Diagnosis, prognosis and treatment of severe falciparum malaria in African children
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Chapter 10

General discussion and conclusions
General discussion

This thesis describes studies performed between 2005 and 2011 on a number of important issues concerning the diagnosis, prognosis and treatment of severe falciparum malaria in African patients with a focus on children. The AQUAMAT study was the largest clinical trial to date in hospitalized patients with severe malaria, and provides the definite answer that artesunate is the best antimalarial treatment of severe malaria. The studies on plasma PfHRP2 in African children provide a method to define malaria-attributable disease in children with severe febrile illness in high transmission settings. The study on rapid diagnostic tests shows that these are a reliable and easy to use alternative to microscopy for the diagnosis of severe malaria in African children and highly suitable for this setting. The pharmacokinetic studies define a practical dosing regimen for artesunate and underline the need for a quinine loading dose, both particularly especially important for small children. The descriptive clinical studies provide a novel predictive severity score for children with severe malaria and show the different clinical presentation of severe malaria in case of HIV coinfection, which are of importance to the treating clinician. This chapter discusses in some detail the key findings of this thesis and aims to put these in perspective, including possible future directions for research.

The WHO estimated 890 000 malaria deaths in 2004, the majority of whom are African children.¹ A recently published systematic analysis, using a wide range of key predictors of malaria mortality into a variety of predictive models, including verbal autopsies, estimated that the total number of malaria deaths in 2004 was almost double as high.² Differences in research methodology aside, these heterogeneous estimates of the worldwide malaria mortality also reflect the challenges in diagnosing severe malaria. Improvements in the diagnosis of severe malaria are needed to quantify the burden of malaria, to define study populations in clinical studies, and to assist the clinician in the management of severe febrile illness including severe malaria.

Diagnosis of severe malaria

The WHO definition of severe malaria is based on the presence of one or more clinical severity criteria and limited laboratory markers in the presence of asexual Plasmodium falciparum parasites on the peripheral blood film.³ This definition was derived from the associated risks of death corresponding to clinical severity signs³,⁴ and was aimed towards optimal sensitivity, since untreated severe malaria approaches a case fatality of
100%. Missing the diagnosis and not treating the patient with a parenteral antimalarial may result in death and therefore sensitive parasitological tests are of utmost importance. Reliable microscopy of the peripheral blood film, the gold standard, is often not available in African health care settings.\textsuperscript{5,6} We hypothesized that malaria rapid diagnostic tests detecting malaria specific antigens would be suitable alternatives to routine microscopy in the diagnosis of severe malaria. Theoretically these tests may well perform better than microscopy, since the plasmodial antigens are freely circulating in the plasma and do not sequester like the malaria parasites.

Various types of RDT have been available, but none have been evaluated for the diagnosis of severe malaria in African children.\textsuperscript{7,8} For the AQUAMAT research project, a rapid and reliable diagnosis of severe malaria, that would be applicable across various levels of health care and transmission settings, was essential. In a substudy, we compared the performance of a \textit{Pf}HRP2- and a pLDH-based RDT versus expert microscopy for the diagnosis of severe malaria in two settings with different transmission intensities (Chapter 4). We found that the \textit{Pf}HRP2-based test was a reliable alternative to routine microscopy with a sensitivity of 96.9\% for parasite densities >100 parasites/\mu L. The \textit{Pf}HRP2 based test was more sensitive than the pLDH-based test. The sensitivity of both tests was positively correlated with the parasite density, with a significantly better performance of the \textit{Pf}HRP2 based test at lower parasite densities. The specificity of the \textit{Pf}HRP2 based was lower which is related to the longer half-life of \textit{Pf}HRP2 versus the parasite enzyme pLDH.\textsuperscript{9,10} Of practical importance, the used \textit{Pf}HRP2 based test was easier to perform, more heat-stable and cheaper than the used pLDH based test.\textsuperscript{11-14}

