

[P30]

Interest of procalcitonin measurement in children with cerebral malaria in southern Benin

Gilles Bognon, Elsa Topanou, Caroline padonou, Florence

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Interest of Procalcitonin Measurement in Children with Cerebral Malaria in Southern Benin

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Abstract

Introduction: Cerebral malaria is a major complication of the *Plasmodium falciparum* infection with a high case fatality rate. The objective of this study was to determine the relationship between cerebral malaria and high serum procalcitonin (PCT) level in children. **Method:** This was a prospective descriptive and analytical cohort study conducted over 12 months, on a series of PCT blood tests in children aged 6 months to 15 years old hospitalized for cerebral malaria in the pediatric wards of four hospitals in southern Benin. The cerebral malaria diagnosis was done based on WHO criteria. Blood samples for PCT measurement were collected on admission, 24 hours and 48 hours after the malaria therapy initiation. Student's test, Pearson's chi² test, Fisher's test and Kruskal-Wallis test were used where appropriate. For all comparisons the difference was significant when p was less than 5%. **Results:** Sixty-five children were included in the study with a sex ratio of 1.41. The age group of children under 5 years was the most represented, at 57%. PCT levels were high in 92.3% of children at admission, 90.8% at 24 hours and 84.6% at 48 hours. Forty-nine children had a positive clinical outcome while 16 died (24.6%). PCT levels were generally high over the three days of hospitalization, but higher at admission in case of death (p = 0.000). The association between PCT level and parasitemia at admission was significant (p = 0.04). **Conclusion:** In the view of the results, blood PCT level measured at admission could be predictive of the disease outcome in children with cerebral malaria.

Keywords

Procalcitonin Cerebral Malaria, Cerebral Malaria Case Fatality Rate

1. Introduction

Cerebral malaria is a major complication of the *Plasmodium falciparum* infection. In 2018 according to WHO, 24 million children were infected with *Plasmodium falciparum* in sub-Saharan Africa, where cerebral malaria accounted in average for 10% of severe malaria cases with 3,000 children deaths occurring daily, despite the existence of a well-coded treatment [1] [2]. In a study conducted in Benin in 2012, cerebral malaria cases represented 12.2% of the 653 children with severe malaria, with a case fatality rate of 14.5% [3]. It is defined by a positive thick blood smear associated with altered consciousness or extreme weakness [4]. Despite the implementation of WHO recommendations on severe malaria management, the case fatality rate for cerebral malaria remains high. One of the most important challenges in the management of cerebral malaria is to identify markers that predict complications or death in sick children requiring intensive care [5]. Procalcitonin is thought to be an important clinical marker of septicemia and systemic inflammatory responses due to pathogens such as bacteria, fungi and parasites including *Plasmodium falciparum* [6] [7]. Recent studies showed that procalcitonin levels also correlate with parasitemia and malaria severity in adults and children [7] [8] [9]. The search for a reliable and accessible predictive tool of the course of cerebral malaria motivated this study with the aim of reducing deaths in affected children.

2. Method

Study type and inclusion criteria

This was a prospective descriptive and analytical cohort study conducted over a 12-months period in the pediatric wards of the National Teaching Hospital HKM of Cotonou, the Zone Hospital of Abomey-Calavi and Sô-Ava, the Saint-Luc Hospital of Cotonou, and the Departmental Teaching Hospital of Ouémé-Plateau in Porto-Novo. The study focused on the determination of PCT in children aged 6 months to 15 years old hospitalized for cerebral malaria in these hospitals. The diagnosis of cerebral malaria was made on the basis of a positive thick or thin blood smear for *Plasmodium falciparum*, associated with a deep coma with a Blantyre score less than or equal to 2 for small children and a modified Glasgow score less than or equal to 9 for children over 5 years old, and/or associated with convulsions occurring at least twice a day, and/or associated with prostration [4].

