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Procalcitonin, Pentraxine 3, and Molecules of the Vascular Endothelium are Hallmark of Pathogenicity and Predict Coma and Mortality Among Children with Severe and Cerebral Malaria

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Research Article

Keywords: Cerebral Malaria, Biomarkers, sICAM-1, EPCR, PTX3, PCT, suPAR

Posted Date: July 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-734440/v1

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Abstract

Background: Malaria-related deaths could be prevented if prompt diagnosis and prognostic biomarkers are available to allow rapid adequate treatment. Understanding of the mechanisms implicated in the progression from *P. falciparum* asymptomatic and uncomplicated malaria infections to severe life-threatening disease is necessary to identify such indicators.

Methods: Using quantitative ELISA, we assessed the plasma concentrations of Procalcitonin, Pentraxine 3, Ang-2, sTie-2, suPAR, sEPCR, and sICAM-1 in a cohort of 337 Beninese children who presented with pediatric malaria to investigate the potential association with clinical manifestations and outcomes.

Results: The molecules showed higher levels in children with severe or cerebral malaria compared to those with uncomplicated malaria. Plasmas concentrations of PTX3, PCT and the soluble receptors suPAR, sTie-2, sICAM-1 were significantly higher in children with deep coma as defined by a Blantyre Coma Score < 3 (P <0.001 for PTX3, suPAR, sTie-2, p=0.004 for PCT and p=0.005 for sICAM-1). Moreover, the concentrations of PTX3, suPAR and sEPCR were higher among children who died from severe malaria compared to those who survived with (p=0.037, p=0.035, and p=0.002 respectively).

Conclusion: Our findings indicate the ability of these seven bioactive molecules to discriminate among the clinical manifestations of malaria and therefore, given their potential utility as prognostic biomarkers for severe and fatal malaria, they might be useful to improve severe cases management.

Background

Plasmodium falciparum malaria is associated with a global annual mortality of 380, 000 with 94% deaths occurring among African children [1]. The diverse clinical presentations of malaria ranging from mild uncomplicated malaria to severe life-threatening disease reflect distinct underlying pathogenic mechanisms involved in each disease state making often a challenge for the clinicians to provide reliable diagnosis and appropriate treatments [2]. Although, the factors associated with the transition from uncomplicated to severe forms of malaria remain not fully understood, several studies showed immune dysfunction and excessive endothelial activation in acute severe cases [3, 4]. Specifically, the types of cytokines and chemokines produced by the host play a critical role in the progression of uncomplicated malaria towards cerebral malaria and other severe complications. For instance, increased plasma levels of pro-inflammatory Tumor-Necrosis-Factor-alpha (TNF-α), Interferon-gamma (IFN-γ), and Interleukin-1-beta (IL-1β) [4–6], as well as a decreased levels of anti-inflammatory cytokines such as Interleukin 10 (IL-10) and Transforming-growth-factor-beta-1 (TGF-β1) [4, 7, 8], are hallmarks of severe malaria. Furthermore, several cytokines are known to take part in the endothelial dysfunction associated with leucocytes and infected red blood cells sequestration via the induction of Intercellular-Adhesion-Molecule-1 (ICAM-1) and Endothelial-protein-C-receptor (EPCR) expression on the cell surface and also by modulation of their shedding in the blood circulation resulting in an increased level of soluble EPCR and ICAM-1 (sEPCR,sICAM-1) in children admitted to hospital for severe malaria [9–12]. Macrophage/Monocytes mediators including neopterine, the Monokine-induced by IFN-γ (MIG), and other molecules such as the pattern-recognition-glycoprotein-pentraxine (PTX3), and the soluble urokinase-plasminogen-activator-receptor (suPAR), were also found to differentiate between uncomplicated and severe malaria. These results however were obtained on a limited number of malaria conditions missing fatal cases in some studies [13–15]. In addition, angiopoietin 2 (Ang-2) and its tyrosine-kinase-receptor 2 Tie-2) were found to be increased and associated with retinopathies in Malawian children with cerebral malaria. In combination with clinical parameters, Ang-2 improved mortality prediction among these children [16]. The results obtained so far highlights the interest to further investigate the potential of these molecules as biomarkers for accurate prognostic of patients at risk of dying from severe malaria and therefore to help improve case management. In this study, we assessed in a cohort of 337 Beninese children presenting various clinical manifestations of malaria, the level of four soluble receptors (suPAR, sEPCR, sICAM-1, and sTie-2 with its ligand protein Ang-2) and other bioactive molecules including PTX3 and the calcitonin-precursor-hormone procalcitonine (PCT).

Material And Method

Study design and participants

In this cross-sectional study carried out from December 2017 to July 2019, data were collected from children under 6 consulting at hospitals including Centre-Hospitalier-Universitaire Mère-enfant, de la Lagune, Centre-Hospitalier-Universitaire of Suruléré, and Ménontinin Hospital, in Cotonou, Benin, l'Oueme/Plateau Hospital in Porto-Novo, and Ouidah Hospital in Ouidah. Porto-Novo is 41.2 km east of Cotonou, while Ouidah is 39 Km west of Cotonou, both settings share the same climate as Cotonou.

