Short Report: Prevalence and Implications of Cerebrospinal Fluid Leukocytosis in Papua New Guinean Children Hospitalized with Severe Malaria

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Abstract. Cerebrospinal fluid (CSF) leukocytosis in severe malaria was assessed in 87 children in Papua New Guinea participating in a detailed longitudinal observational study who had undergone lumbar puncture for further investigation of altered consciousness and/or convulsions. After rigorous exclusion of non-malarial infection, 16 (20.5%) of 78 children with *Plasmodium falciparum* mono-infection but 0 of 9 with *P. vivax*/mixed-species malaria had a detectable CSF leukocytosis, which was unrelated to prior, including complex, seizures. There were eight children with a CSF leukocyte density > 10 cells/ μ L (9.2% of the total sample), half of whom had cerebral malaria (4 of 22, 18.1%). Cerebrospinal fluid leukocytosis is infrequent in severe pediatric malaria, especially in children with *P. vivax* infections, and it is generally mild. Its presence in a blood slide-positive child should prompt consideration of alternative diagnoses and empiric antibiotic therapy.

Studies reporting cerebrospinal fluid (CSF) leukocytosis in cases of pediatric cerebral malaria have been conducted mainly in sub-Saharan Africa where *Plasmodium falciparum* mono-infections predominate. Approximately 10% of children with cerebral malaria and no bacteriologic evidence of acute bacterial meningitis have CSF pleocytosis of > 10 cells/ μ L in this setting.¹ *Plasmodium vivax* is increasingly recognized as a cause of severe malarial illness in Oceania and parts of Asia and South America.

There is limited evidence that CSF leukocytosis can also be found in patients with *P. vivax* malaria and altered consciousness,^{2,3} but these studies did not rigorously exclude co-infections with bacterial and, as in studies in Africa of cerebral malaria caused by *P. falciparum*,¹ viral, or fungal pathogens. Febrile seizures caused by non-malarial infections may also cause CSF leukocytosis in some children⁴ and are a common feature of pediatric severe malaria, thus further exacerbating diagnostic uncertainties when a severely ill child seeks treatment in a malaria-endemic setting. Therefore, there is a need for a prospective study that determines whether severe pediatric malaria caused by *P. falciparum* or *P. vivax* can cause CSF leukocytosis after other infective causes of encephalopathy have been excluded and after taking prior febrile seizures into account.

We studied hospitalized children in Papua New Guinea who were enrolled in a detailed observational study of severe pediatric infections conducted in coastal Madang Province where there is transmission of multiple *Plasmodium* species. The study was approved by the Papua New Guinea Institute of Medical Research Institutional Review Board and the Medical Research Advisory Committee of Papua New Guinea (MRAC 10.08), and parental written consent was obtained before recruitment in all cases. To rule out acute bacterial meningitis in children in Papua New Guinea, routine lumbar puncture is usually performed if a child has impaired consciousness or after febrile seizures but has no clinical evidence of increased intracranial pressure.⁵ Cerebrospinal fluid leukocytes at presentation were quantified by microscopic exami-

nation using the Neubauer improved chamber (BoeCo, Hamburg, Germany). When erythrocytes were present, an adjusted leukocyte count calculated as leukocytes – [erythrocytes/100] was used⁵ (Table 1). Semi-quantitative CSF glucose and protein levels were obtained by using a dipstick method (Acon Laboratories, San Diego, CA).

After excluding children who did not have an admission lumbar puncture and those with a primary or co-incident non-malarial illness caused by locally prevalent bacterial, viral, or fungal pathogens as confirmed by blood/CSF culture, and/or specific antigen, serologic, and/or polymerase chain reaction testing, as well as Indian ink staining,^{5–7} children with blood slide-positive malaria confirmed by polymerase chain reaction were assigned to one of three sub-groups: 1) cerebral malaria (Blantyre coma score ≤ 2 ⁸ and > 1,000 *P. falciparum* or > 250 *P. vivax* asexual parasites/ μ L); 2) malaria with cerebral involvement (parasitemia as in sub-group 1 but a Blantyre coma score of 3 or 4 and/or complex febrile seizures); and 3) malaria admissions (any parasite density and a normal level of consciousness with or without other signs of severity,⁸ including single convulsions). Complex seizures were defined as multiple (≥ 2 episodes), prolonged (≥ 15 minutes), or focal (unilateral).

Over a 30-month recruitment period, 638 children were enrolled and 87 (13.6%) had confirmed malaria without co-infection, as well as CSF examination. Of this latter group, nine had *P. vivax* or mixed *Plasmodium* species infections and 78 had *P. falciparum* mono-infection. Twenty-two had cerebral malaria, 43 had malaria with cerebral involvement, and 22 had malaria admissions (Table 1). The three children with *P. vivax* mono-infection had multiple convulsions. Of the six children with mixed-species infections, one was in a deep coma after multiple convulsions, one had two convulsions before admission, two had a single prolonged convulsion, one had a single short convulsion and associated severe anemia (hemoglobin level = 48 g/L), and one was admitted because of severe anemia (hemoglobin level 39 g/L). All 87 children were treated according to Papua New Guinea national treatment guidelines and none of the children died.

