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Quantification of *Plasmodium falciparum* Histidine-Rich Protein-2 in Cerebrospinal Spinal Fluid from Cerebral Malaria Patients

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Abstract. A cerebrospinal fluid (CSF) biomarker for cerebral malaria (CM) has not been validated. We examined the detection, semiquantification, and clinical use of the *Plasmodium falciparum* histidine-rich protein-2 (PfHRP-2) as a parasite antigen biomarker for CM. The PfHRP-2 was detected in archival CSF samples from CM patients from Tanzania both by a newly developed sensitive and specific immuno-polymerase chain reaction (72 of 73) and by rapid diagnostic tests (62 of 73). The geometric mean PfHRP-2 CSF concentration was 8.76 ng/mL with no differences in those who survived (9.2 ng/mL), those who died (11.1 ng/mL), and those with neurologic sequelae (10.8 ng/mL). All aparasitemic endemic and nonendemic control samples had undetectable CSF PfHRP-2. In a separate group of 11 matched plasma and CSF cerebral malaria patient samples, the ratio of plasma to CSF PfHRP-2 was 175. The CSF PfHRP-2 reflects elevated plasma PfHRP-2 rather than elevated CM-specific CSF ratios, falling short of a validated biomarker.

INTRODUCTION

Comatose children with parasitemia are usually considered to have cerebral malaria (CM) initially, but Taylor and others[1] reported that 23% of children diagnosed clinically as CM in endemic areas actually died of other causes including pneumonia, Reye's syndrome, and head trauma. A clinical approach to show evidence of parasitized red blood cell (RBC) sequestration in the vasculature in CM is to find malaria retinopathy by ophthalmoscopy. The sensitivity and specificity were reported as 90% and 95% compared with autopsy.[2,3] The clinical skill and training required for accurate fundoscopic eye examination limits the widespread routine use of ophthalmoscopy in malaria-endemic regions. Examination of the cerebrospinal fluid (CSF) in CM cases typically shows normal parameters with normal protein, glucose, and low cell count.[4–6] A CSF biomarker for cerebral malaria has not been identified.

Plasmodium falciparum histidine-rich protein 2 (PfHRP-2) is an abundant protein with 34% histidine residues with multiple repeats of $[AHHAHHAA(D,Y,N)]_n$.[7,8] The detection of PfHRP-2 is the basis of several rapid diagnostic tests (RDT) for malaria.[9] An enzyme-linked immunosorbent assay (ELISA) is a validated method for detection of PfHRP-2 in blood, serum, and saliva of malaria patients.[10] The lower limit of detection of RDT and ELISA is ~100 to 1,000 pg of PfHRP-2 in any fluid.[11]

Immuno-polymerase chain reaction (PCR) assay combines ELISA and PCR resulting in improved conventional antigen detection sensitivity.[12] Immuno-PCR has been applied as a diagnostic test to detect several viral antigens (e.g., antigens of hepatitis B, Rotavirus, human immunodeficiency virus [HIV], and avian influenza virus) and bacterial antigens and toxins (e.g., *Staphylococcus aureus*, Group A *Streptococcus*, and *Escherichia coli*).[13] In this study, we developed the immuno-PCR assay for PfHRP-2 and tested for the presence of CSF PfHRP-2 in CM patients.[14] We sought to test if there were elevated levels of CSF PfHRP2 compared with plasma in CM, and if elevated CSF PfHRP2 was associated with clinical outcome in this archival study of CM.".

MATERIALS AND METHODS

Study subjects. In total, 73 CSF samples from Tanzanian children, 6 months to 9 years of age with *P. falciparum* parasitemia and Blantyre Coma Score (BCS) of ≤ 2, who satisfied the 1990 World Health Organization (WHO) clinical case definition of CM[15,16] were collected from 1994 to 1995 at Muhimbili Medical Center, Dar es Salaam, as previously reported.[14] Data from this study included clinical parameters, CSF white blood cell (WBC), RBC, glucose, and protein at the time of collection and malariometrics like parasitemia. The CSF samples from nine Tanzanian children who were microscopy negative for *P. falciparum* in peripheral blood, were also collected at the same site from 1994 to 1995.[14] A total of 24 CSF samples were selected from archived CSF samples obtained from patients with neuroimmunological or neuroinflammatory conditions at the Johns Hopkins Hospital Department of Neurology. Matched plasma and CSF samples were obtained from 11 additional Tanzanian children with CM[17] who satisfied WHO 1990 and 2000 criteria for CM,[15] with BCS ≤ 2. Data on ophthalmoscopy was not able to be obtained. All the samples were cryopreserved at –80°C.