Children were recruited in the study if they presented a positive rapid diagnostic test for *P. falciparum* (DiaQuick-Malaria-*P. falciparum*-Cassette, Dialab; Hondastrasse, Austria) and meet the World Health Organization clinical malaria definition criteria (WHO; 2011). All malaria cases were defined as a microscopically confirmed *P. falciparum* mono infection. Cerebral-malaria-group (CM) had Blantyre Coma Score (BCS) < 3, with the exclusion of any other causes of coma. The severe-non-cerebral-malaria-group (SM) were children presenting with one or more of the following symptoms; pulmonary edema, acute respiratory distress syndrome, acute kidney failure, abnormal liver function, hemoglobinuria, or severe anemia with BCS>2. The uncomplicated-malaria-group (UM) had *P. falciparum* parasitemia infection with fever, without signs of life-threatening malaria and evidence of vital organ dysfunction.

Ethics approval and consent to participate:

Ethical clearance was obtained from the Comité National d'Ethique pour la Recherche en Santé (CNERS), Cotonou, Benin (No 50, 25th October 2017, IRB00006860). For all the participants a signed informed consent was obtained from parents or legal guardians.

Blood and data collection

Up to 4 ml peripheral blood was collected into citrate-phosphate-dextrose-adenine containing tubes from children at admission to the hospital. Clinical, biological and demographic data of patients were captured in a questionnaire and entered in an ad-hoc file for further analysis. All patients were treated according to the guidelines of the Beninese Ministry of Health.

Quantification of plasma bioactive molecules

Plasma samples were assayed in duplicate blindly. Standard ELISA experiments were performed according to the manufacturer's instructions (R&D Systems®, Minneapolis, MN) for PTX3, Ang-2 and soluble receptors. Plasma samples were diluted to ½ for Ang-2, 1/4 for sTie-2, 1/10 for PTX3, suPAR, and sICAM-1, and 1/20 for sEPCR. For PCT quantification, the one-step enzyme-linked fluorescent immunoassay (VIDAS BRAHMS bioMérieux, Lyon, France) was performed using 200µl plasma. In all experiments, analyte concentrations were calculated according to standard curves obtained by the assessment of specific recombinant human proteins which systematically included negative control sera. The final results were expressed as ng/ml.

Statistical analysis

All statistical analysis was conducted with Stata software v15 and GraphPad Prism v9. Continuous variables and categorical variables were compared between the three clinical groups (CM, SM, and UM) using the Kruskal-Wallis test in conjunction with Dunn's multiple comparison post-hoc tests and chi-square test respectively. The levels of analytes were compared between the groups, UM vs SM/CM, survived vs died and deep coma vs no deep coma among severe cases using Mann-Whitney U-test and binary logistic regression. Linear regression was used to examine the relationship between analyte levels and platelets, hemoglobin, leucocytes, glycemia, parasitemia, and age. Significance levels was set at P-value <0.05.

The validity of each molecule as biomarker for malaria related severity, coma and fatality was assessed by Receiver Operating Characteristic (ROC) curve analysis, a two-dimensional measure of classification performance where the area under the ROC curve (AUROCC) accurately measures discrimination, i.e., reflects the power of a quantified parameter to distinguish between two clinical groups. The greater the AUROCC, the better the test is [17].

Results

Cohort description

Blood samples were collected from 337 children aged between 4 and 66 months who arrived at the hospitals for consultation or were admitted to intensive care. Among them, 228 had severe malaria, including 95 with cerebral malaria having deep coma with BCS< 3. Thirty children also had impaired consciousness with BCS=3 accompanied by other symptoms of severe malaria, while 103 children had severe malaria without deep coma and were all ranked in the group of SM. One hundred and nine children suffered from UM. Severe anemia was very common among children and was detected in 39% of children with SM and 41% of those with CM. Thirty-eight children (11.3%) died including 26 (68.42%) among the CM group and 12 (31.57%) among the SM group, 10 of them having consciousness alteration with BCS of 3, and 2 suffered from SM without neurological impairment with BCS of 5 (Table:1).

Table 1

Biological, clinical and parasitological characteristics of children enrolled in the study at admission. Kruskal-Wallis test in conjunction with Dunn's multiple comparison post-hoc tests was used for comparison between the clinical groups.

	Severe Cerebral Malaria (CM) (n=95)	Severe non-Cerebral Malaria (SM) (n=133)	Uncomplicated malaria (UM) (n= 109)	P-Value
Age (months), [IQR]	30 [5-60]	36 [4-60]	36 [5-66]	
Sex ratio (female/male)	43/51	66/62	42/58	
Temperature,median [IQR]	38,7 [36,3-41,4]	38,3 [36,5-41,5]	38,5 [36-40,8]	
Parasitaemia (P/µl), median [IQR]	44000 [240-196875]	65457 [275-349650]	64533,5 [218-992000]	
Haemoglobin (g/dL), median [IQR]	5,2 [2,3-12,9]	1,92 [0,6-12,8]	9,215 [4,8-15,1]	<0,0001
Blantyre coma score, median [IQR]	[0-2]	[3 - 5]	5	
Severe Malaria Anemia (%)	39 (41%)	53 (39,84%)		
Number of deaths	26	12	0	0 ,0005

Relationship between the analytes and other blood components parasitemia and age

In the linear regression analysis, increased levels sTie-2, Ang-2, suPAR and sICAM-1 were associated with reduced level of hemoglobin with a significant p-value of (p<0.001), (p=0.042) (p=0.031), (p=0.004) respectively. Elevated sTie-2 level was also associated with a reduction in platelets count (p=0.025) and

glycemia (p=0.0001). However, elevated levels of Ang-2 and sTie-2 were associated with increased leucocytes count with a significant p <0.001 and p=0.036 respectively. There was no relationship between analytes levels and parasitemia or age (Table:2).

