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SHORT REPORT

Red Cells and Iron

Increased erythroferrone levels in malarial anaemia

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Summary

We assessed the diagnostic potential of erythroferrone as a biomarker for iron homeostasis comparing iron deficiency cases with anaemia of inflammation and controls. The dysregulation of the hepcidin axis was observed by Latour et al. in a mouse model of malarial anaemia induced by prolonged *Plasmodium* infection leading to increased erythroferrone concentrations. In line with that, we found significantly higher erythroferrone levels in cases with malaria and anaemia in an African population, compared to asymptomatic controls. Therefore, our findings extend the previous ones of the mouse model, suggesting also a dysregulation of the hepcidin axis in humans, which should be further corroborated in prospective studies and may lay the basis for the development of improved treatment strategies according to ERFE concentrations in such patients.

KEYWORDS
anaemia, malaria, iron biochemistry

Iron homeostasis is dependent on a complex interplay of physiological mediators[1,2] and is highly sensitive to inflammatory diseases, such as malaria (e.g. *Plasmodium falciparum* malaria).[3] Severe malaria disease is frequently accompanied by anaemia due to haemolysis of parasitized erythrocytes.[4] In the acute situation, this contributes to the disease burden since tissue oxygenation and energy metabolism are impaired. In the longer term, chronic malarial anaemia and concomitant stress erythropoiesis may impair recovery, pose an additional risk for complications in pregnant women,[5] and for failure to thrive in children and adolescents.[6] Knowledge of the mechanisms behind malarial anaemia guides the appropriate treatment approach and helps to determine the optimal timing, for example iron supplementation in case of additional iron deficiency.

Erythroferrone (ERFE) is a modulator of hepcidin and a marker of iron requirement of erythroid precursors in the bone marrow.[7] In acute iron deficiency, for example from blood loss, ERFE is secreted and primarily binds bone morphogenetic proteins (BMPs), namely heterodimers of BMP2 and BMP6, blocking their constitutional signal promoting hepcidin expression.[2,8]

Latour et al. studied the pathophysiological changes caused by severe malarial anaemia in a mouse model (*Plasmodium berghei* K173). According to their findings, a dysregulation of the hepcidin axis was observed. A marked

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increase in hepcidin concentration during the first acute-phase reaction was followed by a decline of erythrocyte indices and haemoglobin. Subsequently, an increase in ERFE expression markers coincided with a reduction in hepcidin levels.[9]

Therefore, in order to initially verify this in human, we assessed ERFE within a case–control study in a malaria-prone region in Africa.

For this study, subjects from two trials were selected in whom complete data such as anthropometric, clinical and in vitro parameters were available as well as corresponding preserved plasma samples for further analysis of ERFE. Inclusion was further restricted to the wet season, the peak season of malaria transmission, which is between June and December (cf. Figure S1). The PALUBAC trial (Burkina Faso, ClinicalTrials.gov Identifier: NCT02669823) served for symptomatic cases, a study in febrile patients with or without chronic disease or malaria. Controls (asymptomatic) were obtained from the NOVAC trial (Burkina Faso, ClinicalTrials.gov Identifier: NCT03176719), a field study in randomly included healthy volunteers from the Nanoro Health and Demographic Surveillance System. Participants were further characterized as symptomatically inflamed according to a body temperature of equal to or greater than 38.5°C at enrolment, which categorizes the study participants into controls without fever ($n = 455$, all from NOVAC) and cases with fever ($n = 140$, all from PALUBAC). Fever was due to acute or chronic diseases such as bacterial, viral or malaria infection (mostly uncomplicated).

ERFE was quantified in EDTA-anticoagulated plasma by an enzyme-linked immunosorbent assay (Intrinsic LifeSciences, La Jolle CA, USA), which was programmed on a DSX device (Dynex, Denkendorf, Germany). It uses a sandwich technique with a monoclonal antibody against ERFE immobilized on 96-well plates and a detection antibody bound to horse-radish peroxidase facilitating a chromogenic reaction with tetramethylbenzidine which is proportionately oxidized and quantified by photometric readings at 450 nm.

Haemocytometry (XN-30, Sysmex, Kobe, Japan) had been used to assess total haemoglobin and parasite density in whole blood. The latter was possible due to a specialized malaria channel newly developed in the XN-30 device. This channel allows for the counting of dyed parasitized red blood cell with the aid of an integrated blue laser. According to the age-related cut-offs recommended by the World Health Organization,[10] we categorized haemoglobin concentrations into the non-anaemic or anaemic groups. Accordingly, we considered the following situations as anaemic: children below 59 months with a haemoglobin below 11.0 g/dL, children between 5 and 11 years with a haemoglobin below 11.5 g/dL, children between 12 and 14 years below and female above 15 years with a haemoglobin below 12.0 g/dL, and male above 15 years with a haemoglobin below 13.0 g/dL. Since pregnancy was not registered, a specific cut-off could not be considered. For parasitaemia, we used a cut-off, which had been established for the XN-30,[11,12] to divide into parasitaemia-negative (P–) or parasitaemia-positive (P+) categories, that is, 20 parasites or more per microlitre were deemed positive.