RDT. The Binax NOW Malaria Test (Alere Inc., Waltham, MA), which detects both PfHRP-2 and panspecies aldolase was performed according to the manufacturer's instructions using 10 μΛ of CSF. The RDT results were interpreted as positive or negative according to kit instructions and were read by two physicians, independently. Although subjective, a weak positive was weaker than the control line and a strong positive was equal or stronger than the control line according to two other previously reported RDT studies.[18,19]

Synthesis of the antibody-oligonucleotide conjugate. We used 65 bp mumps oligonucleotide (5′-GAC AGC GTA CGA CCA ACC TGC CGG GTC TGC TGA TCG GCG ATT TGC GAA ATA CCA GCA GCA AGG TC-3′) as a

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reporter oligonucleotide. For coupling of this oligonucleotide to monoclonal mouse antibody 3A4 to PfHRP-2 (oligo-HRP2 antibody), we used Antibody-Oligonucleotide All-in-One Conjugation Kit (Solulink, San Diego, CA) and performed according to the manufacturer's instructions. The final concentration of oligo-HRP2 antibody was 0.4 mg/mL.

IMMUNO-PCR assay. To optimize the immuno-PCR conditions, especially to reduce the background noise because of high sensitivity,[13] we adjusted the washing reagents, washing times, blocking reagents, and the concentrations of first and second antibody, and found the following optimized condition. The monoclonal antibody 3A4 specific to PfHRP-2 and PfHRP-3 detection described previously was used as the capture antibody.[20] Antibody 3A4 was diluted in coating buffer (0.1 M $NaHCO_3$, pH 9.5) and 20 μL of diluted 3A4 (5 μg/mL) was added into a 96-well microplate, Nunc Immuno Plate U96 MaxiSorp (Thermo-Fisher Scientific, Cambridge, UK). After overnight incubation at 4°C, the wells were washed with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS-T), and blocking solution (5% fetal bovine serum and 5% nonfat powdered milk in 1 M $NaHCO_3$ pH 9.0) was added for 2 hours. After removal of the blocking solution, duplicate 5 μL of CSF samples with 15 μL of blocking solution was added, and incubated overnight at room temperature (RT). Recombinant PfHRP-2 was used as a standard. After washing three times with 200 μL of PBS-T, 20 μL of the 1:20,000 diluted oligo-HRP2 antibody was added with 5% nonfat powdered milk and 5% bovine serum albumin (BSA) in PBS-T, and incubated 2 hours at RT. After washing the wells two times with 200 μL 5% BSA in PBS-T and two times with 200 μL PBS-T, 20 μL of PCR grade water was added into the wells. After sealing the microplates, the plates were put into boiling water for 10 minutes to denature the antigen-antibody-oligonucleotide complex.

The real-time PCR reactions were run in 9 μΛ volumes comprising triplicates 4 μΛ from the 20 μΛ immunosorbent heated water, 100 μM of each forward primer (5′-GAC AGC GTA CGA CCA ACC T-3′), reverse primer (5′-GAC CTT GCT GCT GGT ATT TC-3′), Texas red probe (5′-CCG GGT CTG ATC GGC GAT-3′), and 4.5 μΛ iQ Multiplex Powermix (Bio-Rad, Hercules, CA). The protocol of real-time PCR had been optimized for iQ Multiplex Powermix (Bio-Rad) according to the manufacture's instruction as follows; annealing cycles comprised an initial 3-minute denaturation step at 95°C, followed by 39 cycles of denaturation at 95°C for 10 seconds, annealing at 55°C for 30 seconds. The real-time PCR amplifications were performed in a C1000 Thermal Cycler (Bio-Rad). All the samples from each CSF well, standards, and negative controls, were done in triplicate PCR reactions using 12 of 20 μΛ from ELISA. The triplicates were all within three threshold cycle counts of each other. We converted pg per well from 5 or 10 μL of sample to ng/mL. The standard deviation for PFHRP-2 determinations of individual CSF patient immuno-PCR from triplicate PCR measurements on independent duplicate CSF samples averaged < 30% of the value before conversion to ng/mL.

