yaoundé, du 28 Janvier au 30 Juin 2020. La recherche du plasmodium par le test de diagnostic rapide (TDR), la goutte épaisse (GE) et le frottis sanguin a été effectuée dans les poches de sang des donneurs et chez les receveurs après la transfusion sanguine. Les données ont été analysées à partir du logiciel SPSS version 23. Les associations étaient statistiquement significatives pour $p < 0,05$.

Résultats : L'incidence d'une parasitémie positive dans les poches de sang était de 11,5 % (n=12) par les techniques de TDR et GE. Chez les receveurs, le TDR et la GE étaient positifs chez les receveurs respectivement à 18,3% (n=19) et à 11,5% (n=12). Tous Les enfants avec une GE positive avaient développé les symptômes du paludisme. Toutes les poches de sang avec une parasitémie positive avaient été conservées moins de 10 jours. Une GE positive dans les poches de sang était associée au développement d'une parasitémie chez les receveurs ($p < 0,05$).

Conclusion : Une parasitémie positive chez le donneur constitue un risque de paludisme post transfusionnel.

Mots-clés : paludisme, transfusion sanguine, enfant, Yaoundé.

Abstract

Introduction: Plasmodium has been found in the blood of asymptomatic donors, putting recipients at risk of developing post-transfusion malaria. Our aim was to

assess the incidence of post-transfusion malaria in children and the associated factors.

Methodology: We conducted an analytical cross-sectional study involving 104 children in whom malaria was excluded prior to blood transfusion at the Mother and child center of Yaounde, from January 28 to June 30, 2020. Plasmodium testing by rapid diagnostic test (RDT), thick drop (TD) and blood smear was performed in donor blood bags and in recipients after blood transfusion. Data were analysed using SPSS version 23 software. Associations were statistically significant for $p < 0.05$.

Results: The incidence of positive parasitaemia in blood bags was 11.5% (n=12) by both RDT and GE techniques. In recipients, RDT and TD were positive in 18.3% (n=19) and 11.5% (n=12) respectively. All TD-positive children had developed malaria symptoms. All parasitaemia-positive blood bags had been stored for less than 10 days. Positive TD in blood bags was associated with the development of parasitaemia in recipients ($p < 0.05$).

Conclusion: A positive parasitaemia in the donor constitutes a risk of post-transfusion malaria.

Keywords: malaria, blood transfusion, child, Yaoundé.

Introduction

Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium*, responsible for a severe acute febrile state that is potentially fatal in the absence of prompt and appropriate management [1]. Worldwide, in 2019, WHO reported 228 million cases of malaria, the majority (93%) in the African region. Malaria was responsible for 405,000 deaths, 67% of which occurred in children under the age of 5. The species most responsible for these deaths was *Plasmodium falciparum.* Although malaria is mainly transmitted by the bite of the female Anopheles, transmission can also occur from mother to child in utero, or by blood transfusion [2]. The latter is an urgent and necessary treatment for severe anemia, which is a major cause of hospitalization and infant mortality. Like many blood-borne micro-organisms, Plasmodium can be transmitted from an infected donor to a recipient [3] all the more so as it can withstand a temperature of 4˚C for at least three weeks when storing blood bags [4]. In Cameroon, efforts are being made to exclude blood bags in which HIV, hepatitis B and C, syphilis are detected from the blood donation circuit in the event of positivity. [3] This is not the case for malaria, despite the country being an endemic zone. Only an interrogation is carried out in search of symptoms suggestive of malaria, but this is not enough, as there are also healthy carriers. The parasite has been documented in the blood of asymptomatic donors [5]. The prevalence of the parasite in collected blood bags varies from region to region and from period to period [6] from 0.67% to 55% in Kenya and Nigeria respectively [7] in Cameroon, it ranges from 6.5% to 11.75 in Yaoundé and Douala respectively [8,9]. This would justify the systematic administration of antimalarial drugs after blood transfusion in certain departments, despite the absence of national recommendations. The incidence of malaria in transfused children remains poorly known, hence the interest of our work, whose aim was to assess the incidence of post-transfusion malaria in these vulnerable children and the factors associated with it.