Statistical analysis was performed using R version 4.3.1 (The R Foundation for Statistical Computing, Vienna, Austria). The ANOVA was used for comparison of means after log transforming the data to achieve a normal distribution, and the subsequent test for departure from normality was not significant. Otherwise, the Kruskal–Wallis test was employed. Pearson's chi-squared test was used to compare categorical variables. Univariate and multivariate analyses for the assessment of possible predictors of a continuous response (ERFE concentration) were carried out through linear regression using Beta estimates to quantify the relationship. Interactions were assessed through linear regression. p-values less than 0.05 were considered statistically significant.

Baseline characteristics of cases and controls are summarized in Table 1. Sex distribution did not differ significantly between groups ($\chi^2(3) = 6.33$, $p = 0.11$). However, a significant difference between cases and controls in the distribution of age ($\chi^2(3) = 20$, $p < 0.001$) was found. Haemoglobin was significantly lower and anaemia was more prevalent in cases than in controls while plasma ERFE concentrations were significantly higher in cases ($\chi^2(3) = 49$, $p < 0.001$).

To explore this further, we tested the differences in parasitaemia subgroups outlined in Figure 1A and observed a significant difference for elevated mean ERFE concentrations (ANOVA, $F(3, 468) = 6.793$, $p < 0.001$). The same was true for ERFE with a significant impact on different anaemia groups outlined in Figure 1B (ANOVA, $F(3, 468) = 11.03$, $p < 0.001$). This is also evident in the linear model described in Table S1. In this model, parameters without a significant association with ERFE concentrations, as assessed by previous multivariable regression without the interaction term, were excluded from the calculation. These were age (Beta = −0.02, CI_{95}: −0.05 to 0.01, $p = 0.2$) and sex (Beta = −0.28, CI_{95}: −1.3 to 0.78, $p = 0.6$). Furthermore, an interaction term between fever and anaemia was included. Independent from malaria parasitaemia, anaemia in combination with fever had a highly significant impact on ERFE with higher concentrations in cases than in controls (Beta = 6.3, CI_{95}: 3.3–9.2, $p < 0.001$; Table S1). In contrast, the multivariate analysis did not show a significant interaction effect for ERFE concentration when fever and parasitaemia coincided (Table S2).

Haemolysis during severely symptomatic malaria disease stimulates haemato- and particularly erythropoiesis. Therefore, the increased ERFE represents the increased proliferation of red cell precursors due to erythropoietin stimuli and their immediate demand for iron.[13,14] Similar observations of possible ERFE action, judging from the lowered hepcidin concentrations, have been made before in studies of highly inflamed children[15] while other authors describe complex kinetics of hepcidin in malarial anaemia,[16] which hint at the interference from ERFE. Furthermore, Latour et al. describe a decreased parasite density in ERFE knockout mice, which could correspond to the restricted iron supply. The lack of correlation of ERFE with parasitaemia in our

TABLE 1 Baseline characteristics of study population and statistical comparison between all four groups.

Characteristic	Controls		Cases		*p*-value
	Aparasitaemic, *N* = 209	Parasitaemic, *N* = 246	Aparasitaemic, *N* = 90	Parasitaemic, *N* = 50	
Female sex[a,c]	120 (57%)	118 (48%)	44 (49%)	21 (42%)	0.11
Age (years)[b,d]	7 (4, 29)	10 (6, 14)	19 (3, 54)	3 (2, 21)	<0.001
Temperature (°C)[b,d]	36.5 (36.2, 37.0)	36.5 (36.2, 36.8)	38.0 (38.0, 39.0)	38.0 (38.0, 39.8)	<0.001
ERFE (μg/L)[b,d]	2.2 (0.3, 5.9)	2.1 (0.0, 5.9)	5 (2, 11)	8 (3, 13)	<0.001
Hepcidin (μg/L)[b,d]	7 (3, 14)	10 (4, 19)	NA (NA, NA)	NA (NA, NA)	<0.001
Ferritin (μg/L)[b,d]	33 (15, 66)	43 (27, 81)	124 (18, 726)	263 (91, 999)	<0.001
Haemoglobin (g/dL)[b,d]	11 (11, 12)	11 (10, 12)	10 (7, 12)	7 (5, 10)	<0.001
Anaemia[a,c]	110 (53%)	151 (61%)	68 (76%)	45 (90%)	<0.001
MCV (fl)[b,d]	81 (76, 86)	80 (76, 85)	81 (73, 86)	82 (76, 90)	0.4
MCH (pg)[b,d]	26 (25, 28)	26 (24, 28)	26 (23, 28)	26 (24, 30)	0.3
MCHC (g/dL)[b,d]	33 (32, 34)	32 (31, 34)	32 (31, 33)	32 (30, 34)	0.004
Reticulocytes (10^4/μL)[b,d]	6.1 (5.0, 8.4)	7.3 (5.6, 9.0)	4.6 (2.7, 6.8)	7.4 (4.8, 11.8)	<0.001

[a]*n*(%).