Western blot analysis. Ten μΛ of matched CSF and plasma (1:100 diluted with PBS) samples from two CM patients were mixed with 2 μΛ of 6 X sodium dodecyl sulfate (SDS) loading buffer and boiled for 5 minutes at 95 C°. The samples were loaded onto a 4–20% Mini-Protean TGX Gel (Bio-Rad) for Western blotting. After electrophoresis, proteins were transferred to the 0.45 μm nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% powdered milk in TBS (20 mM Tris-HCl [pH 7.6], 137 mM NaCl) for 2 h at RT. The membrane was washed with TBS and 3A4 (2 mg/mL) was added to the membrane at a dilution ratio of 1:5,000, and incubated overnight at 4°C. After washing with TBS, a secondary goat antimouse IgG + IgM (H+L) (Jackson Laboratories, Bar Harbor, ME) antibody coupled with horseradish peroxidase was added to the membrane at a dilution ratio of 1:10,000, and incubation was continued for 1 h at RT. The membrane was exposed on Amersham Hyperfilm ECL (GE Healthcare, UK) using ECL Western blot detection reagents (GE Healthcare, Buckinghamshire, UK).

Statistical analysis. Results of assays of CSF HRP2 and parasitemias were log transformed. The Student *t* test was performed on the log transformed data. Geometric means were converted back from the log transformed averages.

Ethics. Approval of this study was obtained from the College Research and Publications Committee at Muhimbili Medical Center, the Institutional Review Boards of Hubert Kairuki Memorial University, National Medical Research Institute, Tanzania, Duke University Medical Center, the Durham VA Medical Center, University of Utah, and Johns Hopkins University. Informed consent was obtained from parents or guardians of all patients; human experimentation guidelines of the U.S. Department of Health and Human Services were followed.

RESULTS

There were no significant differences in parasitemia, age, and CSF parameters among those who had complete recovery

Table 1
Clinical characteristics and laboratory findings on cerebral malaria (CM) patients

	CMCR	CMD	CMNS
Number of patients	45	20	8
Male/female/unknown	20/24/1	12/6/2	5/3/0
Mean age (years ± SD)	3.5 ± 1.8	3.5 ± 1.6	3.1 ± 2.1
CSF protein (mg/dL)	20.0 ± 10.5	19.3 ± 7.9	22.7 ± 8.6
Opening pressure (cm H_2O)	18.3 ± 7.2	19.5 ± 6.1	22.7 ± 8.6
RBC in CSF (No.) > 5/μL	19	9	1
> 1,000/μL	4	1	0
WBC in CSF (No.) > 5/μL	8	3	1
> 50/μL	0	0	0
Coma duration (days)	11.9 ± 15.3	14.7 ± 11.8	*26.6 ± 29.5
Coma score 0	6	7	0
1	14	5	4
2	25	8	4
Parasitemia (geomean/μL)	25,359	77,365	7,037
CSF RDT strong positive	24	14	5
Weak positive	13	2	3
Negative	8	4	0
PfHRP2 in CSF geomean (ng/mL)	9.2 ± 3.6	11.1 ± 4.0	10.3 ± 2.3
(median, range)	(9.7, 0.05–90.8)	(10.4, 0.6–318.2)	(9.8, 3.3–47.1)

*Only duration of coma between CMCR and CMNS has a significant *P* value (0.037) with other group comparisons had a $P > 0.05$.

CMCR = cerebral malaria with complete recovery; CMD = cerebral malaria with death; CMNS = cerebral malaria with neurological sequelae, CSF:CSF; RBC = red blood cell; WBC = white blood cell; RDT: rapid diagnosis test; PfHRP2 = *Plasmodium falciparum* histidine-rich protein 2.

Parasitemia and PfHRP-2 in CSF comparisons by student *t* test of log transformation individual quantity between CMCR and CMD, CMCR and CMNS, and CMCR and CMNS all have a $P > 0.05$.

from CM, those who died, and those who had neurologic sequelae in this subsampling of available patients and CSF samples. Duration of coma was prolonged in those with neurologic sequelae. Demographic and clinical details of CM patients[14] in which archival CSF was available for analysis are shown in Table 1.