Table 2

Relationship by linear regression model between PTX3, PCT, Ang-2, sTie-2, suPAR, sEPCR, sICAM-1 levels and Hemoglobin, Platelets, Leucocytes, Glycemia, Parasitemia and Age in children at admission.

Molecules	Hemoglobin	p-value		p-value	Leucocytes	p-value	Glycemia	p-value	Parasistaemia	p-value	Age
			Plateletes								
PTX3	0.605(-0.524,1.733)	0,292	-0.003 (-0.041,0.036)	0,894	-0.214 (-0.883, 0.455)	0,523	-0.235 (-0.575,0.106)	0,173	-9.3e-7(-9.8e-6,8.0e-6)	0.836	0.01 0.19
Ang-2	-0.115 (-0.225,-0.004)	0,042	-0.000 (-0.003,0.003)	0,770	0.110 (0.051, 0.170)	<0.001	-0.026 (-0.054,0.002)	0,073	6.0e-7(-1.2e-6, 2.4e-6)	0.516	-0.0 0.02
sTie-2	-0.649 (-0.9391,-0.359)	<0.001	-0.011 (-0.021,0.001)	0,025	0.177 (0.012, 0.342)	0,036	-0.143 (-0.227,-0.059)	0,001	-6.2e-7(-4.8e-6, 3.5e-6)	0.770	-0.0 0.01
suPAR	-0.971 (-1.855,-0.088)	0,031	0.008 (-0.017, 0.032)	0,506	0.940 (-0.203, 2.084)	0,100	-0.172 (-0.394,0.051)	0,120	2.6e-6(-4.5e-6, 9.7e-6)	0.472	-0.0 0.05
PCT			-0.050 (-0.132,0.032)	0,212	0.064 (-2.257, 2.386)	0,954	-0.234 (-1.316,0.848)	0,654	-7.2e-5(-1.9e-4, 4.8e-5)	0.239	-0.4 1.20
sEPCR	3.042 (-3.227,9.310)	0,340	-0.051 (-0.414,0.312)	0,780	0.481 (-4.384, 5.347)	0,845	1.763 (-1.586,5.114)	0,298	-1.1e-5(-9.5e-5, 7.4e-5)	0.807	0.11 1.18
sICAM-1	-20.496 (-34.575, -6.669)	0,004	0.268 (-0.510, 1.046)	0,477	-6.91 (-25.844, 12.428)	0,470	-0.761 (-7.336, 5.815)	0,810	1.4e-4(-1.1e-5, 2.8e-4)	0.069	0.44 2.90

Immune and endothelial activators are associated with malaria related severity, coma and mortality.

Geometric mean comparisons between the clinical groups including UM versus SM or CM and between SM versus CM showed that the levels of all tested analytes were higher in children with SM and CM compared to those with UM, all P< 0.0001 (Figure:1 A-G), while only suPAR, sTie-2, sICAM-1, PTX3, and PCT were increased in children with CM as defined by deep coma (BCS <3) compared to those with BCS>2 among children with SM. Besides, the levels of sEPCR, sICAM-1, sTie-2, Ang-2 and suPAR did not differ between children with deep coma BCS<3 and those with altered consciousness with BCS=3. However, the level of sEPCR and sICAM-1 was higher in children with BCS=3 who died than those who survived with p-value of 0.035 and 0.04 respectively (Figure:2 A-G). Furthermore, the levels of suPAR, sEPCR and PTX3 were higher in children who died from SM or CM than those who survived to these pathologies (Figure:3 A-C).

The logistical regression analyses summarizing the Odds Ratios calculations are presented in Table 3. In these analyses we defined disease severity (SM and CM versus UM), presence versus absence of deep coma and mortality versus survival among severe cases, as outcome variables while the effect described was independent of covariates such as ethnicity, age, parasitemia and sex.

Table 3

Relationship between PTX3, PCT, Ang-2, sTie-2, suPAR, sEPCR, sICAM-1 level and malaria outcomes, Odds Ratios for various outcomes when testing 7 analytes as potential predictors of severity, coma and mortality.

