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Challenges in the Determination of Early Predictors of Cerebral Malaria: Lessons from the Human Disease and the Experimental Murine Models

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Key Words
Cerebral malaria · *Plasmodium berghei* ANKA · Murine models

Abstract
Cerebral malaria (CM) is a life-threatening complication of malaria caused by *Plasmodium falciparum*, and it claims around two million lives a year, mainly those of children in sub-Saharan Africa. A number of works, particularly in murine models of CM, showed that several mediators are involved in the development of the disease, including monocytes, T lymphocytes, cytokines, chemokines, platelets, nitric oxide scavengers and heme, among others, but a comprehensive understanding of the pathogenesis of this complication is still lacking. This overview critically analyzes and discusses the definition, clinical features, neurocognitive outcomes and pathogenesis of human CM. We focused on the relationship between clinical and laboratory features and the diagnosis and prognosis of the complication showing indicators of poor prognosis and emphasizing the need of establishing predictive scores to estimate, on admission, the likelihood of any malarial patient to develop neurological complications. The potential development of a mathematical model for early prediction of CM through neurological assessment using the SHIRPA protocol in *Plasmodium berghei* ANKA-infected susceptible mice is shown. High positive predictive values (>89%) on days 5 and 6 of infection, observed for some generated SHIRPA scores, indicate the possibility of early detection of mice with a high probability of developing CM.

Introduction

Malaria is the most important parasitic disease in the world, being a health problem in more than 100 countries and killing more people than any other communicable disease except tuberculosis and HIV/AIDS [1]. It is estimated that over 40% of the world's population (2.4 billion people) live in malarious areas, and the incidence of the disease worldwide is estimated to be 300–500 million cases each year [2]. About 60–80% of malaria cases occur in sub-Saharan Africa and about 25% in Southeast Asia [3, 4], being mostly a disease of the poor. However, the

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number of malaria cases in some developed countries, mainly imported by travelers and immigrants, is increasing [4–6].

Presently, it is accepted that 5 species of *Plasmodium* can cause the disease in humans: *Plasmodium malariae, Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* [7] and, more recently, it was reported that *Plasmodium knowlesi,* previously known as a simian parasite, can also infect human beings [8, 9]. Although all 5 species of *Plasmodium* can theoretically cause life-threatening disease [10–18], *P. falciparum* malaria has the greatest capacity to complicate [19]. The global proportion of *P. falciparum* malaria is between 35 and 43% [20], and in sub-Saharan Africa it accounts for 14–17% of pediatric admissions to some hospitals [21]. *P. falciparum* infections can rapidly progress to renal failure, abnormal bleeding, hemoglobinuria, intractable vomiting, high fever, hyperparasitemia, circulatory collapse (shock), pulmonary edema (respiratory distress), placental dysfunctions, anemia, acidosis, hypoglycemia, jaundice, cerebral malaria (CM), impaired consciousness and several others neurological manifestations [22–28] causing a clinical picture called severe (life-threatening) malaria that kills more than 2 million people annually [3]. Children under 5 years old and pregnant women in endemic areas and nonimmune individuals are at greater risk of developing severe malaria [29, 30]. The World Health Organization (WHO) suggested a definition of severe malaria based on clinical and laboratorial features [31] that requires *P. falciparum* parasitemia and a measure of severe disease, such as impaired consciousness or severe anemia, and also produced a handbook outlying its management [32].

Among the complications of *P. falciparum* infection, CM is one of the most life-threatening with an incidence of 1.12 cases per 1,000 children per year, a 7–18.6% mortality rate despite rapid administration of chemotherapy [33, 34], and accounting for 10% of pediatric admissions in some sub-Saharan hospitals [21]. The great majority of deaths in children with CM occurs within the first 24 h [33], before they can benefit from the full effect of antimalarials [35]. Clinicians agree with the need for adjunctive therapy to rescue these patients from death [36], but the majority of the therapeutic measures evaluated so far have not resulted in improved outcome [37]. CM is a multifactorial disease. Although cytoadhesion of parasitized red blood cells (pRBCs) to the brain microvessels seems to be the main finding in the pathology, several immunological factors (leukocytes, cytokines and chemokines), platelets, nitric oxide (NO) scavengers and heme, among others, are involved in the development of the disease [38–43]. These factors integrate a systemic inflammatory response during the clinical course of a malarial infection that acts in the brain and is responsible, at least in part, for the neurological symptoms and signs presented by the patients [36]. However, it is not known to what extent and timing each factor contributes to the pathogenesis and interferes with the prognosis of the disease.