Methodology

Study population, location and duration: We conducted a cross-sectional analytical study over a 5-month period from January 28 to June 30, 2020. It took place at the Mother and Child Center of the Chantal BIYA Foundation in Yaoundé. This is a pediatric referral hospital located in Yaoundé, the political capital of Cameroon. It attracts not only the surrounding population, but also people from the suburbs and outlying areas, and records more than 10,000 emergency consultations and over 500 blood transfusions a year.

Selection criteria and sampling: we used consecutive, non-exhaustive sampling. We included patients aged between 3 months and 17 years who had received a blood transfusion, whose EW and RDT had been negative prior to the blood transfusion, and whose parents had agreed to participate in the study. Patients with incomplete data who had received antimalarial treatment after transfusion were excluded.

Data collection procedure and tools: we identified patients requiring blood transfusion in whom the GE and malaria RDT were negative, and performed these tests on the blood bags to be transfused. The same tests were carried out on children transfused 24 to 48 hours after transfusion. The blood to be analyzed was taken from the pulp of the finger.

For malaria RDT

The malaria RDT is a diagnostic test that detects antigens produced by malaria parasites in the patient's blood. We used WHO-compliant RDT kits detecting P. falciparum antigens (HRPf) and those of other species (LDH). falciparum (HRPf) and other species (LDH) antigens (Pf/pan) from the careStart and SD Bioline brands. After writing down the patient/blood bag identification number and date, a drop of blood was pipetted into well S and 3 drops of buffer solution into the corresponding well, results were read and interpreted 15-20 minutes later, ensuring that the results were valid (control band C clearly visible, no blood in the reaction zone). The result was positive if two or three bands of the same color appeared on the membrane, and negative if only the control line was present.

About the thick drop

After identifying the slide, a drop of blood was collected from the 4ème finger/big toe after disinfection, and 3 small drops of blood were placed on the slide and spread out in a circle using a 2ème slide in 6 rapid, continuous, concentric movements of 1 cm in diameter, then dried while protecting it from contamination and stained with Giemsa. GE detects parasitaemia ranging from 10 to 20 parasites /mm3.

For blood smears

A drop of blood was taken simultaneously with the GE and placed on the slide. It was fixed with May Grumwald Giemsa for 8 to 10 minutes, then gently rinsed with water in an upright position, and the slide was dried on the drip tray or rack, spread side down.

To minimize the risk of errors, the slides were read by the principal investigator, a laboratory technician and a medical biologist successively, using an electron microscope with an objective of 100 after oil immersion. Slides were run over at least 100 fields. A result was declared negative when no parasites were observed after counting at least 500 leukocytes on the thick drop slide. The sensitivity of the blood smear is 150 to 200 parasites per mm3.

The blood smear was used to diagnose the species, how the slide was prepared for the smear and how it was read. It should also be described as a RDT and GE.

The recipient's information was collected from the parents, while the donor's information was collected from the blood bank after tracing, and recorded on a pre-determined data sheet.

The variables of interest were: for the recipient: age, sex, post-transfusion clinical signs, post- transfusion anti-malarial treatment, GE and RDT before and after transfusion; and for the donor: age, sex, use of a LLIN, anti-malarial treatment in the last month, GE and RDT.

Operational definitions of terms

Post-transfusion malaria: parasitaemia and/or positive RDT for Plasmodium 24 or 48 hours after a blood transfusion with or without clinical signs in a patient whose initial tests were negative.

However, we considered patients with positive parasitaemia after blood transfusion to be malaria patients, as residual antigenemia may have been present in the donor (indicating a long-standing infection).

Data management and analysis: data were entered into CS Pro 7.0, then imported into SPSS version 23.0 for analysis. Chi-square and Fisher tests were used for measures of association between categorical

variables. The significance level was set at 10% and the CI at 95%.

