

Artesunate treatment of severe pediatric malaria: A review of parasite clearance kinetics and clinical implications

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Severe malaria causes an estimated 1.24 million deaths every year, mostly in children in sub-Saharan Africa (1). Canadian pediatric infectious diseases practitioners may encounter life-threatening malaria among children emigrating from or returning from travel to the tropics. There were 195 reported cases of severe malaria in Canada between 2001 and 2012 (2), and the recent death of a malaria-infected adult in Alberta provided a reminder of the potential severity of the disease. Two large, multicentre, randomized controlled trials conclusively demonstrated a mortality benefit for the use of artesunate over quinine, the centuries-old standard, for children and adults with severe malaria (3,4). As a result, intravenous artesunate is now the treatment of choice for severe malaria, as reflected in guidelines from the WHO and the Canadian Committee to Advise on Tropical Medicine and Travel (5,6).

Severe malaria in children most often manifests as either a single, or a combination of three clinical syndromes: severe malarial anemia, cerebral malaria and/or respiratory distress. In addition, patients with a high parasite density are at elevated risk for progression to severe disease and death. In travellers from low-transmission settings, such as Canada, the parasitemia threshold suggested for close monitoring, hospitalization and parenteral therapy is >2% (6). It should be emphasized that circulating parasites represent only a fraction of the sequestered parasite biomass in severe malaria, such that this threshold is imprecise.

Artesunate is a water-soluble, semi-synthetic derivative of artemisinin, the active antimalarial component of the herb *Artemisia annua* (qinghaosu). Its use in clinical practice is expanding in malaria-endemic areas of Africa and Asia, replacing quinine as the mainstay of severe malaria treatment (7). It is available across Canada, 24 h per day, through the Canadian Malaria Network and Health Canada's Special Access Program (8). If, despite national efforts to ensure its accessibility, artesunate cannot be obtained in a timely manner for a patient with severe malaria, alternative agents (eg, atovaquone-proguanil or quinine) should be initiated pending arrival of artesunate.

Although the weight of evidence and international consensus favour artesunate treatment, some countries continue to list quinidine or quinine for severe malaria as they await regulatory approval of artesunate (9,10). Clinicians consulting widely available online (9,11) or print (10,12) treatment guidelines for severe malaria may be puzzled by the United States (US) Centers for Disease Control and Prevention's listing of quinidine as first-line therapy in the US, or quinine in the United Kingdom (Table 1). WHO-prequalified artesunate (Guilin Pharmaceutical, China) was used in major clinical trials with excellent efficacy (3); however, it has not yet obtained Health Canada, US Food and Drug Administration or European Union regulatory approval, creating a gap between the preferred evidence-based regimen and the

regimen approved by regulatory authorities. High-quality artesunate from the Walter Reed Army Institute of Research (Maryland, USA) is, nonetheless, widely used in Canada and the US, made available to Canadians through the Canadian Malaria Network (2) and to US hospitals through a Food and Drug Administration-approved investigational new drug protocol (9).

In addition to reducing mortality in patients with severe malaria, artesunate treatment also results in remarkably rapid parasite clearance. The kinetics of parasite elimination from the peripheral circulation under artesunate treatment are summarized in Table 2. Parasite clearance kinetics link the three themes discussed in the present article: postartemisinin-delayed hemolysis (PADH), a newly recognized adverse event and consequence of rapid parasite clearance; pharmacokinetics and dosing in young children who exhibit slower parasite clearance; and artesunate resistance, heralded by slow parasite clearance.

POSTARTEMISININ-DELAYED HEMOLYSIS

The safety profile of parenteral artesunate in the largest randomized trials to date was excellent, with essentially no serious drug-related adverse effects reported. Compared with quinine, the incidence of post-treatment hypoglycemia and neurological progression (new seizures, development of coma) was lower (3,4). Artesunate is, therefore, preferred not only for its superior efficacy over quinine, but also its lack of acute toxicity.