To determine the detection limit of RDT and immuno-PCR for PfHRP-2, we generated a standard curve using dilutions of recombinant PfHRP-2 in PBS. The detection limit of RDT was 10 ng/mL solution, or 100 pg per test. We determined that the limit of quantification of PfHRP-2 with immuno-PCR was 1 pg/well, or 0.2 ng/mL for a 5 μL CSF

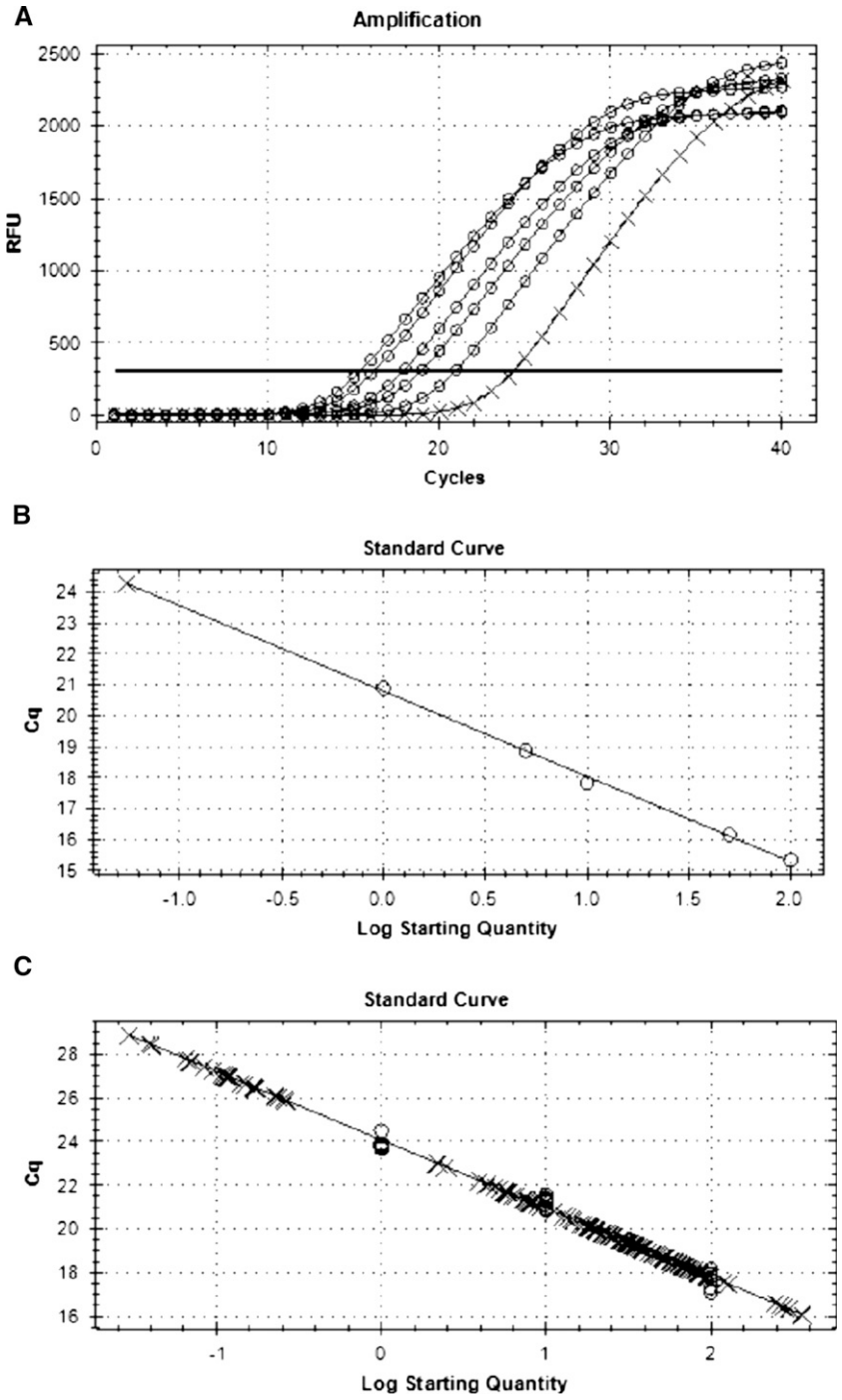

FIGURE 1. Development of Immuno-polymerase chain reaction (PCR) for *Plasmodium falciparum* histidine-rich protein-2 (PfHRP-2) detection (**A**) Real-time immuno-PCR amplification of PfHRP-2 after enzyme-linked immunosorbent assay (ELISA). Amplification plots of relative fluorescence unit (RFU) intensities versus the PCR cycle number are displayed for samples from microplates with addition of 100, 50, 10, 5, 1 pg/well of recombinant PfHRP-2 (O) left to right, respectively, and PBS (X) as a negative control. (**B**) Standard curve showing the correlation between the log starting quantity of PfHRP-2 (O) in pg per well and PBS (X), and the threshold cycle number (Cq). (**C**) Quantitative standard curve with positive and negative CSF samples (X) in duplicate from a representative immuno-PCR plate. The negative control samples consistently from all plates analyzed had a three cycle separation from any of the positive controls. This corresponds to at least a log separation of negative samples from positive detections of PfHRP-2.

sample (Figure 1), and we determined the limit of detection to be 0.1 pg/well. Immuno-PCR analysis of CSF showed a significant threshold gap at least three cycles between positive samples and negative samples.

The RDTs were performed on 73 CSF samples from CM Tanzanian children (Table 1). A strong positive PfHRP-2 signal was evident in 43 of 73 (58%) samples, a weak positive PfHRP-2 signal was in 18 of 73 (25%) samples, and 12 of 73 (16%) were negative. Of the 43 strong positive PfHRP-2 CSF samples, only three were also positive for *P. falciparum*-specific aldolase suggesting that the repetitive PfHRP-2 persisted in