	Severity: CM/SM versus UM		Coma : BCS<3 versus BCS>2 among SM and CM group		Mortality:Survivors versus Dead	
Molecules	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
PTX 3	1.03 (1.01,1.05)	0,002	1.04 (1.03,1.06)	<0.001	1.02 (1.00,1.04)	0,0453
PCT	1.04 (1.02,1.07)	<0.001	1.00 (1.00,1.00)	0,975	-	-
Ang-2	1.51 (1.17,1.94)	0,002	1.05 (0.94,1.19)	0,353	1.08 (0.94,1.23)	0,302
sTie-2	1.21 (1.13,1.30)	<0.001	1.08 (1.03,1.14)	0,001	1.04 (0.97,1.12)	0,314
suPAR	1.06 (1.03,1.09)	<0.001	1.04 (1.02,1.07)	<0.001	1.03 (1.01,1.05)	0,014
sEPCR	1.00 (1.00,1.00)	0,461	1.00 (1.00,1.00)	0,648	1.02 (1.0-1.03)	0,003
sICAM1	1.01 (1.00,1.01)	<0.001	1.00 (1.00,1.01)	0,004	1.0 (1.00,1.01)	0,199

The odds ratios show an increase of 51% for Ang-2 and 21% for sTie-2 in children with CM or SM compared to UM indicating that SM is 1.51 and 1.21 times more likely to occur with a unit increase in Ang-2 and sTie-2. While a slight increase in the odds (between 1.03 and 1.06) for suPAR, PCT, and PTX3 was noticed with a statistically significant difference between the two groups. Except for sICAM-1 for which the increase in the odds was low close to zero but significant, the odds for sTie-2, PTX3, and suPAR levels were 1.09, 1.04, 1.04 respectively times higher in children with BCS<3 compared to those with BCS>2.

In addition, there was 1.03 1.02, 1.03 times increase in the odds of suPAR, PTX3 and sEPCR respectively in fatal cases compared to survivors from CM or SM pathologies which was statistically significant.

Predictive accuracy of analytes to discriminate between the various clinical groups of malaria

The ROC analysis showed by the area under the ROC curve (AUROCC) of [0.83] for PCT, [0.78] for sTie-2, with P<0.001 showing a good diagnosis performance for malaria severity. However, the AUROCC for Ang-2, PTX3, suPAR and sEPCR were lower (Figure:4). For deep coma the highest diagnostic performance was shown by the AUROCC values of [0.78] P<0.001, [0.71] P [0.01-0.001] for sICAM-1 and PTX3 respectively (Figure:5), while the highest diagnostic performance for mortality was obtained for sEPCR with AUROCC [0.76] P<0.001 (Figure:6).

Discussion

Our findings showed the importance of four soluble receptors including sEPCR, sICAM-1, suPAR, and sTie-2 with its ligand Ang-2, and two other molecules PCT, and PTX3, as informative biomarkers of malaria disease severity, coma and mortality.

The plasma levels of sEPCR, sICAM-1, sTie-2 and suPAR were significantly higher in children with CM or SM compared to children with UM using both logistical regression analysis **(Table:3)** and non-parametric tests **(Figures:1,2,3)**. These molecules have also been found to be increased in patients with other infectious or chronical diseases such as sepsis, HIV-1-AIDS, tuberculosis, rheumatoid-arthritis and various cancers with suPAR, sEPCR and PTX3, often predicting poor clinical outcome as in our present study [12, 18-20]. Furthermore, sEPCR, suPAR and PTX3 were associated with both deep coma and fatality.

sEPCR, sTie-2/Ang-2 and sICAM-1 are involved in cytoprotective activity to maintain micro-vessels endothelial barrier functions. The soluble as well as the membranous form of EPCR bind with similar affinity to activated C-reactive-protein (APC) to maintain endothelium integrity through the activation of protease-activator-1 [21, 22] promoting anticoagulation and anti-inflammation. During malaria, EPCR has been found to bind the *P. falciparum* erythrocyte-membrane-protein-1 to the same region as APC does thus, decreasing anticoagulant activity of APC and promoting thrombosis and obstruction of blood circulation [11, 23]. Our data supports the hypothesis that increased sEPCR in severe cases contributes for maintaining APC anticoagulant activity in these patients preventing acute thrombosis.

Similarly, the level of Ang-2 and its receptor sTie-2 was higher in SM or CM groups with sTie-2 highly increased in coma cases suggesting a pathophysiological role during CM. Interestingly, Ang-2 and Ang-2/Ang-1 ratio were shown as independent predictors of metabolic acidosis, coma and mortality [16, 24, 25]. The Ang1/Ang-2-Tie-2 system is a paramount regulator of endothelial integrity with Ang-1 signals through Tie-2 to maintain vascular quiescence. This is antagonized by Ang-2 resulting in endothelial dysfunction [26]. The sTie-2 contains the ligand binding domain that binds angiopoietins and inhibit their interaction with cellular Tie-2. Given the importance of sTie-2 in regulating angiopoietins availability it contributes to vascular pathology when its level is impaired [27]. Therefore, the high production of sTie-2 may thwart the antagonizing effects of Ang-2 which was highly expressed in CM and SM.

Moreover, the enhanced level of Ang-2 and sTie-2 leads to sICAM-1 expression which was significantly higher in children with CM or SM as well as in children with coma **(Figure:1 C, D, E) (Figure:2 B, C)**.

We also found that suPAR level was significantly higher in children with SM or CM compared to those with UM, in children with BCS<3 compared to those with BCS >2 and in children who died from SM or CM making this molecule a potential marker of severity, coma and fatality. Produced by activated monocytes and endothelial cells, suPAR plays several roles in innate immune defence and inflammation. It acts in the recruitment of effector cells (monocytes/macrophages) and clearance of pathogens at infection sites [28]. In addition to the uPA/suPAR binding which triggers the plasminogen activation system, suPAR also binds integrins and other receptors to activate different intracellular signaling pathways implicated in cell proliferation, invasion, angiogenesis, and metastasis.