Defining early signs of neurological involvement and indicators of poor outcome in malaria patients can allow the improvement of accuracy of the diagnosis and the prompt establishment of therapeutic approaches. This overview discusses the clinical features, neurocognitive outcomes, pathogenesis and indicators of poor prognosis of human CM and in the murine experimental model.

Definition and Differential Diagnosis

CM is always a diagnosis of exclusion, mainly in malaria endemic areas. In the past, the term 'cerebral malaria' was applied to a wide range of neurological manifestations of malaria with potentially disparate pathophysiological mechanisms and outcomes [30]. More recently, the WHO has defined it as a clinical syndrome characterized by coma (inability to localize a painful stimulus) persisting for at least 1 h after termination of a seizure or correction of hypoglycemia that occurs during a *P. falciparum* infection without the presence of other causes of encephalopathy [31]. This definition excludes people with transient postictal and hypoglycemic coma, common features during severe malaria, but a lumbar puncture must be done to exclude other causes of encephalopathy [44–46]. CM should also be distinguished from viral encephalitis (herpes simplex, HIV, enterovirus, mumps, and arboviruses such as West Nile), bacterial meningoencephalitis (pyogenic and rarely tuberculous), fungal and protozoal meningoencephalitis (African trypanosomiasis), cerebral typhoid, brain abscess, heat stroke, cerebrovascular events, hypertensive encephalopathy and intoxications with drugs and poisons [23]. In addition, central nervous system involvement in severe malaria has a large spectrum ranging from prostration and irritability to impaired consciousness that must be differentiated from CM [21, 47]. Despite the strict definition, in a study with Malawian children using postmortem analysis, it was shown that 23% of cases that fulfill the WHO criteria for CM

had other causes of coma like Reye's syndrome, ruptured arteriovenous malformation and hepatic necrosis [48]. For practical purposes, any patient with impaired consciousness or other neurological sign and a diagnosis of malaria should be treated as severe malaria with parenteral antimalarials [23, 30, 49].

The WHO definition of CM is very useful for works in the field allowing investigators to get similar results even if the studies are carried out at different periods [34], but it has been criticized as being too narrow [50]. In practice, the clinical diagnosis of CM, severe malaria and even malaria infection in endemic areas is difficult [51–53]. A study in Tanzania showed that 54% of people treated for severe malaria had no parasitemia [53], revealing a high sensitivity and low specificity for the definition of 'severe malaria'. Accordingly, in another Tanzanian study, from the total cases diagnosed as 'cerebral malaria' on admission, only 19.7% had detectable parasitemia, giving a positive predictive value (PPV) of 13.3% [30]. A high rate of overdiagnosis was also obtained in Nigeria [54]. CM misdiagnosis and overdiagnosis is thought to derive from various factors like scarcity of material and of trained technicians to perform the blood smears, low sensitivity of the rapid diagnostic tests [55], inability of some hospitals to exclude other causes of coma in a parasitemic child [51], high number of asymptomatic individuals in holoendemic areas, low specificity of the clinical features of the disease [56, 57], and the concomitant occurrence of other organ dysfunctions and metabolic changes present in severe malaria [21, 33]. In addition, the WHO definition of severe malaria itself was derived from estimates of the associated risks of death, and it is biased towards sensitivity [29]. Together, these data show that, although the WHO definition of CM is adequate in research, it is not routinely applied in practice.

Although it could be argued that the benefits of overtreating outweigh the risks of not treating a true case, especially where diagnostic facilities are limited or of uncertain quality [58], increasing data argue that overdiagnosis leads to dangerous unnecessary treatment [59, 60], insufficient investigation of other possible diagnoses, high mortality rates [30, 34, 54], and the development of resistance by the parasite [61].