Ethical considerations:

Study authorization was obtained from the Institutional Ethics Committee of the University of Yaoundé 1 and granted by the administrative services of the Mother and Child Center and the Yaoundé Central Hospital. The parents/guardians of eligible children were clearlý informed of the purpose of our study and their consent obtained. The information collected waś treated with the utmost confidentialitý, in strict compliance with patients' rights and will be used for scientific purposes only. The tests were carried out free of charge to all patients, and the results were communicated to parents and the medical team. All children detected positive had received anti-malarial treatment after transfusion, but in the event of refusal of consent, no consequences took place on the patient's care in the department.

Results

Seven hundred and fifty-nine children received a blood transfusion during the study period, 312 of whom were eligible. 447 were parasitaemia positive. 208 were excluded for the following reasons: anti-malarial treatment during the week, failure to perform a thick drop test prior to blood transfusion, refusal to participate in the study. **Figure 1**

Socio-demographic characteristics

Our study population consisted of 104 children, including 65 boys and 39 girls, giving a sex ratio of 1.6. The median age of transfused children was 4.5(2-10) years, with a minimum of 4 months and a maximum of 17 years. The most represented age group was under 5 (50%). The median age of donors was 27, with a minimum of 17 and a maximum of 57, and a sex ratio of

2.25 in favor of males. Donors living in urban areas accounted for 81.7% (n=85). Among the latter, 67.9% (n=57) used long-acting mosquito nets, 66.6% (n=38) of them permanently. 10.3% (n=9) had received anti-malarial treatment 2 weeks prior to blood donation. **Table 1**

Prevalence of blood bags transfused and found positive for plasmodium falciparum / Incidence of post-transfusion malaria

Positive parasitaemia for *Plasmodium falciparum* was found in 11.5% (n=12) of blood bags. Parasite density was 171.3 ± 106.2 trophozoites/µl [16-380]. After blood transfusion, the percentage of malaria infection in transfused children was 11.5% (n=12) by thick blood smear and 18.3% (n=19) (10.6-26) by rapid diagnostic test . Parasite density on thick film was 177.9

±143.5 trophozoites/µl. The plasmodium species found was *plasmodium falciparum*.

Ten of the twelve children detected as positive for *Plasmodium falciparum* had received implaludinized blood and had the classic symptoms of malaria: fever in 83.3%, asthenia in 16.7%, polyarthralgia, headache and chills in 8.3%.

The shelf life of blood bags tested positive for Plasmodium falciparum was 4.75 ± 3.05 days. Two children developed post-transfusion malaria when they received blood tested negative for RDT and EW. 2 children who received malaria-negative blood did not develop signs of the disease and had a negative parasitaemia; they had received ACT-based antimalarial treatment. **Table 2**

Factors associated with post-transfusion malaria

The only factor statistically associated with post-transfusion malaria was a positive parasitaemia/ RDT in the donor's blood bag (p-value<0.001*). There was a weak positive correlation (r=0.2) between donor and recipient parasite loads. Table 3