As artesunate use expanded to include nonimmune returning travellers with severe malaria, reports emerged of hemolytic anemia occurring weeks after treatment (13). This late sequela of artesunate treatment was not reported in earlier studies because they only had a three-day median for follow-up of hemoglobin levels (14).

Anemia with malaria is multifactorial, and can be due to parasite and host factors, in addition to antimalarials. For example, severe anemia (hemoglobin <50 g/L) is the most common pediatric presentation of severe malaria (15), and severe acute intravascular hemolysis ('blackwater fever') occurs in the acute phase in both quinine- and artesunate-treated patients (3). During recovery, protracted anemia was observed in up to 7% of patients even before the introduction of artesunate (16), which may be caused by bone marrow suppression and/or hemolysis due to autoimmune and nonimmune processes (17-19). Drug-induced hemolytic anemia related to glucose-6-phosphate dehydrogenase (G6PD) deficiency may also contribute to anemia.

PADH has recently been recognized as a clinically and mechanistically distinct entity that only occurs in patients with severe malaria treated with artesunate. PADH occurs well after parasite clearance and resolution of clinical symptoms, and is not consistently associated with markers of autoimmune hemolysis (eg, positive direct antiglobulin test)

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TABLE 1
Guideline confusion: summary of recommendations for treatment of severe malaria from several national guidelines

Country	Source	Statement
Global	World Health Organization (6)	Treat adults and children with severe malaria with parenteral artesunate. (Strong recommendation, high quality evidence)
Canada	Canadian Committee to Advise on Tropical Medicine and Tropical Medicine and Travel (CATMAT) (2)	"Parenteral artesunate is recommended as first-line treatment for severe <i>Plasmodium falciparum</i> malaria, with parenteral quinine as an alternative." (Good evidence from at least one properly randomized controlled trial)
United States	United States Centers for Disease Control and Prevention (9)	"Since 1991, quinidine gluconate has been the only parenterally administered antimalarial drug available in the United States." "Artesunate is recommended by the World Health Organization in preference to quinidine for the treatment of severe malaria and has been used worldwide for many years."
United Kingdom (UK)	Public Health England British Infection Association (11)	"In the UK, the treatment of choice for severe or complicated malaria is currently an infusion of intravenous quinine." "Intravenous artesunate reduces high parasite loads more rapidly than quinine and is more effective in treating severe malaria in selected situations. It can also be used in patients with contraindications to quinine. Intravenous artesunate is unlicensed in the EU."

EU European Union

or erythrocyte susceptibility to oxidative stress (eg, G6PD deficiency) (20,21). Although artesunate may cause reversible bone marrow suppression, this mechanism likely contributes minimally to postartesunate anemia (22). Instead, PADH involves a distinct mechanism of hemolytic anemia, discussed below (22).

Clinically, several patterns of anemia following artesunate treatment of severe malaria are observed, including a 'rising' hemoglobin profile following early nadir, 'persistent' anemia and late-onset extravascular hemolysis beyond day 8 (PADH pattern) (22). To distinguish PADH from the myriad causes of anemia in severe malaria, a specific case definition of PADH has been proposed, and includes: a nonrecurrent hemolytic episode occurring >7 days after initiation of treatment, with artesunate associated with a >10% decrease in hemoglobin level; haptoglobin <0.1 g/L; and either an increase in lactate dehydrogenase to >390 IU/L or a >10% rise (23).

The incidence of PADH is difficult to quantify with currently available data because of the variability in case definitions used in the literature since its relatively recent description. A systematic review of the literature up to 2014, revealed 37 cases of hemolysis and estimated an incidence rate of 13% among patients with severe malaria treated with artemisinin derivatives. However, this report did not use the optimized case definition of PADH; application of the more specific case definition would result in a lower incidence rate (22,24). The majority of these cases involved nonimmune adult returning travellers receiving intravenous artesunate. Reports of PADH from North America are surprisingly uncommon relative to Europe (24). The incidence among children with severe malaria in an endemic area has been estimated to be 7%; however, the reduction in hemoglobin in this report was <10 g/L in all but one patient (25). Again, applying the more specific definition of PADH would result in a lower incidence rate estimate.