Clinical Features

The clinical features of CM differ between children and adults, and it is not yet clear if these differences are related to immunity or age [44].

In children, CM begins with a 1- to 4-day history of fever, littleness, anorexia, irritability, vomiting, cough and one or more convulsions before coma is established [62]. During coma other neurological manifestations that can occur include symmetrical upper motor neuron syndrome (increased muscle tone, brisk tendon reflexes, ankle clonus, extensor plantar responses), absent abdominal and other superficial reflexes, clenching of the jaws and grinding of the teeth ('bruxism'), or a brisk jaw jerk reflex, decorticate and decerebrate posturing, opisthotonos, dysconjugate gaze, nystagmoid eye movements, abnormal respiratory patterns (hyperventilatory, ataxic and periodic breathing), changes in pupils size, and absence of brainstem reflexes (oculocephalic, oculovestibular, pupillary and corneal) [22, 26, 44, 49]. Besides the neurological manifestations, the children are febrile and, in some cases, can also present tachycardia, tachypnea, stertorous breathing, dehydration, jaundice, hepatomegaly and splenomegaly in the physical examination [62]. Retinal changes appear in over 60% of CM children and include retinal and vessel whitening, Roth's spot-like hemorrhages, cotton wool spots and, more rarely, edema, exudates and papilledema [63–68]. Seizure is another prominent clinical feature affecting 60% of children and being characterized by tonic eye deviation, nystagmus, salivation, hypoventilation, hypoxia and acidemia [26, 69, 70]. Ictal activity usually originates on the posterior temporoparietal regions and spreads to one or both hemispheres [70]. Peripheral blood parasitemia greatly varies in CM children ranging from subpatent levels (parasites not found on the initial blood film) to 50% or more [62]. Concomitant complications common in children with CM include severe anemia, hypoglycemia, metabolic acidosis, dehydration, transient impairment of renal function and concomitant bacterial infections [21, 34, 62, 71, 72].

In adults, CM is part of a multisystemic syndrome with associated severe complications like renal failure, hepatic dysfunction, pulmonary edema, severe anemia, and disseminated intravascular coagulation occurring in 60% of the cases [44, 73, 74]. The incidence of CM in adults is higher in low and moderate transmission areas of Southeast Asia [75, 76] and Papua New Guinea [77], and in areas of varying endemicity in Africa [74, 78–81]. This is explained by the development of immunity in children living in malaria hyper- and holoendemic areas, limiting severe morbidity in adults in much of Africa [30]. The clinical course of the disease begins gradually with fever, vomiting, diarrhea, headache, malaise,

anorexia, psychosis, delirium, trismus, seizures, joint and body aches, and neurological deficits like symmetrical upper neuron lesion, dysconjugate eye deviation, and extrapyramidal, decorticate and decerebrate rigidity [30, 44, 73, 80]. Neck stiffness can rarely be present making the differential diagnosis more difficult [82]. Patients generally decline after this prodromic period developing coma in the end. Retinal changes are rare in adult CM, but 15% of patients develop retinal hemorrhages [44, 83].

Clinical features of a given disease are determined based on the association of each symptom and/or sign with the disease. However, similar symptoms and signs may be present in different diseases making the differential diagnosis a difficult issue. This situation is particularly true for CM which presents a clinical picture similar to many other neurological conditions [23]. The experienced physician learns empirically with time how to differentiate CM from the neurological conditions that are prevalent in the area. However, this 'predictive power' in adults has only 65% sensitivity and specificity [30]. One way of improving the clinical diagnosis of CM is to rationalize the diagnosis process by constructing clinical algorithms. It has previously been shown that this approach can increase the accuracy of the diagnosis of malaria [52, 61, 84–92], and of anemia in a malaria-endemic area [93]. Tangpukdee et al. [94] proposed a score to estimate the risk of uncomplicated *P. falciparum* infection to develop into a severe form of malaria using the same approach. Mathematically determining the predictive power (sensitivity and specificity) of each sign and symptom to diagnose CM in both children and adults is the first step to make these algorithms. To our knowledge, this has been made only for the retinal changes in children and revealed a sensitivity of 95% and a specificity of 90% [48]. However, the number of cases in the study was low (<15 per group), the values were not validated in other studies, and the findings can be used only for the diagnosis and not as early signs to predict CM. It was shown that a past history of seizures, fever lasting for 2 days or less, metabolic acidosis, delayed capillary refill time, and hypoglycemia are independently associated with neurological involvement, but not specifically with CM, in children with *P. falciparum* malaria [27]. The same work found that severe malarial anemia morbidity is associated with absence of neurological involvement.