There were 16 children with leukocytes detected in CSF and this was pleocytosis in 12 (75%). None of the nine children with either a *P. vivax* or mixed-species malarial infection had any CSF leukocytosis compared with 16 of the 78 with *P. falciparum* mono-infection ($P = 0.20$, by Fisher's exact test).

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TABLE 1
Clinical and laboratory characteristics of children in sub-groups of rigorously defined severe malaria, Papua New Guinea*

Characteristic	Cerebral malaria (n = 22)	Malaria with cerebral involvement (n = 43)	Malaria admissions (n = 22)	P
Male sex	59	63	52	0.71
Age (months)	39.5 (23–60)	35 (27.2–48)	31 (18.9–44.3)	0.73
Axillary temperature (°C)	38.4 (37.4–39)	37.9 (37.8–38.1)	37.9 (37–38.5)	0.55
Pre-hospital antipyretic use	59	54	65	0.049
<i>Plasmodium falciparum</i> / <i>P. vivax</i> / mixed-species malaria	21/0/1	37/3/3	20/0/2	–
Neurologic manifestations				
Cerebral malaria	100	0	0	< 0.001
Impaired consciousness	0	44	0	< 0.001
Multiple seizures	36	61	0	< 0.001
Prolonged seizures	14	19	0	0.32
Focal seizures	0	5	0	0.35
CSF leukocyte count/ μ L	0 (0–0.35)	0 (0–0)	0 (0–0)	0.26
0	72.7	81.4	91.3	–
< 5	0	0	0	–
5–9	9.1	14	0	–
10–20	13.6	4.6	8.7	–
> 20	4.6	0	0	–
CSF protein level $\geq$ 1 g/L	9	0	9	0.51
CSF glucose level < 5 mmol/L	0	0	0	–
Deaths	0	0	0	–

* Values are medians (interquartile ranges) or percentages. CSF = cerebrospinal fluid.

There were eight children with a CSF leukocyte density > 10 cells/ μ L (9.2% of the total sample), half of whom were in the cerebral malaria group (4 of 22, 18.1%). There was no significant difference in total CSF leukocyte counts in children with multiple seizures compared with those without seizures ($P = 0.60$, by Mann-Whitney U test).

The present data show that children in Papua New Guinea who are hospitalized with severe *P. falciparum* malaria and have indications for lumbar puncture can have a CSF leukocytosis that is not explained by co-incident central nervous system infection or febrile convulsions. The percentage of the cerebral malaria patients (all but one with *P. falciparum* mono-infection) with a CSF leukocyte density > 10 cells/ μ L (18.1%) was, given limited number of patients in this group, broadly consistent with that in a larger study in Africa of children of similar ages with cerebral malaria (9.2%).¹ Cerebral microvascular sequestration of parasitized erythrocytes containing mature forms of *P. falciparum* may promote a low-grade local inflammatory response,⁹ which promotes CSF leukocytosis. Consistent with this hypothesis, there is no evidence that *P. vivax* sequesters in the brain of patients with severe *P. vivax* or mixed-species malarial infections and altered consciousness,¹⁰ and a lack of sequestration-associated inflammation could explain why all such patients in the present series had no CSF leukocytosis. In addition, all but one of these patients had at least one convulsion, and we could not find any association between multiple seizures and CSF leukocytosis in the series as a whole. These observations are consistent with those of a recent systematic review, which showed that CSF pleocytosis occurs in < 6% of children with a febrile convulsion,⁴ and which found insufficient data to support the notion that complex and/or prolonged convulsions cause higher rates of CSF pleocytosis than simple febrile convulsions.

The present study had limitations. The group with severe *P. vivax* or mixed-species infections was small, consistent with a low incidence of complications relative to patients with *P. falciparum* malaria.¹¹ However, even in areas with hyper-endemic transmission of *P. vivax*, large-scale studies may not

provide an adequate sample size.² We did not have detailed data relating to the interval between seizures and when the lumbar puncture was performed, a potentially important variable in interpretation of the relationship between seizure activity and CSF pleocytosis.⁴ Nevertheless, the lack of a relationship between complex seizures and CSF leukocytosis in the present study suggests that even when a lumbar puncture is performed in the immediate post-ictal period, there is unlikely to be an increased likelihood of CSF pleocytosis.

The present data have several important clinical implications in geoepidemiologic situations similar to coastal Papua New Guinea. First, most pediatric patients with cerebral malaria associated with *P. falciparum*, and an even greater majority, if not all, of those with altered consciousness caused by severe *P. vivax* infections, have no leukocytes in the CSF. In resource-poor settings such as Papua New Guinea, where blood and CSF cultures, as well as facilities to enable appropriate clinical and laboratory monitoring may not be available, the presence of CSF pleocytosis should prompt consideration of diagnoses other than severe malaria and empiric antimicrobial therapy in addition to antimalarial therapy. Depending on the clinical situation,¹² there may even be a case for withholding such empiric therapy in children with malaria parasites on a peripheral blood smear or a positive rapid diagnostic test result and no CSF leukocytes, and monitoring the response to antimalarial therapy alone. Second, an increased CSF leukocyte density should not be attributed to seizures, whether caused by fever or malaria, even if they are complex. Last, lumbar puncture remains a safe and important diagnostic tool in the evaluation of altered consciousness in a severely ill child in even basic healthcare settings.

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