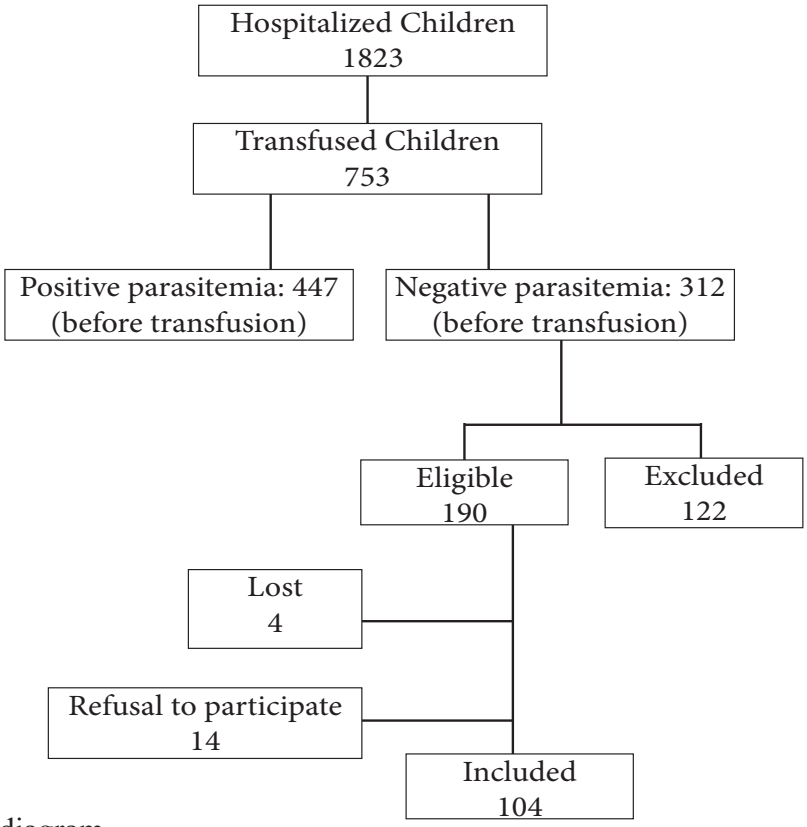


Figure 1: Inclusion diagram

Table I: sociodemographic data of the study population (transfused patients and donors)

Variables	Number (N = 104)	Percentage (%)
Age groups of transfused patients		
< 5 years	52	50
5 – 10 years	24	23.1
10 – 15 years	26	25
> 15 years	2	1.9
Sex of transfused children		
Feminine	39	37.5
Male	65	62.5
Age group of donors		
< 20 years	22	21.2
[20 – 40[	72	69.2
[40 – 50[	8	7.7
> 50 years	2	1.9
Sex		
Male	72	69.2
Feminine	32	30.8
Residence area		
Urban	85	81.7
Semi urban	19	18.3
Rural	00	0
Donor status		
Family	86	82.7
Volunteer	17	16.3
Paid	1	1

Table II: prevalence of detectable malaria parasitemia in transfused blood bags and in transfused children

Variables	Number (N = 104)	Percentage (%) (5.8 – 18.3)
Transfused blood bags		
RDT Malaria		
Positive	12	11.5
Negative	92	88.5
Thick drop		
Positive	12	11.5
Negative	92	88.5
Transfused children		
RDT Malaria		
Positive	19	18.3
Negative	85	81.7
Thick drop		**95% CI (5.8 – 18.3)**
Positive	12	11.5
Negative	92	88.5

Table III: factors associated with post-transfusion malaria

Variables	P-value	OR (CI=95%)	Adjusted P-value	OR (CI=95%) adjusted
Age of recipients (in years)				
<4.5	1	1 (0.3 – 3.33)		
> 4.5				
Child gender				
Male	0.03*	0.25(0.07 – 0.91)	0.1	0.14 (0.01 – 2.74)
Feminine				
Age of donors				
< 27 years old	0.67	1.3 (0.39 – 4.34)		
> 27 years old				
Donor sex				
Male	0.75	0.72 (0.18 – 4.34)		
Feminine				
Use of an LLIN by the donor	0.04*	0.27 (0.08 – 0.97)	0.23	0.12 (0.01 – 3.83)
Shelf life of the blood bag				
< 10 days	0.26	1.23 (-3.23 – 3.12)		
> 10 days				
GE of donor				
Positive	<0.001*	225(28.5 – 1775.9)	0.1*	<0.001
Negative				

*statistically significant

Discussion

The aim of this study was to determine the incidence of post-transfusion malaria. It found that 11.5% of blood bags intended for transfusion were positive for plasmodium. This parasitemia was associated with the development of parasitemia in 83.4% of recipients.

At Covid 19, work was carried out in the midst of the pandemic, which had a dampening effect on the workforce. Indeed, due to the confinement, we noticed a drop in attendance. We also mentioned a possible bias in the production of the Thick Drop, whose reading can vary depending on the operator.