Outcome

CM in hospitalized African children receiving proper antimalarial treatment has a mortality rate of approx. 20% [95]. The factors associated with poor outcome (death) in CM are pretreatment at home with antimalarials, abnormal respiration pattern, a cold periphery, rapid pulse rate, coma score of 0 or 1, malnourishment, hyperpyrexia (axillary temperature >40°C), hyperparasitemia (parasite count >500,000/μl), jaundice, absence of corneal reflexes, age under 3 years, hyperleukocytosis (white blood cell count >10,000/μl), raised intracranial pressure, low RBC deformability, multiple and prolonged seizures, hypoglycemia, increased levels of some cytokines and/or chemokines (IL-1ra, IP-10, TNF-R1, TNF-R2, Fas-L, and sFas among others), and abnormal aspartate aminotransferase, lactate and urea levels [34, 96–99]. In addition, shock and decorticate, decerebrate and opisthotonic posturing, although rather rare, are also associated with a poor prognosis [34, 100]. Hypoglycemia seems to be the most important predictor of death [25, 98, 101]. Molyneux et al. [69] described a predictive score of poor outcome for CM children based on 4 neurological parameters (witnessed seizures, coma score of 0, signs of decerebration, absent corneal reflexes) and 3 laboratory parameters (blood glucose level below 2.2 mmol/l, parasitemia $>10^6$ ring forms per microliter, total leukocyte count $>15 \times 10^9$/l). The presence of 4 or more of these parameters has a PPV of 83% for poor outcome (death or neurologic impairment), but sensitivity was only 66%. Jaffar et al. [98] found that a high pulse rate, a cold periphery, deep coma, hypoglycemia, and lactate, aspartate aminotransferase and urea levels were the best predictors of fatal outcome, but these authors do not propose a predictive score. Predictors of poor outcome [21, 24, 102] as well as a predictive score of poor prognosis for severe malaria [103] have also been published.

Most children that survive CM have a full recovery, but 10–25% develop long-lasting neurological and cognitive impairments after infection [104–107]. Impairments have been described in hearing, visual, motor, speech and language, memory, attention and other cognitive functions [44, 106–111]. It is not clear whether neurologic sequelae reflect the severity of the past disease or whether it is derived from a distinct pathologic process [98, 105, 108]. Factors associated with an increased neurological and cognitive impairment in children are a history of previous seizures, raised intracranial pressure, deep and prolonged coma, hypoglycemia, multiple

seizures during hospitalization, severe anemia, and neurological deficits on discharge [44, 98, 105]. Low coma score, multiple convulsions during hospital stay and long duration of unconsciousness seem to be the best predictive factors for persisting neurological and cognitive impairments [105, 108]. In adults, the incidence of neurological and cognitive impairment is lesser than in children (<5%), but the range of types of sequelae is much greater, including cranial nerve lesions, neuropathies, extrapyramidal disorders, focal epilepsy, poor dichotic listening, personality change, depression, and subclinical mixed anxiety-depression syndrome [112–114].