Therefore, suPAR has been described to be increased in cancers, in coronary heart diseases (CHD) and in infectious diseases including malaria [15]. An immunohistochemistry analysis of CM patients brain showed an accumulation of suPAR in macrophages/microglial cells in Durks granuloma adjacent to petechial haemorrhages, as well as in astrocytes, and in endothelial cells while the expression of suPAR was low in normal brains suggesting the association of suPAR expression with tissue damage of the blood brain barrier during CM [14, 29, 30]. High suPAR level was associated with disease severity and poor prognosis in cancers, CHD and infectious diseases including SARS-CoV-2 [31-34].

We found that PCT came out as the best biomarker for diagnosing malaria severity, with an AUROCC of [0.83]. This result is consistent with previous findings [35-37]. This molecule able to discriminate between viral and bacterial, fungi or parasitic infections is used in guiding antibiotic treatments in patients [38].

Interestingly, PCT was higher in children with BSC<3 than those with BCS>2 (P=0.004) and showed moderate predictive value for coma with an AUROCC of [0.65] which is to our knowledge a novel finding. Of note, PCT in combination with CRP, chitinase-3-like-protein, and S100beta was recently described to be a promising biomarker in determining the presence, location, and extent of traumatic intracranial lesions [39]

The level of PTX3 was higher in children with SM or CM compared to children with UM, in children with BCS<3 compared to those with BCS >2 and in children who died compared to those who survived with significant diagnosis performance for severity, coma and mortality as shown by the significance of the AUROCC analyses **(Figure:4D,5A,6B)**.

PTX3 is involved in the humoral innate immunity by recognizing microbial moieties and damaged tissues and in regulating inflammation and autoimmunity. PTX3 is stored in specific granules in neutrophils and is released in response to microbial recognition and inflammatory signals. Increased levels of plasma PTX3 was associated with the clearance of apoptotic cells by dendritic cells resulting from high immune response and endothelial dysfunction in cardiovascular disorders [40, 41]. PTX3 was investigated as biomarker in infectious diseases and has emerged as strong independent predictor of mortality as recently described in SARS-CoV2 syndrome [42]. However, this study is only the second one that investigated PTX3 as potential biomarker during malaria and revealed the potential of this molecule at distinguishing SM and CM from UM cases, deep comas from mild and conscious cases and fatal cases from patients who survived. This result highlights the unique interest of PTX3 as biomarker to assess the progression of the disease and response to treatment in hospitalized patients.

Nine (75%) of the fatal cases among the SM group were children who had consciousness alteration with BCS=3. In these children the levels of sEPCR, sICAM, sTie-2, Ang-2 and suPAR were similar to those with CM BCS<3. This finding suggests that SM children with (BCS=3) who died are probably children with CM misclassified in the SM group for compliance with the WHO definition of CM conditioned by the BCS <3 criterion. It might also reflect an underestimation of the BCS evaluation in these children by the clinicians.

In addition, sEPCR and sICAM -1 levels were higher in children with BCS = 3 who died than in those who survived emphasizing the interest of sEPCR as marker of mortality from malaria supporting earlier findings **(Figure: 2 F, G)** [12].

The enhanced levels of sTie-2, Ang-2, suPAR and sICAM-1 were significantly associated with reduced hemoglobin with sTie-2 also associated with low platelets count and hypoglycemia and sTie-2, Ang-2, associated with high leucocytes count. These results emphasize a deleterious effect of these analytes during severe malaria as the fall of hemoglobin, platelets count and glycemia are hallmarks of SM and CM pathologies [43].

Beyond the observed association between the elevated levels of the analytes and SM and CM manifestations, these molecules might be implicated in the processes that control blood parameters and deserve to be fully investigated.

Conclusion

Our findings show that in Beninese children with malaria suPAR, sICAM-1, sEPCR, sTie2, Ang-2, PCT, and PTX3 differentiate children with SM and CM from children with UM, with PCT and sTie-2 providing the highest diagnosis performance for severity. sICAM-1, sTie-2, suPAR and PTX3 were higher in children with BCS<3 with PTX3 and sICAM-1 presenting the best diagnosis performance for coma. In addition, sEPCR, PTX3 and suPAR levels discriminate between children who died from SM and CM and those who survived to these pathologies with the highest predictive value for sEPCR.

Overall, the expression of these molecules during malaria, either at the onset triggering the inflammatory response like suPAR and PTX3 or resulting from an acute inflammatory response like sICAM-1 and sEPCR, contributes to pathogenesis of SM in which the site of initiation of this response and its intensity play a major role. Therefore, further researches on the mechanisms of action of these molecules are needed to better understand their role in the pathogenesis of SM and CM and explore their potential in therapeutics.

Declarations

Authors' contributions

RT, NN and CR designed the study and got the funding for its accomplishment, RA, AM, contributed to the sample collection and preparation, MJA, AA and CP were responsible of patient's enrollment in the study and patient's treatment, BT, LR, PT, LD, BA, HL and SOB carried out all the lab experiments, BAM performed the statistical analysis, SP facilitated PCT assessment. BT, RT, wrote the manuscript. All the authors contributed, read corrected and approved the submitted version of the manuscript.