The age of these patients ranged from 4 months to 17 years, with a median of 4.5 years. The under-5 age group accounted for 50% (n=52). There was a clear male predominance (62.5%) (n=65), with a sex ratio of 1.6. A study by Ali *et al.* in Sudan had shown that 44% of the transfused population was represented by children under 5 years of age [10]. This may be due to the fact that children under 5 are more vulnerable to infectious pathologies (respiratory infections, diarrhoea) and more prone to nutritional deficiencies. Donor age ranged from 17 to 57, with a median of 27. Males were the most represented (69.2%) (n=72), with a sex ratio of 2.25. These results are similar to those of Ndo *et al.* who, in a study conducted in Douala in 2016, reported an average age of 28.27 ± 7.27 years [9] and those found by Iro *et al.* in Niger, with a sex ratio of 3.6 [11]. This trend can be explained by the fact that young people are the most aware of the need to donate on the one hand, and on the other that there are several exclusion criteria for women in the selection process for female blood donors, including pregnancy, breastfeeding and menstruation.

Plasmodium falciparum was found in 11.5% (5.8-18.3) (n=12). These results are identical to those of Ndo *et al.* who found a *P. falciparum* infection rate in blood bags of 11.75%. [9]but different from those of Noubouossie *et al.* in Yaoundé in 2007, who reported a lower prevalence of 6.5%. [8]. These differences could be explained by differences in malaria epidemiology. The presence of *plasmodium falciparum* in donor blood bags has been described in all regions where malaria is endemic, such as Sudan, India, South Africa, Benin or Cameroon. In Yaoundé, Cameroon, where the work was carried out, transmission is perennial and intense all year round, but differences in prevalence are due to the seasons (rainy or dry), as well as to epidemiological facies. Higher prevalences are observed during peak periods on equatorial facies (Central Africa) and tropical facies (West Africa), and in the rainy season, when anopheline multiplication is greater. The study by Noubouossie et al. was carried out during the mesoendemic period (October-November), when transmission is less intense, while that by Kinde *et al.* in Benin, who conducted the study during the rainy season, found a prevalence of 33.1% [12]. On the other hand, this difference is due to the technical methods of analysis (different sensitivities) and the experience of the slide reader. GE is operator-dependent, depending not only on the quality of the blood spread on the slides, but also on the experience of the slide reader. Other studies have been carried out in Africa: Ali *et al.* in Sudan used only the thick drop as a screening technique, and found a prevalence of *Plasmodium falciparum* carriage of 6.5%. [10] . Other authors reported higher prevalences, notably Bassandja *et al.* in the Democratic Republic of Congo, who used the thick drop and blood smear techniques with a technician trained in microscopy by the national malaria control program, Epidi *et al.* in Nigeria and Diop *et al.* in Senegal, with prevalences of 28.3%, 51.1% and 65.3% respectively [13-15].

With regard to post-transfusion malaria, we noted that 12 children had developed a positive parasitaemia for *Plasmodium falciparum*, i.e. a prevalence of 11.5% (5.8-18.3), and 19 children had a positive RDT, i.e. 18.3% (10.6-26). Children with positive parasitaemia were distributed as follows:

- 10 had received blood bags that tested positive, for a percentage of 83.3%.
- 2 had received blood bags that tested negative, i.e. a percentage of 16.7%.

On the other hand, 2 children who received blood bags that tested positive did not develop clinical or biological signs of malaria, representing 2.17% of the children who tested negative. These children had received empirical antimalarial treatment immediately after the blood transfusion. This incidence is higher than that of Ihéonu *et al*. in Nigeria and Ali *et al.* in Sudan, who found prevalences of 2.4% and 3.5% respectively [10,16]. Our study was carried out in Yaoundé, where malaria transmission is stable and intense, and also due to the fact that the method used, in particular the thick drop and blood smear, depends not only on the spreader but also on the reader's experience. A study by Owusu-Ofori et al. found a prevalence of 2%; in addition to the thick blood smear, they used PCR, which showed a concordance between the parasite in the bag and that in the transfused patient. [17].