Laboratory procedures

The blood samples for the thick blood smear and for the PCT level measurement were collected at admission (Do), at 24 hours (D1), and at 48 hours (D2) after the initiation of the malaria therapy in all included children. The diluted Giemsa staining technique was used for the realization of the thick blood smears in these four hospitals. The slides were proofread at the reference central laboratory of the National Teaching Hospital Hubert K Maga in Cotonou by an experienced technician. The PCT level measurement was performed on the Mindray

CL-1000i analyzer at the multidisciplinary laboratory of Mahouna Clinic in Cotonou. The principle consisted of performing the PCT level measurement on a Mindray CL series device; it is two-step immunoenzymatic assay to determine the PCT level. In the first step, the sample, some paramagnetic microparticles coated with anti-procalcitonin (mouse) monoclonal antibodies, and an anti-procalcitonin (mouse) monoclonal antibody-alkaline phosphatase conjugate were added to a reaction vessel. After incubation, the PCT in the sample bound to both the microparticles coated with anti-procalcitonin antibodies, and to the anti-procalcitonin antibody-alkaline phosphatase conjugate, to form a sandwich complex. The microparticles were magnetically captured, while other unbound substances were removed by washing. In the second step, the substrate solution was added to the reaction vessel. It was catalyzed by the anti-procalcitonin antibody (mouse)-alkaline phosphatase conjugate in the immune complex retained on the particles. The resulting chemiluminescence reaction was measured in relative light units (RLU) by a photomultiplier inside the system. The amount of PCT present in the sample was proportional to the relative light units (RLU) produced during the reaction and was determined using a calibration curve. The normal value ranged between 0.5 and 2 ng/mL. The children included in the study also had a full blood count performed by an XN 550 analyzer using a combination of flow cytometry and light diffraction.

Variables and analysis

The management of the included children was done according to the department protocol for the management of severe malaria drawn from the national protocol which is in turn adapted from the WHO 2015 recommendations for the management of severe malaria [10]. The variables studied were sociodemographic characteristics (age, sex, parents' socioeconomic level, use of long-lasting treated mosquito nets), treatment before admission, physical examination data (vitals, anthropometric parameters, severity signs, neurological status, hemodynamic status), paraclinical examination data (full blood count, thick blood smear, serum PCT level), treatment (oxygen, anti-malarial drugs, antipyretic, nursing), and outcome data (duration of hospitalization, complications, sequelae, and death). Data were coded, entered and analyzed using CSPRO® and SPSS 22 software. Quantitative variables were expressed as mean with standard deviation, while qualitative variables were expressed as frequency. Means were compared with Student's t test and frequencies with Pearson's χ^2 or Fisher's exact tests as appropriate. The nonparametric Kruskal-Wallis test was used to compare the distribution of small samples. A multivariate analysis according to the logistic regression model by stepwise iterations was used by introducing in the model all the variables, whose p-value in univariate analysis is lower than or equal to 20%. For all comparisons, the difference was considered significant for a p-value lower than 5%. The approval of the ethics committee of the Faculty of Health Sciences of Cotonou was obtained under the number: 01319/UAC/FSS/CER.SS. The study was carried out in accordance with good clinical practices with strict respect for the anonymity and confidentiality of the information collected. Writ-

ten informed consent was obtained from the parents before the children were included.

3. Results

Socio-demographic characteristics

Sixty-five children were included in the study. The mean age of the children was 6 years with a standard deviation of 4.04. The majority were younger than 5 years (37/65; 57%). The sex ratio was 1.4.

Clinical, biological characteristics and outcome

On admission, the main clinical signs were fever (55/65; 84.6%), convulsion (51/65; 78.5%), coma (48/65; 73.5%) and prostration (44/65; 67.7%). Overall, the minimum PCT level was 0, 13 ng/mL and the maximum level was 100 ng/mL. At D0, the PCT ranged from 0.13 to 100 ng/mL with a peak between 11 and 29 ng/mL. The level was greater than 2 ng/mL at D0 in 60 children (92.3%), at D1 in 50 children (90.8%) and at D2 in 41 children (84.6%). The PCT levels distribution at D0, D1 and D2 is shown in **Figure 1**.

The children's hemoglobin levels ranged from 2 g/dL to 14.7 g/dL with a mean of 7.3 g/dL and a standard deviation of 2.8. Parasitemia ranged from 48 parasites/ μ L to 412,470 parasites/ μ L of blood with a median value of 9043 p/ μ L. The evolution was marked by a complete recovery in 46 children (70.8%); three children (4.6%) recovered with sequelae such as deafness and hearing disorders. Almost a quarter of the children died (16/65; 24.6%).