CSF, whereas other *P. falciparum* proteins like the glycolytic enzyme aldolase do not persist in CSF. All the negative control samples were negative for aldolase detection. The RDTs showed negative results on all nine endemic control CSF samples from non-malaria Tanzanian children and all 24 non-endemic control CSF samples obtained from patient donations to the Johns Hopkins School of Medicine CSF repository. The results showed a RDT sensitivity of 84% for this CM sample collection and a specificity of 100% for the 33 non-malaria CSF samples.

Immuno-PCR tests were performed on 73 CSF samples from CM Tanzanian children. Seventy-two of 73 (99%) were positive on immuno-PCR (72 of 73; 99%). The geometric mean PfHRP-2 concentration in CSF was 9.6 ng/mL with a median of 9.9 ng/mL and range 0.1 to 318 ng/mL for all the samples. Comparing the immuno-PCR PfHRP-2 values to negative, weak, and strong RDT results revealed PfHRP-2 geomeans of 1.5, 6.7, and 19.4 ng/mL, respectively (Figure 2A). The clearly strong positive RDT ranged from 4.4 to 318.2 ng/mL. The weakly positive RDT ranged from 2.1 to 36.8 ng/mL. The RDT negative CM positive samples and immuno-PCR positive samples ranged from 0.2 to 7.9 ng/mL. One CM sample was negative by both immuno-PCR and RDT with both 5 and 10 μL of CSF input to immuno-PCR. The 33 endemic and nonendemic control samples ranged from 0.004 to 0.08 ng/mL with a geometric mean of 0.02 ng/mL.

Segregating the patients into CM with complete recovery, those who died, and those who had neurologic sequelae revealed no significant differences (P values < 0.05, Student t test) in geometric mean CSF PfHRP-2 concentrations (9.1, 11.1, and 10.8 ng/mL) (Table 1 and Figure 2B). All nine CSF samples from Tanzanian children who were microscopy negative for malaria and 24 archival CSF samples from Johns Hopkins School of Medicine patients were negative on immuno-PCR. The results showed an immuno-PCR sensitivity of 98.6% for this CM sample group and a specificity of 100% for the 33 non-malaria CSF samples.