The severity of anemia in reports of PADH is variable. In a systematic review of mostly adult cases, the median reduction in hemoglobin level was 60 g/L, and 73% of patients required blood transfusions (24). One study involving children revealed a median reduction in hemoglobin of 8 g/L, although one patient was noted to have a decrease of 42 g/L (25). No fatal outcome has been reported in the literature to date.

The mechanism of PADH has been elucidated in recent studies. Malaria parasites killed by artesunate are removed from erythrocytes by the spleen, leaving behind once-infected erythrocytes (o-iEs). These o-iEs have a 'pitted' appearance, remaining in circulation with a considerably decreased life span of seven to 21 days (26,27). This reduced life span correlates with the timing of the onset of PADH, and may account for the higher risk for PADH among patients with higher initial parasite densities in whom higher numbers of o-iEs were observed. Therefore, PADH is a predictable consequence related

to the life-saving effect of artemisinins: patients with high parasite burden rapidly cleared with artesunate, who may otherwise have succumbed to the acute infection, are left with high numbers of residual short-lived pitted erythrocytes.

PEDIATRIC PHARMACOKINETICS AND DOSING

Inspection of artesunate-clearance half-life across clinical studies illustrated slower parasite clearance in children relative to adults (Table 2). Quantitative measures of parasite clearance are affected not only by parasite genetics and drug susceptibility (28,29), but also by host determinants including patient age, organ function, drug metabolism and immunity. Therefore, lack of acquired partial immunity (premunition) in pediatric cohorts and/or age-dependent pharmacokinetic differences (30) may explain, at least in part, the slower parasite clearance in children relative to adults (31,32). Differences in pharmacokinetics, leading to lower exposure to the active metabolite dihydroartemisinin, has led to revised dosing recommendations in the 2015 WHO Guidelines for the Treatment of Malaria (6): children weighing <20 kg should receive 3.0 mg/kg/dose of artesunate, rather than the dose for larger children or adults of 2.4 mg/kg/dose (30,33).

EMERGING ARTEMISININ RESISTANCE

Artemisinin-resistant *Plasmodium falciparum* has recently emerged and spread in Southeast Asia, threatening the efficacy of first-line treatment regimens for severe and uncomplicated malaria worldwide (34-37). Resistance to artemisinins manifests as a slower parasite clearance rate in vivo (28,34,38). The parasite-clearance half-life, derived from the log-linear decline in parasite density during treatment, is now widely accepted as the best pharmacodynamic index of *P falciparum* sensitivity to artemisinins (39-43). Parasite clearance time and clearance half-life are compared across studies in Table 2. Nonsynonymous single-nucleotide polymorphisms in the propeller domain of a kelch (K13) gene are generally accepted as the major genetic determinant of artemisinin resistance in *P falciparum* (35,40,43,44). The mechanism involves increased expression of unfolded protein response pathways (eg, chaperone complexes), as well as decreased expression of proteins involved in DNA replication among mutant parasites. These K13 polymorphisms have emerged independently in multiple geographic locations in Southeast Asia, suggesting that selection pressure may drive the selection of resistant isolates as artemisinin treatment is distributed worldwide (44). To date, only limited numbers of infections with K13 mutants have been detected in Africa (45-47). One report has linked K13 mutations to delayed parasite clearance in African children treated with artesunate for severe malaria (48).