Associated factors

Parasitemia ranged from 48 p/ μ L to 412,470 p/ μ L (median 9043 p/ μ L) in children who recovered, from 700 p/ μ L to 8042 p/ μ L (median 4540 p/ μ L) in children who recovered with sequelae, and from 180 p/ μ L to 341,105 p/ μ L (median 46,976 p/ μ L) in deceased children. Comparison of the medians in the three groups showed a statistically significant difference ($p = 0.040$). At D0, the PCT

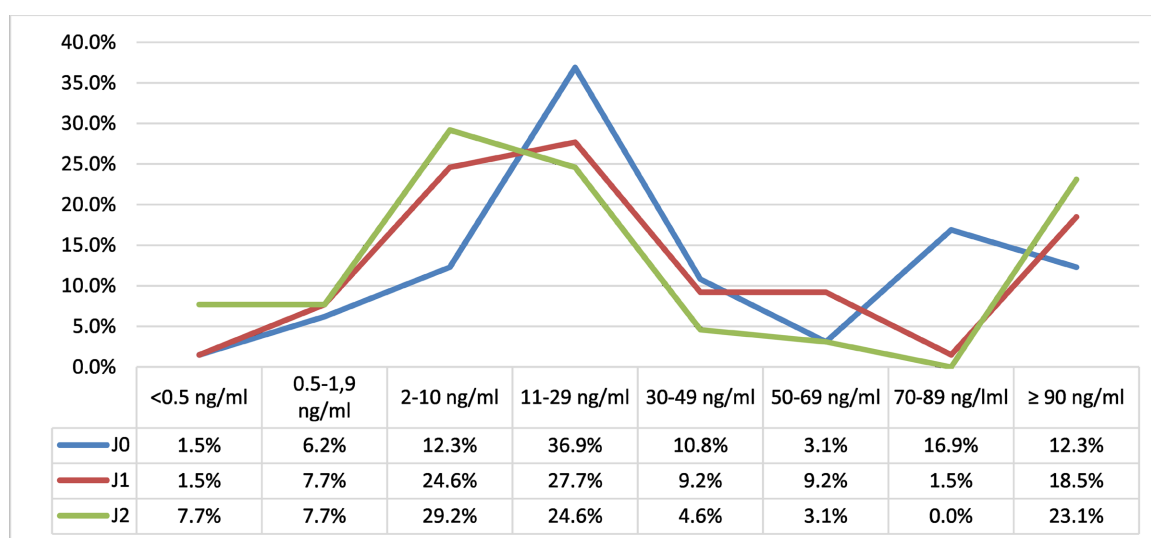


Figure 1. Distribution of procalcitonin levels over 72 hours.

level was greater than 2 ng/mL in all deceased children compared with 82.6% and 66.7% in children who completely recovered and those who recovered with sequelae, respectively. The median PCT level in the group of children who recovered was around 20 ng/ml compared to 80 ng/ml in the deceased group ($p = 0.001$) (Figure 2).

There was no correlation between parasitemia at D0, procalcitonin at D0 and the child clinical outcome at discharge.

4. Discussion

Sixty-five children with cerebral malaria were included in this study. More than two out of five children were between six months and five years old. Severe manifestations of malaria, including cerebral malaria, mostly affect and take a heavy toll on children under 5 years old [7] [11]. The sex ratio was equal to 1.4 as reported in a study in India [11]. The frequent clinical manifestations found in these children were hyperthermia, convulsion, coma and prostration (extreme weakness) as a consequence of the massive and simultaneous release of merozoites associated with the destruction of parasitized red blood cells, which contribute to worsen the cerebral lesions due to severe malaria, the manifestations of which are convulsion, coma and prostration. These neurological signs are due to anoxia related to the obstruction of cerebral capillaries by parasitized red blood cells. Like other authors, the quantitative method used to determine PCT levels would limit all sources of interference with PCT measurement [5] [8]. Procalcitonin levels measured at D0 ranged from 0.13 to 100 ng/mL with a peak between 11 and 29 ng/mL. It was high (≥ 2 ng/mL) in more than 9 out of 10 children with cerebral malaria. PCT could be a diagnostic tool to identify patients with or at risk for neurological disorders during a severe malaria. A study in Italy showed that PCT could be used to predict severe malaria; the results noted that children