To compare ratios of plasma to CSF PfHRP-2, we also performed immuno-PCR on 11 matched CSF and plasma samples from a separate CM study in Tanzania (Figure 2C).[17] In the set of matched plasma and CSF samples, the geometric mean ratio of plasma to CSF PfHRP-2 was 175. In these matched samples, *P. falciparum* aldolase was present in 10 of the 11 plasma samples but absent from all of the CSF samples. The average protein content of CSF in these matched samples was 20 mg/dL (0.2 mg/mL) and all 11 had zero erythrocytes recorded for CSF cell count. Other CSF parameters in these 11 samples were opening pressure average of 19 cm water (range 17–22), white blood cell (WBC) = 9 (range 0–52) × 10^9/μL and glucose 74

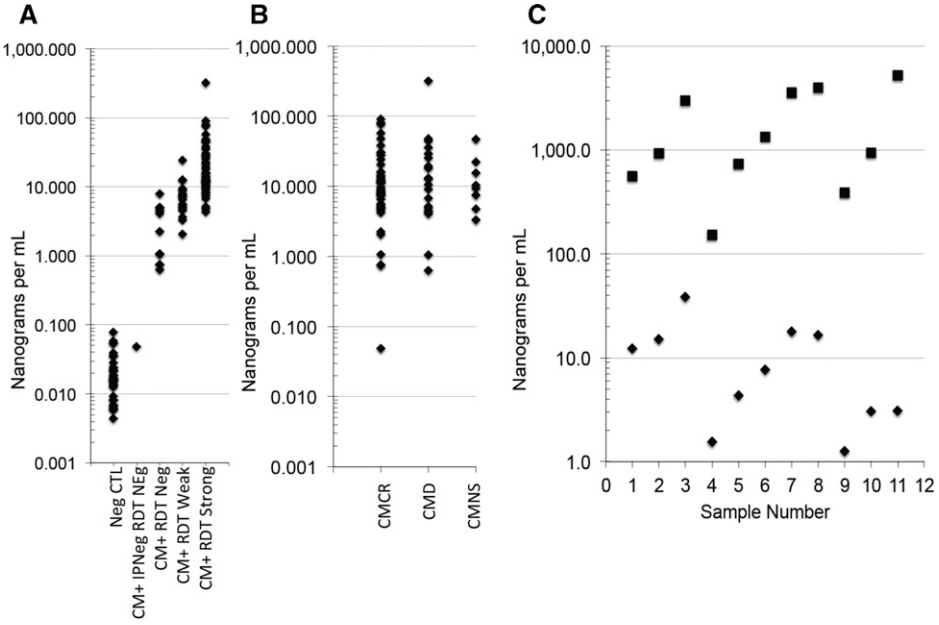

FIGURE 2. PfHRP-2 concentrations in cerebrospinal fluid (CSF) samples. (**A**) Segregated by strong, weak, and negative rapid diagnostic test (RDT) intensity. The clearly strong positive RDT (CM + RDT Strong) had a geometric mean of 19.4 ng/mL. The weakly positive RDT (CM + RDT weak) had a geometric mean of 6.7 ng/mL. The RDT negative cerebral malaria (CM) positive samples and immuno-polymerase chain reaction (PCR) positive samples (CM + RDT Neg) had a geometric mean of 1.5 ng/mL. One CM sample was negative by both immuno-PCR and RDT (CM+ IP Neg RDT Neg). The 33 endemic and nonendemic control samples had a geometric mean of 0.02 ng/mL. (**B**) Segregated by CM categories. Patient CSF samples into the CM categories of complete recovery (CMCR) (N = 45), death (CMD) (N = 20), or neurologic sequelae (CMNS) (N = 8) revealed no differences (P values > 0.05) in CSF PfHRP-2 with geometric means of 9.2 + 3.6, 11.1 ± 4.0 ng/mL, and 10.3 ± 2.3 ng/mL, respectively. (**C**) Separate clinical study samples from plasma and CSF 11 matched pairs. The geometric mean of CSF PfHRP-2 (◆) was 6.7 ng/mL and plasma (■) was 1176.4 ng/mL. The matched plasma to CSF ratios ranged from 46 to 307 with a geometric mean of 175.