Artemisinin resistance has alarming implications for malaria control globally. Artemisinin combination therapy is the first-line agent for uncomplicated falciparum malaria, and parenteral artesunate is the

TABLE 2
Parasite clearance kinetics for artesunate in recent clinical trials

Country	Study population	n	Disease severity	Treatment	Median parasite clearance time, h	Median clearance half-life, h	Reference
Uganda	Children 1–10 years of age	71	Severe	IV artesunate*	44	4.6	Hawkes et al (39)
Malawi and Gabon	Children 0.5–10 years of age	171	Hospitalized	IV artesunate	36	NR	Kremsner et al (49)
Mali	Children 0.5–15 years of age	215	Uncomplicated	PO artesunate (4 mg/kg/day)†	24	1.9	Lopera-Mesa et al (31)
Sudan	Adults (60%) and children (40%)*	47	Severe	IV artesunate*	16.5	NR	Abdallah et al (50)
Kenya	NR	171	NR	NR	NR	2.5	Flegg et al (51)
20 sub-Saharan African countries	Predominantly children <6 years of age (77%)	15,017	Uncomplicated	PO artesunate-amodiaquine	31	2.5‡	Zwang et al (52)
Bangladesh	Adults	27	Severe	IV artesunate*	48	2.5	Maude et al (53)
Laos	Youth and adults ≥10 years of age	44	Uncomplicated	PO artesunate (2–4 mg/kg/day)	23	1.93	Mayxay et al (54)
Thailand	Adults ≥15 years of age	591	Severe	IV artesunate*	62.5	NR	Newton et al (55)
Thailand (Wang Pha)	Adults >16 years of age*	20	Uncomplicated	PO artesunate (2 mg/kg/day)*	54	2.2	Dondorp et al (34)
Thailand (Wang Pha)	Adult patients ≥18 years of age	80	Uncomplicated	PO artesunate (6–8 mg/kg/day)†	48	3.4	Amaratunga et al (28)
Cambodia (Pursat)	Youth and adults >10 years of age	168	Uncomplicated	PO artesunate (4 mg/kg/day)	78	5.9	Amaratunga et al (28)
Cambodia (Pailin)	Children 5–16 years of age (28%) and adults*	20	Uncomplicated	PO artesunate (2 mg/kg/day)*	84	5.4	Dondorp et al (34)
Cambodia (Pailin)	Children 6–18 years of age (28%) and adults	79	Uncomplicated	PO artesunate (6–8 mg/kg/day)†	78	6.0	Amaratunga et al (28)

*From the placebo arm of a randomized controlled trial; Data derived from patients in the study treated with intravenous (IV) artesunate as sole antimalarial agent; †Oral (PO) artesunate was used as monotherapy for the first 72 h of treatment, the time during which kinetic parameters were calculated; ‡Not directly reported, but calculated from published results. NR Not reported

treatment of choice for severe malaria, highlighting our current reliance on this drug class. Alternative effective and safe agents are limited, and artemisinins are believed to have contributed to the decline in malaria deaths globally over the past decade.

Nonetheless, clinicians managing patients with severe malaria acquired in Southeast Asia should continue to use artesunate for the time being. Safety advantages of artesunate over quinine favour its continued use in clinical practice. It should be emphasized that the phenotype of resistant *P falciparum* clones described to date is delayed parasite clearance, rather than nonresponse or increased mortality. Slow-clearing parasites may require longer courses of therapy or alternative antimalarial regimens. Serial quantitative determination of parasite density (eg, daily peripheral blood smear), which is the standard of care in *P falciparum* infection, will reflect parasite response to treatment. Note that the presence of gametocytes (sexual-stage parasites) alone in a peripheral smear does not imply treatment failure because this stage is less sensitive to artesunate and is not associated with symptoms or disease.

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CONCLUSION

We have summarized three relevant or emerging practice points for pediatric infectious diseases clinicians managing children with severe malaria using artesunate. First, as an unintended consequence of rapid parasite killing, clinicians should monitor for delayed hemolysis, with hemoglobin measurement weekly, up to four weeks after treatment. Second, pediatricians should be aware of the need for a higher weight-based dose in young children due to pharmacokinetic differences in artesunate metabolism, resulting in slower parasite clearance in young children. Third, artemisinin resistance associated with slow parasite clearance is emerging in Southeast Asia; nonetheless, clinicians should continue to use artesunate while monitoring for parasite clearance with serial blood smears.

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