[b]Median (IQR).

[c]Pearson's chi-squared test.

[d]Kruskal–Wallis; ERFE, erythroferrone; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NA, not applicable.

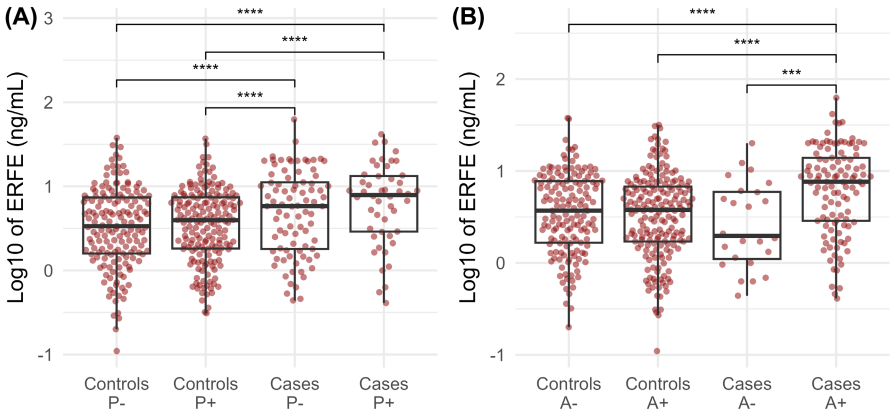

FIGURE 1 Logarithmic erythroferrone levels, ANOVA was applied as a global test to guard against false-positive observations in pair-wise comparisons. (A) Data divided into controls (afebrile) without (P–, $n = 209$) or with (P+, $n = 246$) malaria parasitaemia, and cases (febrile) without (P–, $n = 90$) or with (P+, $n = 50$) malaria parasitaemia (ANOVA, $F(3.468) = 6.793$, $p < 0.001$); (B) data divided into controls without (A–, $n = 194$) or with (A+, $n = 261$) anaemia, and cases without (A–, $n = 27$) or with (A+, $n = 113$) anaemia (ANOVA, $F(3.468) = 11.03$, $p < 0.0001$). Post hoc pair-wise comparisons were performed with Wilcoxon–Mann–Whitney *U*-tests ($p > 0.05$ = non-significant (not indicated), $p < 0.001$ = ***, $p < 0.0001$ = ****).

cohort might be attributable to pro- and anti-inflammatory stimuli beyond parasitaemia. While parasitaemia in the peripheral blood is not the only marker of disease activity in malaria, inflammation due to disease other than malaria might add to the ERFE secretion through a so far unknown mechanism.

According to our data, the ERFE level is increased in human malarial anaemia similarly to the previously described mouse model by Latour et al. While ERFE is one of the inhibitors of hepcidin expression and influences iron absorption, this is a promising finding, since it could possibly help to find suitable timing for therapeutic interventions like iron supplementation. The importance of careful and selective iron therapy has been established in a rodent model.[17] While iron supplementation can ameliorate iron deficiency anaemia and anaemia of inflammation, its success is dependent on the severity of inflammation.[17] Iron is being withheld from circulation and tissues by enterocytes and macrophages as long as hepcidin is ineffectively suppressed. This is pathophysiologically[13] and clinically[14] relevant. Concerning concomitant iron deficiency in malaria patients, ERFE and hepcidin are prospective biomarkers in the diagnostic work-up. However, hepcidin levels were not available in all the case subjects of this study to investigate its

relationship between ERFE in malaria and anaemia further. ERFE concentration in plasma may be a potential biomarker to optimize the timepoint for iron supplementation, that is, to start earlier than in current daily practice, where this therapy is recommended only after cured inflammation.[13] Additionally, such an improved regime could contribute to lowering side-effects such as increased oxidative stress, gastrointestinal distress due to ineffective absorption or exacerbation of underlying condition. As Latour et al. hypothesized, a treatment strategy with controlled iron restriction for symptomatic malaria illness may be beneficial to better overcome the disease. We here corroborated previous findings of the above-mentioned mouse model, that ERFE levels are increased also in humans infected with malaria and suffering from anaemia. Our data should be confirmed in prospective studies before ERFE could serve as a potential decision biomarker to improve therapeutic interventions such as optimal timing of iron supplementations.