Acknowledgements

The authors gratefully acknowledge all children and their parents for participating in this study. We are grateful to the pediatricians and nurses in CHUMEL, CHU of Suru-Léré, l'Oueme/Plateau in Porto-Novo, Ménontin, and Ouidah Hospitals for their support at sample collection.

Funding:

This work was supported by the Merieux Institut Research Grant (MRG) from Institut Mérieux at Lyon France; The Institut de Recherche pour le Développement (IRD) at Marseille France, the SCAC – Cultural and Cooperation Department - French Embassy in Ghana, and The Danish International Development Agency (*DANIDA*).

Competing interests:

All the authors declare no competing interest

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Figures

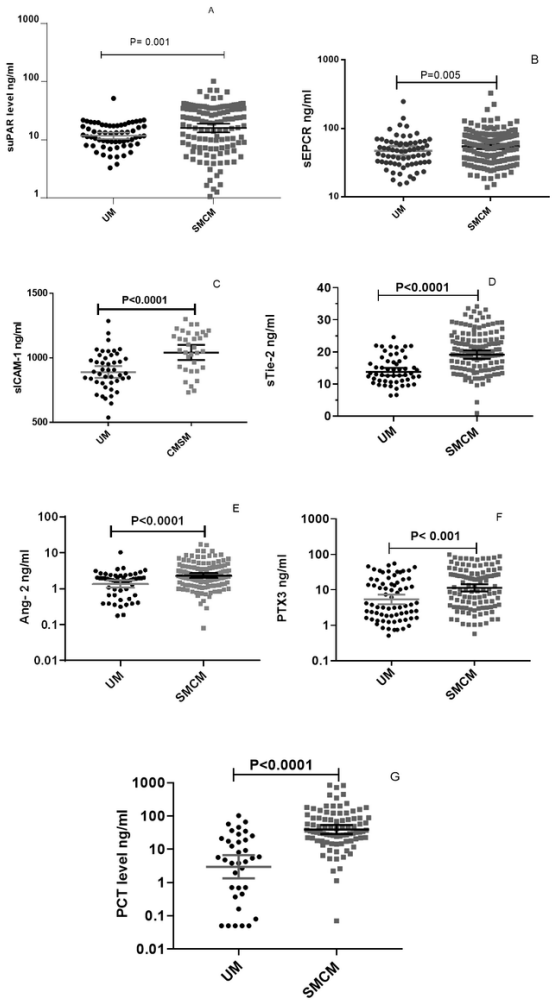


Figure 1

(A-G). The levels of suPAR, sEPCR, sICAM-1, sTie-2, Ang-2, PTX3 and PCT in children with uncomplicated malaria (UM) (black dots) and severe or cerebral malaria (SMCM) (grey squares) at day of admission to hospital as measured by ELISA. Data are presented as dot plots with geometric mean and (95% CI). The Mann Whitney U test was performed for each comparison.

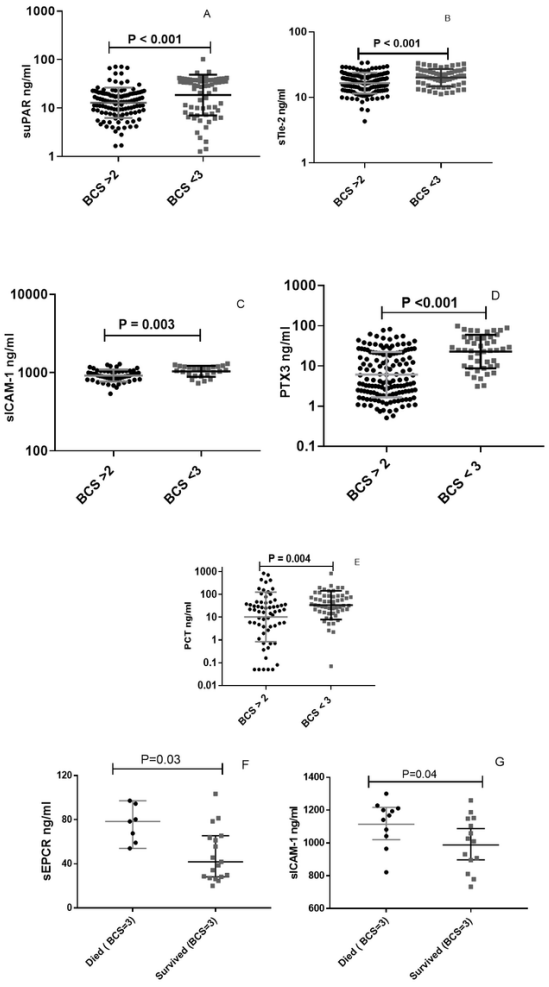


Figure 2

(A-G) The levels of suPAR, sTie-2, sICAM-1 and PTX3 in children with deep coma BCS<3 (grey squares) compared to those with BCS>2 (black dots). (F-G) shows the levels of sEPCR and sICAM-1 in children with BCS=3. Black dots are children who died and grey squares are children who survived. The molecules were measured by ELISA at day of admission to the hospital. Data are presented as dot plots with geometric mean and (95% CI). The Mann Whitney U test was performed for each comparison.