Pathophysiology

The pathogenesis of CM has been studied in the past decades by diverse and potentially complementary approaches like clinical and genetic predisposition studies in malaria-endemic areas (clinical case series and case-control studies), postmortem surveys, studies with animal models, and in vitro studies [44, 115]. These approaches indicate that the disease is a result of a complex process that involves human and malaria parasite genetics, nutritional and immunological status, and intercurrent infections, among other factors [7, 44, 115–118]. However, despite the high amount of information generated about the disease, some central questions remain unanswered. For example, it is not clear whether the pathogenesis of CM is the same in children and in adults [115]. Nowadays, it is accepted that there are two nonexclusive dominant hypotheses to explain the pathological process: the sequestration (or mechanical) hypothesis and the inflammatory hypothesis [40, 119–121]. Some evidence supporting these hypotheses is based on the use of mouse models of CM, the most widely used model being the infection of different mouse strains by *P. berghei* ANKA [122]. *P. berghei* ANKA-infected susceptible mice develop a lethal neurological syndrome 6–12 days after infection with a cumulative incidence of 50–100% [123–125]. Comparisons can be made with other mouse strains that are resistant to the cerebral complications of the infection [1, 126] or with parasite strains that do not cause CM [127, 128]. Although it became accepted that the behavioral changes, histopathology and immunological manifestations in murine and human CM are similar, there is at least one major difference between these conditions that must be noticed: while in human CM pRBC are the main cells sequestered in brain vessels, in murine models the main cells are leukocytes [119].

The sequestration hypothesis was first proposed by Marchiava and Bignami in 1894 [36]. This hypothesis is based on the fact that *P. falciparum* pRBC adhere to the capillary endothelial cells of the major organs of the body, predominantly the brain, heart, lungs and submucosa of the small intestine [121]. This phenomenon, called sequestration, could cause obstruction of blood flow, anoxia of the brain tissue and decreased removal of waste products, causing the neurological picture of the disease [44, 129]. Sequestration occurs as a consequence of the expression of *P. falciparum* adhesion proteins on the membrane of infected erythrocytes, which can bind to ligands expressed or upregulated in the endothelial cells during the infection [130–132]. Parasitized and unparasitized RBC become less deformable during the infection, mechanically plugging the capillaries, which is associated with a poor outcome [99]. However, there are concerns about the sequestration hypothesis, such as the low correlation between parasitemia and mortality, low rates of neurological deficits after recovery from coma [133, 134], and the increasing number of case reports of CM by *P. vivax*, which does not sequester [13, 17].

The inflammation hypothesis was first proposed in 1948 by Maegraith [36] and revived by Clark (reviewed in Clark and Cowden [134]) and postulates that malaria parasites cause a systemic inflammatory response that induces multiorgan failure and death. Briefly, when pRBC lysis occurs, parasite toxins with pathogen-associated molecular patterns, presumably glycosylphosphatidylinositol, together with host intracellular molecules like hemoglobin, are freed in the blood. Pathogen-associated molecular patterns can be recognized by pattern recognition receptors [135] of the innate immune system and activate monocytes and neutrophils to secrete proinflammatory cytokines like TNF-α, IFN-γ and LT-α that act by recruiting other immune cells like CD4+ and CD8+ T cells, upregulating the expression of adhesion molecules and activating metabolic changes in endothelial cells [36, 115, 123, 136]. It is thought that this inflammatory response is beneficial at first reducing parasite growth and activating catabolic pathways to eliminate parasite toxins and host molecules that can be dangerous when present in high amounts like free heme. For example, TNF-α, LT-α and IFN-γ activate heme enzymes like indoleamine 2,3-dioxygenase, cyclooxygenase-2, inducible NO synthase, and heme oxygenase-1, improving metabolic pathways