In terms of concordance between blood bag positivity and malaria in recipients, we found a percentage of 83.3%, in contrast to Ihéonu *et al*. and Ali *et al.* in Sudan, who had reported that 100% of patients who had received malaria-infected blood had been detected as positive for Plasmodium and that 01 patient had developed parasitaemia after receiving negative blood [10,16]. This difference is probably due to the fact that, in our study, patients detected negative after receiving malaria-infected blood had received post-transfusion anti- malarial treatment empirically 24 hours later, and this percentage would probably have been 100% if this treatment had not been undertaken. The presence of detectable parasitaemia in the bag, in addition to the patient's weakened organism, would contribute to the development of malaria. The association between the receipt of Plasmodium-positive blood and the development of post-transfusion malaria in recipients proved to be statistically significant (P value < 0.001*).

Parasite density in our study was low. It ranged from 16 to 500 trophozoites/µl, with an average of 177.9 ± 143.5 trophozoites/µl. These results are lower than those of Ihéonu *et al.* whose parasite density ranged from 71 to 4,079 parasites with an average of 908 parasites/µl [16]. These results are due to the difference in parasite load found in the donors, notably an average of 171.3 ± 106.2 trophozoites/µl (16 - 380 TPf/µl) in our study and 525 parasites/µl (30 - 6,029) in their study. This can be explained by the fact that high parasite loads lead to clinical signs that exclude donors from donating at the time of collection. All the bags detected as positive for Plasmodium had been kept for less than 10 days, as shown by Owusu- Ofori *et al.* in Ghana, whose bags were kept for between 1 and 6 days. This suggests that short bag storage times contribute to the persistence of parasitaemia in blood bags. We found a positive correlation (r = 0.2) between the parasitemia of blood bags and those of children, as did Iheonu *et al. Plasmodium falciparum* was the only species found on all thick drop spreads, as shown in the studies by Owusi-Ofori et *al*. [17].

As factors associated with the occurrence of post-transfusion malaria, we identified the receipt of Plasmodium-positive blood and the presence of parasitaemia (P value <0.001*). These results concur with those of Iheonu et al in Nigeria [16].

Conclusion

The risk of malaria through blood transfusion is real when parasitaemia is present in donor blood. We found positive parasitaemia and a relatively low density among donors, with an incidence of 11.5%. Thus, the risk of malaria following a blood transfusion is real, with a prevalence of 11.5% in our study, and the development of parasitemia in the recipient is statistically associated with the positivity of a blood bag received. The factor associated with

the development of parasitemia in the recipient is the presence of parasites in the donor's blood bag. Post-transfusion antimalarial treatment would prevent the occurrence of post-transfusion malaria in the recipient. We recommend systematic screening of donors for malaria as part of transfusion safety strategies, and a systematic thick blood drop after blood transfusion.

Authors' contributions

Alima conceived the project and participated in all stages. Manga and Tony collected and analyzed the results; Epée, Boula, Oloume, Wete, Kamo and Mekone interpreted the results, wrote and proofread the manuscript; Mbassi ensured the scientific validity of the study, from conception to manuscript.

Thanks

The authors would like to express their sincere thanks to the staff of the Centre Mère Enfant and to all the participants.

***Correspondence**

Jeannette Épée Ngoué

jeannette.epee@fmsb-uy1.cm

Available online: November 30, 2023

1 : Institut Supérieur de Technologies Médicales, Cameroon
2 : Centre Mère et Enfant (CME) de la Fondation Chantal Biya, Yaoundé, Cameroon,
3 : Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon
4 : Centre Pasteur du Cameroun
5 : Faculty of Medicine and Biomedical Sciences, University of Garoua, Cameroon

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Conflict of interest : None

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To cite this article :

ANA Yanda, IAM Manga, JE Ngoué, A Boula, M Oloume, EW Kamgueng et al. Post-transfusion malaria: incidence and factors associated at the mother and child center in Yaoundé. Jaccr Africa 2023; 7(4): 223-232