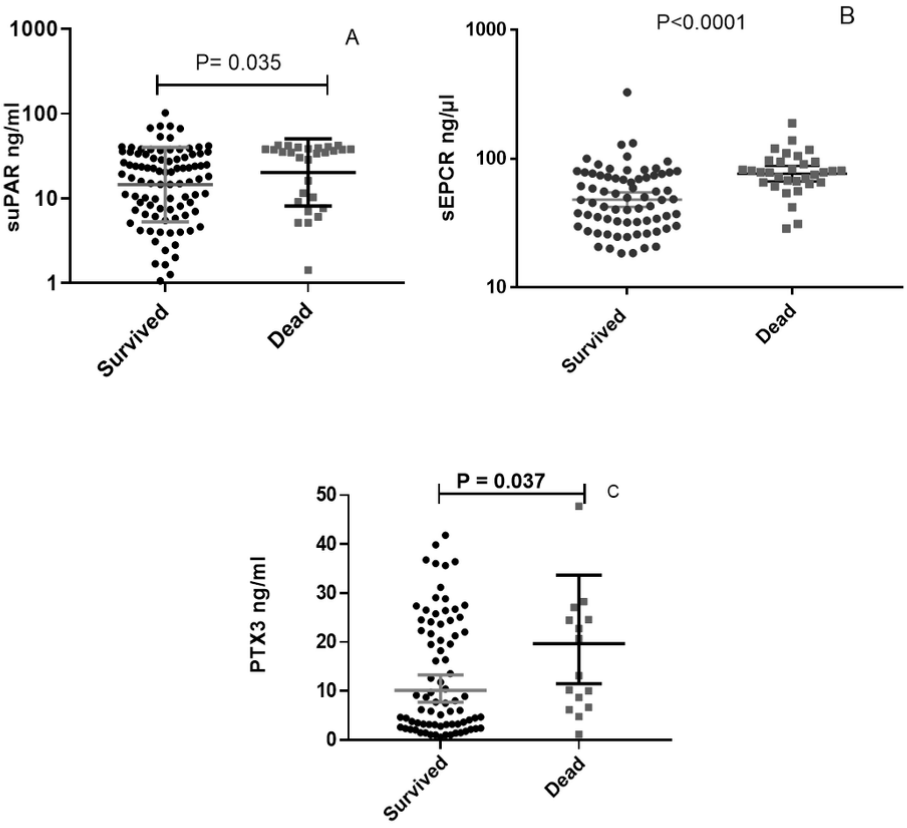


Figure 3

(A-C) The level of suPAR, sEPCR, and PTX3 in children who survived to severe or cerebral malaria (black dots) compared to those who died (grey squares) at day of admission to hospital as measured by ELISA. Data are presented as dot plots with geometric mean and (95% CI). The Mann Whitney U test was performed for each comparison.

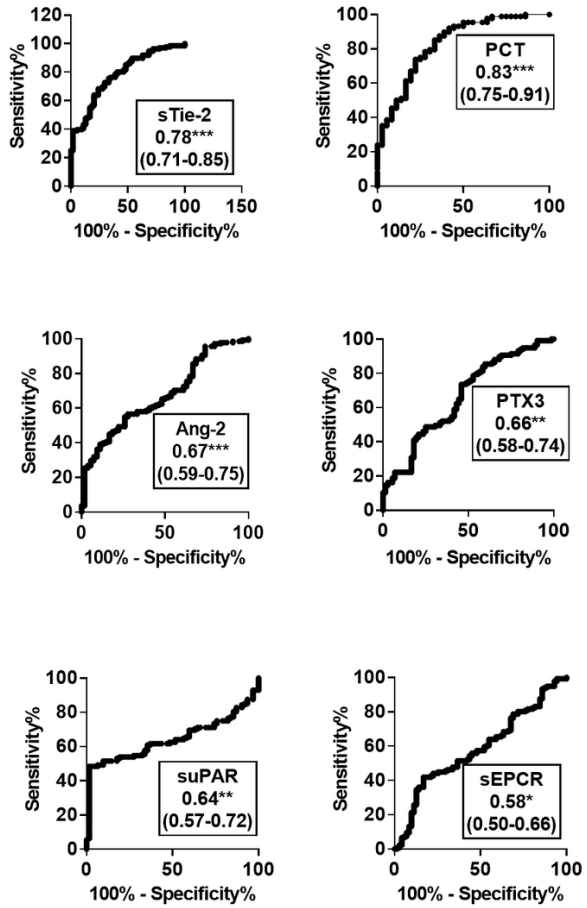


Figure 4

Assessment by ROC curve analyses of the individual prediction performance of sTie-2, PCT, Ang-2, PTX3, suPAR, sEPCR to differentiate for severe cases on the day of admission to hospital. The area under the curve as well as the confidence intervals are indicated in the legend. * indicates 0.01<p value <0.05, **0.001< p value<0.01, *** indicates p value ≤0.001.