(kynurenine, prostaglandins, leukotrienes, and NO pathways), which seem to have protective effects during the infection [115, 120, 137, 138]. However, at later stages this inflammatory response causes damage to the host, and the eliminatory pathways, although stimulated, are not enough to eliminate the high amounts of toxins generated [115, 137]. CD8+ T cells may increase the impairment of the endothelial cell function by perforin-mediated mechanisms leading to blood-brain barrier (BBB) leakage, causing brain edema and allowing cytokines and malaria antigens to enter the brain environment, to activate microglia and to damage astrocytes and neurons [39]. High levels of hemoglobin released in the plasma following parasite replication in RBC scavenge NO leading to endothelial and microcirculatory disturbances [42], and free heme damages the BBB [43]. BBB leakage also induces a stage of cytopathic hypoxia with reduction of high-energy phosphates and elevated brain lactate damaging brain cells. In addition, platelets, γδT cells, low levels of anti-inflammatory cytokines, course of parasitemia, genetic factors, microparticles and prostaglandins produced by the parasite seem to be involved in the pathogenesis [36, 119, 139–144]. The inflammatory hypothesis alone can not fully explain the pathogenesis of CM. High levels of inflammatory cytokines are found in non-lethal *P. vivax* infection [145], anti-inflammatory agents did not improve or even exacerbated the clinical course of the disease in humans [146, 147], and, mainly, there is not a clear definition of the sequence of events during the course of the disease.

Most of this physiopathogenesis scenario has been conceived based on experimental data using murine models of CM, such as *P. berghei* ANKA-infected mice. Although there is much criticism on the relevance of the murine models, they are still considered a valuable resource to understand human CM pathogenesis. It is likely as well that such models can help defining better ways to predict outcome in human CM, in an effort to establish timely interventions. Experiments to understand the pathogenesis of CM using murine models are based on the association of each factor with the development of CM and whether the blockage or addition of a given mediator interfere with the outcome of the disease [119]. These approaches permit to determine that a factor is needed for the pathogenic process, but the precise role of each mediator and the time that is needed to generate CM are very difficult to determine. For example, Carvalho et al. [148] showed that, although CM incidence was 70% overall in their series, all *P. berghei* ANKA-infected CBA mice analyzed presented some degree of histological alteration in the brain on days 6–8 after infection; animals not presenting clinical CM had microhemorrhages, although in a much lower frequency than animals with clinical CM. *P. berghei* ANKA-infected susceptible mice develop neurological signs (ataxia, convulsions, roll over, paralysis and coma) only few hours before death, and the rate of mice succumbing to CM varies among experiments [149]. Different host-parasite factors like genetic background, age, amount of inoculum, course of parasitemia, and clonal variations of the parasite also interfere with the incidence of CM in mice [124, 126, 149]. These variations can be a drawback for some experimental designs, in which it may be necessary to know in advance which mice will develop CM. A way to solve or minimize this problem is to define factors or tests that can discriminate susceptible *P. berghei* ANKA-infected mice that will (CM^+) or will not (CM^-) develop CM and build predictive models using logistic regression. Collete et al. [122] showed that it is possible to discriminate CM^+ from CM^- B10.D2 mice infected with *P. berghei* ANKA by analyzing the TCR-β repertoire using a throughput CDR3 spectratyping method. However, for being sophisticated, expensive and time consuming, the use of this method for purposes of predicting CM seems complicated.

It was previously shown that a temperature below 30°C [150] and changes in locomotor activity [138] are associated with death of *P. berghei* ANKA-infected CBA mice. Lackner et al. [151], using a well-established protocol for behavioral tests in mice, the SHIRPA protocol, showed that CM^+ mice present behavioral alterations 36 h prior to death and that some of those characteristics were strongly associated with a poor outcome. However, the purpose of that study was to provide researchers with a tool for improved performance of clinical assessment of CM mice, not a tool for predicting CM development. We are using the same behavioral assessment with the SHIRPA protocol to develop a mathematical model for early prediction of CM development, and our preliminary results (see below) indicate that this is feasible [Y.C. Martins, G.L. Werneck, L.J.M. Carvalho, T. Mello e Souza, D.O.G. de Souza, C.T. Daniel-Ribeiro, manuscript in preparation].