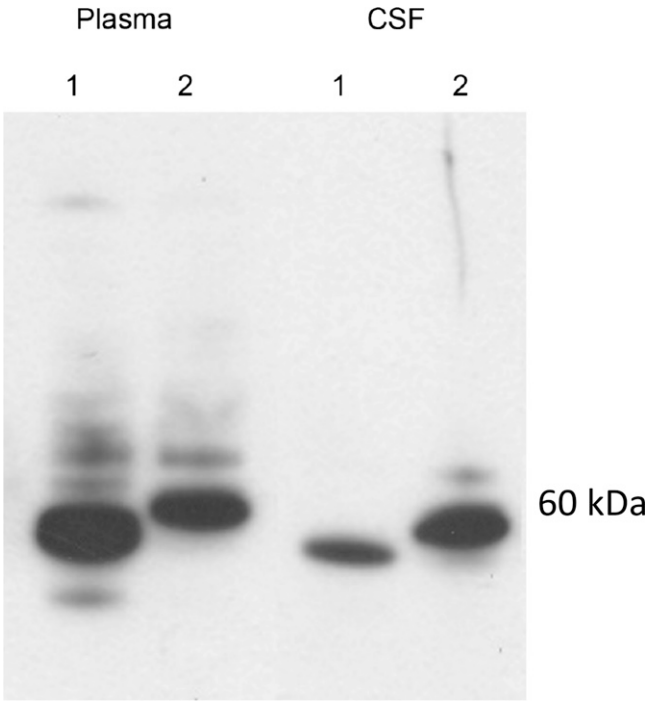

FIGURE 3. The *Plasmodium falciparum* histidine-rich protein-2 (PfHRP-2) in cerebrospinal fluid (CSF) is full length and not degraded (i.e., comparable to plasma PfHRP-2). The diluted plasma and undiluted CSF from two matching patients numbered 1 and 2 without any erythrocytes in CSF both have full length PfHRP-2 with the laddering of multimers more evident in plasma rather than the single band in CSF detected by immunoblot with antibody 3A4 to PfHRP2.

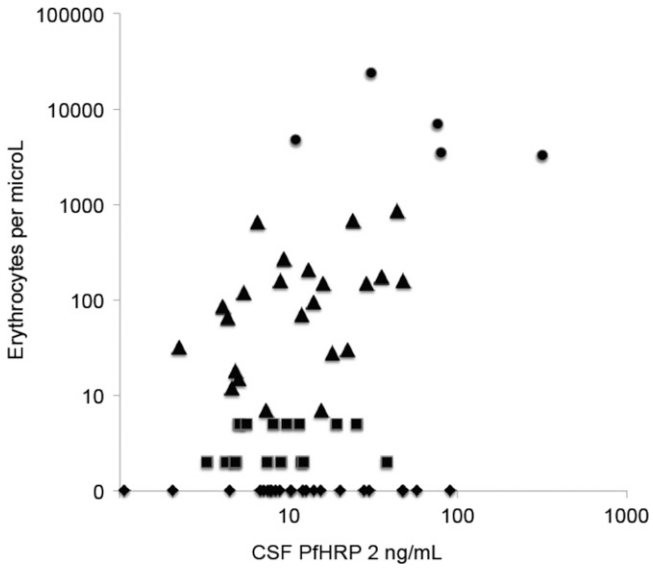

FIGURE 4. Lack of correlation of cerebrospinal fluid (CSF) *Plasmodium falciparum* histidine-rich protein-2 (PfHRP-2) and erythrocyte count. The samples were plotted in groups of zero erythrocytes (◆), 2–5 erythrocytes (■), 7 to 1,000 erythrocytes (Δ), and over 1,000 (●) erythrocytes per μL. The R^2 correlation was 0.19.

(range 39–89) mg/dL. The gram weight per mL ratio of total CSF protein to PfHRP-2 was then 200,000:7 (~20,000). Western blot analysis from two patients matched by CSF and plasma PfHRP-2 have the same molecular mass (60 kD) corresponding to the PfHRP-2 dimer, a finding that suggests intact full-length CSF PfHRP-2 (Figure 3).

Bloody taps containing infected erythrocytes with PfHRP-2 theoretically could contribute to CSF PfHRP-2 amounts. There was no correlation of CSF PfHRP-2 with CSF erythrocyte counts (correlation coefficient R^2 of 0.19) (Figure 4). Of the 73 archival samples with available CSF erythrocyte counts, 27 had zero erythrocytes, and 17 more had normal counts of 2 to 5 erythrocytes (within the normal range). The CSF PfHRP-2 geomean was 8 ng/mL on these 44 samples compared with 10 ng/mL for the 24 samples with erythrocytes/μL of 7 to 1,000 (Student *t* test was $P = 0.45$). Five more samples had erythrocytes/μL over 1,000. None of the 73 cerebral malaria samples has CSF WBC over 50/μL, with only 12 over 5/μL.