AUTHOR CONTRIBUTIONS

PJN performed the statistical analysis, performed the experiments for measuring erythroferrone, wrote the manuscript and, together with BK, collected the data. CTN provided substantial support on statistical analysis and interpretation. ARH and AJV contributed substantial input and took part in the design of the study. CRL and AHL supervised and made substantial contributions to the analysis and reviewed every draft. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocols were approved by the national ethics committee of Burkina Faso (ref 2016-01-006 and 2017-4-036). Written informed consent was obtained from all participants or their parents/legal guardians. Assent was obtained from all participants aged 7–20 years according to the local requirement. Furthermore, the case–control study has been approved by the regional ethics committee for central and northwestern Switzerland (id 2022–00907).

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all participants or their parents/legal guardians. Assent was obtained from all participants aged 7–20 years according to the local requirement.

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REFERENCES

1. Silvestri L, Pettinato M, Furiosi V, Bavuso Volpe L, Nai A, Pagani A. Managing the dual nature of iron to preserve health. International Journal of Molecular Sciences. 2023;24(4):3995.
2. Xu Y, Alfaro-Magallanes VM, Babitt JL. Physiological and pathophysiological mechanisms of hepcidin regulation: clinical implications for iron disorders. British Journal of Haematology. 2021;193(5):882–93.
3. Sandalinas F, Filteau S, Joy EJM, Segovia De La Revilla L, Macdougall A, Hopkins H. Measuring the impact of malaria infection on indicators of iron and vitamin a status: a systematic literature review and meta-analysis. The British Journal of Nutrition. 2023;129(1):87–103.
4. Tiono AB, Konaté AT, Kargougou D, Diarra A, Ouedraogo IN, Ouedraogo A, et al. Hospital-based surveillance of severe paediatric malaria in two malaria transmission ecological zones of Burkina Faso. Malaria Journal. 2023;22(1):6.
5. Roberts SA, Brabin L, Tinto H, Gies S, Diallo S, Brabin B. Seasonal patterns of malaria, genital infection, nutritional and iron status in non-pregnant and pregnant adolescents in Burkina Faso: a secondary analysis of trial data. BMC Public Health. 2021;21(1):1764.
6. Muriuki JM, Mentzer AJ, Mitchell R, Webb EL, Etyang AO, Kyobutungi C, et al. Malaria is a cause of iron deficiency in African children. Nature Medicine. 2021;27(4):653–8.
7. Srole DN, Ganz T. Erythroferrone structure, function, and physiology: iron homeostasis and beyond. Journal of Cellular Physiology. 2021;236(7):4888–901.
8. Camaschella C, Pagani A, Silvestri L, Nai A. The mutual crosstalk between iron and erythropoiesis. International Journal of Hematology. 2022;116(2):182–91.
9. Latour C, Wlodarczyk MF, Jung G, Gineste A, Blanchard N, Ganz T, et al. Erythroferrone contributes to hepcidin repression in a mouse model of malarial anemia. Haematologica. 2017;102(1):60–8.
10. WHO. Centers for Disease Control and Prevention. Assessing the iron status of populations. 2nd ed. Geneva: World Health Organization; 2007. p. 22–3.
11. Pillay E, Khodaiji S, Bezuidenhout BC, Litshie M, Coetzer TL. Evaluation of automated malaria diagnosis using the Sysmex XN-30 analyser in a clinical setting. Malaria Journal. 2019;18(1):15.
12. Post A, Kaboré B, Reuling IJ, Bognini J, Van Der Heijden W, Diallo S, et al. The XN-30 hematology analyzer for rapid sensitive detection of malaria: a diagnostic accuracy study. BMC Medicine. 2019;17(1):103.
13. Lanser L, Fuchs D, Kurz K, Weiss G. Physiology and inflammation driven pathophysiology of iron homeostasis—mechanistic insights into anemia of inflammation and its treatment. Nutrients. 2021;13(11):3732.
14. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. Lancet. 2016;387(10021):907–16.
15. Jonker FAM, Calis JCJ, Phiri K, Kraaijenhagen RJ, Brabin BJ, Faragher B, et al. Low hepcidin levels in severely anemic malawian children with high incidence of infectious diseases and bone marrow iron deficiency. PLoS One. 2013;8(12):e78964.
16. Casals-Pascual C, Huang H, Lakhal-Littleton S, Thezenas ML, Kai O, Newton CRJC, et al. Hepcidin demonstrates a biphasic association with anemia in acute plasmodium falciparum malaria. Haematologica. 2012;97(11):1695–8.

17. de Souza LV, Hoffmann A, Fischer C, Petzer V, Asshoff M, Theurl I, et al. Comparative analysis of oral and intravenous iron therapy in rat models of inflammatory anemia and iron deficiency. Haematologica. 2022;108(1):135–49.

SUPPORTING INFORMATION

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

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