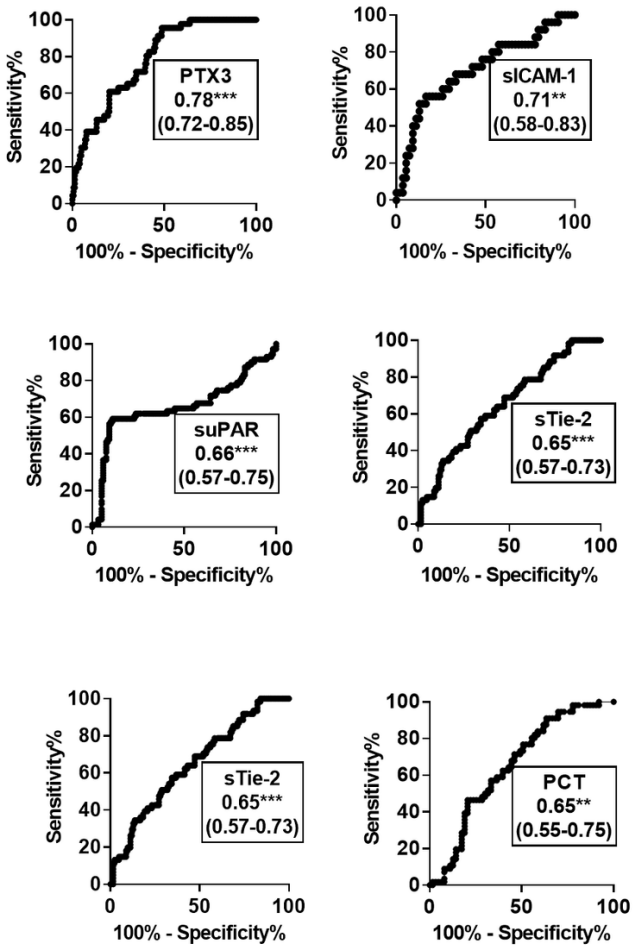


Figure 5

Assessment by ROC curve analyses of the individual prediction performance of PTX3, sICAM-1, suPAR, sTie-2, PCT to differentiate for deep coma cases on the day of admission to hospital. The area under the curve as well as the confidence intervals are indicated in the legend. * indicates 0.01<p value <0.05, **0.001< p value<0.01, *** indicates p value ≤0.001

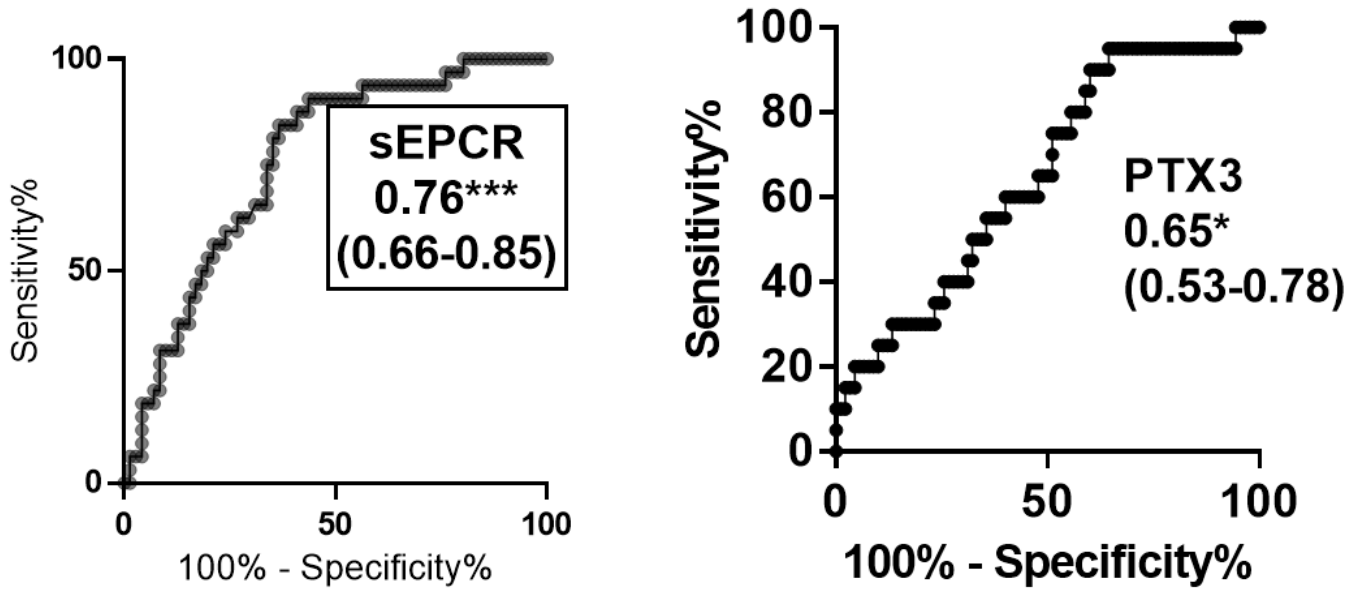


Figure 6

Assessment by ROC curve analyses of the individual prediction performance of sEPCR, PTX3, suPAR and sTie-2 to differentiate for fatal cases on the day of admission to hospital. The area under the curve as well as the confidence intervals are indicated in the legend. * indicates 0.01<p value <0.05, **0.001< p value<0.01, *** indicates p value ≤0.001.