DISCUSSION

This is the first report of a highly sensitive method engineered for low sample volumes for the detection of PfHRP-2 using an immuno-PCR. We used it to quantitate PfHRP-2 in CSF samples of CM patients. A previous study published in 1989 used affinity purified human immune sera with *Plasmodium knowlesi* lysates as capture proteins to show by counter-current electrophoresis unidentified malaria proteins in CSF.[21] Since then, most CM studies have focused on metabolites or cytokines as potential biomarkers in CSF. A recent report detected PfHRP-2 by ELISA of 100 μL in all 12 CM patients and in 6 of 24 patients without CM, including 3 without PfHRP-2 in plasma.[22] They did not report a correlation of plasma to CSF PfHRP-2, possibly because the standards were based on human plasma segregated by parasitemia. Levels of PfHRP-2 in plasma most often do not correlate with parasitemia.[23,24] Here, we identified PfHRP-2 by both an RDT using a minimum volume of 10 μL and by immuno-PCR using an average volume of 5 μL because of limited volume in archival samples. The non-repetitive protein aldolase was mostly absent from CSF by RDT, either from lack of sensitivity of the RDT for aldolase compared with PfHRP-2 in CSF (as is described in whole blood)[25] or because proteolysis between capture and detection epitopes for the RDT makes aldolase in CSF not detectable in the RDT format.

Our results showed that the ratio of plasma to CSF PfHRP-2 is 175. This is comparable to that found for the CSF ratio of total protein (1:200) and albumin (1:100).[26] The CSF levels of 10 to 20 ng/mL of PfHRP-2 multiplied by 175 would translate into plasma values of more than 1,000 ng/mL, levels that have been measured in many severe malaria or CM studies. The PfHRP2 levels in uncomplicated malaria are about 100 to 300 ng/mL.[17,24,27] The PfHRP-2 could enter the CSF by the choroid plexus with its fenestrated endothelium and specialized epitheloid cells that generate a 200-fold reduction in protein as they produce CSF from plasma.[26] Additionally, < 50% of CSF is made from interstitial fluid in brain parenchyma, which drains directly into ventricles. The PfHRP-2 in the CSF, like other proteins, is in dynamic equilibrium with inflow from the choroid plexus and interstitial fluid by the ventricles and outflow resorption through arachnoid villi, which function as unidirectional valves between CSF and dural venous drainage with flow controlled by CSF pressure.[26]

There are some limitations of this study. Because autopsies were not done on these subjects, and our 1994–1995 study antedated the studies showing the diagnostic use of retinal exam,[2] we were not able to compare CSF PfHRP-2 between malaria retinopathy-positive or autopsy-confirmed CM and malaria retinopathy-negative or CM cases with an alternative cause diagnosed at autopsy. It is plausible that some patients had parasitemia and a different cause of coma. We speculate that some of the 16% of children with RDT-negative CSFs may represent this latter category, and future studies comparing PfHRP-2 RDT in malaria retinopathy-positive and -negative children would help discern this. Ideally, CSF samples from malaria patients without neurologic symptoms but with elevated plasma PfHRP-2 should also be characterized for presence of PfHRP-2 in CSF. However, it is generally not appropriate to perform lumbar punctures in children with normal neurologic function or no suspicion of CNS disease, so we did not have access to these types of samples. Third, a bloody tap with CSF erythrocyte counts > 1,000/μL (which was seen in only 5 of 73 CSF samples we tested) could falsely elevate CSF PfHRP-2, but CM with hemorrhage could also have an elevated erythrocyte count with a truly elevated CSF PfHRP-2 level.

The widely used RDTs for malaria may be useful in CSF detection of PfHRP-2 in patients with CM. A total of 62 of 73 samples had at least 1 ng/mL PfHRP-2 concentration detectable, an amount well above the 100 pg limit of detection threshold, if 100 μL is used for RDT. Further studies are needed to determine whether CSF PfHRP-2 is applicable to severe anemia patients with coma, and what role PfHRP-2 may play in CM pathogenesis correlated to malaria retinopathy-positive or malaria retinopathy-negative patients. We did not find a higher ratio of CSF to plasma PfHRP2 compared with CSF to plasma albumin ratios as we first predicted. The CSF PfHRP2 is proportional to plasma such that detection in the CSF is reflective of elevated plasma or whole blood PfHRP2 seen in many studies of cerebral malaria.

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