The primary screen of the SHIRPA protocol is a standard method that provides a behavioral and functional profile by observational assessment of mice with a total of 40 different tests. It is based on a protocol developed by Irwin in 1968 and indicates defects in gait or posture, motor control and co-ordination, changes in excitability

Table 1. Performance of scores based on the first screen of the SHIRPA protocol as predictive models for CM

Score	Day	$_{au}$ROC, %	Se., %	Sp., %	PPV, %
Total	4	56.91	–	–	–
	5	77.82	26.67	96.15	88.89
	6	87.33	57.58	96.00	95.00
Reflex/ Senso.	4	60.83	–	–	–
	5	46.64	–	–	–
	6	87.60	35.14	96.43	92.86
Neuro.	4	61.54	–	–	–
	5	67.11	–	–	–
	6	83.44	41.67	96.30	93.75
Motor	4	56.90	–	–	–
	5	82.16	23.53	100.00	100.00
	6	87.56	64.86	96.55	96.00
Auto. func.	4	57.00	–	–	–
	5	62.65	–	–	–
	6	71.86	35.29	96.55	92.31
Muscle tone	4	61.53	–	–	–
	5	50.51	–	–	–
	6	79.77	35.14	96.67	92.86

Se. = Sensitivity; Sp. = specificity; Total = total score; Reflex/ Senso. = reflex/sensory score; Neuro. = neuropsychiatric score; Motor = motor score; Auto. func. = autonomous function score; Muscle tone = muscle tone score.

and aggression, salivation, lacrimation, piloerection, defecation, analgesia, muscle tone and temperature. It has recently been demonstrated that the SHIRPA protocol is reproducible across time and laboratories [152, 153], and it is a reliable tool for the evaluation of the functional neurological outcome in murine models of human disease [154–160]. We evaluated the predictive performance of the SHIRPA protocol on days 4–6 of infection of *P. berghei* ANKA-infected C57Bl/6 mice. Total score and scores by functional category – reflex/sensory, neuropsychiatric, motor, autonomous function, and muscle tone – were generated as previously described [151, 155], and predictive models for CM were built using logistic regression. The area under the receiver operating characteristic ($_{au}$ROC) curve, sensitivity, specificity and predictive values were used to evaluate the model's performance (table 1).

The sensitivity is the percentage of individuals that truly have a condition among all individuals who are identified as having the condition by a test. On the other hand, the specificity is the proportion of true negative individuals among those that are identified as negative by a test. The $_{au}$ROC curve expresses the relation between sensitivity and specificity indicating the overall predictive accuracy of a test. A test with perfect predictive accuracy has a combined $_{au}$ROC curve of 1.0, whereas a test with no predictive accuracy has an $_{au}$ROC curve of 0.5. $_{au}$ROC curves of all generated scores were very poor on day 4 (<60%), showing that they could not discriminate CM^+ from CM^- mice on that day. However, $_{au}$ROC curves were higher when evaluated on day 6 (>70%) and for total (77%) and motor (82%) score on day 5. As expected, higher PPVs, which are the proportion of CM^+ mice out of all mice predicted as positive, were observed for the total score (89%, 8 true CM mice out of 9 mice) and the motor score (100%, 8 out of 8) on day 5 (table 1). For day 6, all scores gave PPVs higher than 90% (table 1). Higher PPVs indicate the possibility of early selecting mice with a high probability of developing CM. As expected, the predictive power increased with the proximity of the neurological syndrome manifestation, being more powerful within 24 h of death. The construction of new scores based on specific SHIRPA tests or different combinations of them may solve this problem improving the earlier prediction of CM.

Conclusions

Malaria continues to claim two million lives a year, mainly those of children in sub-Saharan Africa. Even if receiving appropriate care and antimalarial treatment, 10–20% of children hospitalized with CM die, and there is a high incidence of cognitive deficits in those who survive. So, there is an urgent need for improved management of CM, including effective adjunctive therapies and tools for improved clinical assessment with earlier definition of neurological involvement. Basic research using animal models of CM is expected to generate data with potential translation to the clinical setting, helping in the development of effective adjunctive therapies and prognostic tools for CM. In this overview of the CM problem, which encompassed from epidemiological and clinical perspectives to physiopathogenesis and research in animal models, we also presented data showing that clinical assessment of mice infected with a CM-inducing strain of *Plasmodia* using a well-established tool, the SHIRPA protocol, is able to predict CM development with considerable specificity. This might be a useful tool in experi-

mental research as well as serve as a basis for the trial and adoption of similar strategies for earlier determination of neurological impairment in humans with *P. falciparum* malaria.

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