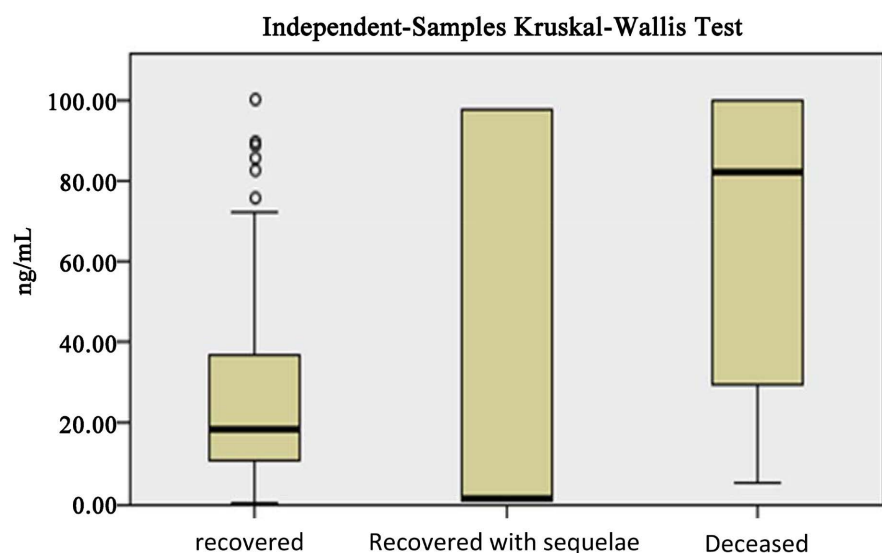


Figure 2. Distribution according to procalcitonin level at D0 and the child clinical outcome at discharge.

with severe *Plasmodium falciparum* malaria had higher parasitemia ($p = 0.0001$) and PCT ($p = 0.0018$) [5]. Another study from Germany showed that PCT levels were also higher than 2ng/ml in all patients with severe malaria [12]. Parasitemia at admission ranged from 48 p/μL to 412,470 p/μL with no correlation between parasitemia and PCT level at D0. Nevertheless, the analysis of the PCT levels variation in children under treatment revealed a decrease in the number of children with a PCT level greater than or equal to 2 ng/mL at D0 compared with D2. The existence of a correlation between high PCT level and parasitaemia was shown in a study where it was observed that the lower the parasitemia during treatment, the more the PCT level decreased till cancellation [13]. A study comparing PCT levels in patients who recovered and in those who died during the course of the disease noted a statistically significant difference in PCT values between the populations of those two groups [8]. In the present study, 92.3% of the children had a high PCT level at D0; this level decreased globally between D0 and D2 in parallel with the disappearance of clinical signs in the children who recovered with a significant difference ($p = 0.000$), which indicated that the elevated PCT levels were correlated with the severity of the disease as found in two other studies [9] [12]. In this study, we recorded 16 deaths, 14 of which occurred between D0 and D2, and two after D2. Of the 14 deaths, 12 had a PCT level higher than 25 ng/mL. These observations were made in a study in which six of the seven deaths had a PCT value higher than 25 ng/mL on D0 [12]. An increase in the PCT value could be thought to be predictive of death in case of malaria infection. A correlation has also been noted between the PCT level at D0 and the child's clinical condition at discharge: the lower the PCT level, the greater the improvement in the clinical condition of the children and the greater the probability of recovery. This means that PCT can be used as a predictive factor for the severity and the outcome of malaria. Two authors had made the same observation [8] [12].

Furthermore, sonographic measurement of optic nerve sheath diameter could be a leading prognostic tool in childhood cerebral malaria management [14].

In the present series, we did not observe any correlation between the hemoglobin level and the PCT level, between the parasitemia and the PCT levels at admission, neither in children who recovered nor in deceased children. Some authors have found the same results, but others have found the opposite, noting a correlation between parasitemia and admission PCT in their study population [8] [9]. In addition, PCT levels remained above 2 ng/mL at D2 despite recovery in 82.6% of the children. As we did not perform assays beyond the 2nd day, it is difficult to say whether the post-cure PCT level at day 2 remained constant, decreased or increased. It would then be interesting to undertake other studies in which the PCT kinetics in malaria would be studied over several days in order to have a better idea of the evolution of PCT during cerebral malaria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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