The prior probability of having severe malaria depends on malaria transmission intensity and the prevalence of alternative diseases causing severe febrile illness. The malaria transmission intensity in the study site of Beira was lower than in Muheza, reflected by the different age and clinical pattern of severe malaria (Chapter 4). The reported HIV-prevalence was much higher; 30\% in sentinel surveys in pregnant women in the city of Beira.\textsuperscript{15} Mozambique was in the top-3 of countries with the highest estimates of mortality in children >5 years and adults in 2000 and 2010.\textsuperscript{2} This was one of the reasons for including adult patients in the AQUAMAT trial in Mozambique (Chapter 7). However, during the almost 5 year study period we recruited only 68 adults with severe malaria, despite extensive screening of clinically suspected severe malaria patients. Although our study was not designed as an epidemiological survey, our observations suggest that the absolute numbers of adults dying from severe malaria are not that high, even though HIV might increase the risk of severe disease and increase the case fatality of severe malaria (Chapter 7). The malaria rapid diagnostic tests performed well in children in this setting.
(Chapter 4, 7), but overdiagnosis of severe malaria in adults was more common. Not restricted to adults, our findings are in line with other studies reporting a low predictive value of clinical signs and symptoms for peripheral blood parasitaemia.\textsuperscript{16} Pneumonia, meningitis, bacteraemia and other HIV-associated severe illnesses may present with similar signs and symptoms to severe malaria.\textsuperscript{17-20}

In malaria-endemic areas, an additional challenge in the diagnosis of severe malaria is the aspecificity of presence and malaria parasites in the peripheral blood film due to the development of host partial immunity early in life.\textsuperscript{21,22} Overdiagnosis of severe malaria is also an important problem in young children in Africa who are at highest risk of death of malaria or other severe febrile illness.\textsuperscript{1,18,23,24}

\textit{Plasmodium falciparum} specific sequestration in the second half of the erythrocytic cycle renders the more pathogenic mature parasites invisible to the microscopist assessing the peripheral blood slide.\textsuperscript{25} Peripheral blood parasite density is therefore not a reliable measure of total parasite burden and severity of disease.\textsuperscript{4,26} \textit{Pf}HRP2 is produced by \textit{Plasmodium falciparum} and approximately 90% is released into the circulation at the moment of schizont rupture.\textsuperscript{27} Since this is distributed through the total plasma volume, quantitative plasma \textit{Pf}HRP2 can be considered as a measure of the total parasite burden of the preceding asexual cycle.\textsuperscript{28} We compared the distributions of peripheral blood parasitaemia versus plasma \textit{Pf}HRP2 concentrations in control, asymptomatic, uncomplicated and severe malaria cases from a community and a nearby hospital in a high transmission setting in Tanzania (Chapter 6). We showed a stepwise increase in plasma \textit{Pf}HRP2 concentrations according to the severity of infection, ranging from asymptomatic parasitaemia, uncomplicated malaria to severe malaria. There was substantially less overlap in plasma \textit{Pf}HRP2 distributions between these groups compared to the distributions of peripheral blood parasitaemia. Notably, peripheral blood parasitaemia distributions were entirely overlapping between patients with uncomplicated and severe malaria. The distinct plasma \textit{Pf}HRP2 distributions were used to model the malaria-attributable proportion of severe disease, in order to distinguish patients with “true” severe malaria from those with coincidental peripheral blood parasitaemia in whom severe febrile illness is caused by an alternative disease. \textit{Pf}HRP2 levels above 1000 ng/ml corresponded to a malaria-attributable fraction of 99% with a sensitivity of 74%. The proportion of malaria-attributable disease declined at lower \textit{Pf}HRP2 concentrations. Below 200 ng/ml an alternative diagnosis was suggested in >10% of patients and below 50 ng/ml in >50% of the patients (Chapter 6).

These results are in strong agreement with the \textit{Pf}HRP2 thresholds identifying high or low probability of alternative disease derived from a mechanistic model based on the malaria-
attributable mortality in the AQUAMAT population (Chapter 5). In the latter study, we found that a \( Pf \)HRP2 concentration >1000 ng/ml corresponded to a malaria-attributable proportion >95%, and that a \( Pf \)HRP2 concentration <174 ng/mL suggested an alternative illness in >10% and was responsible for more than 50% of the mortality. Since >60% of patients with severe malaria in the AQUAMAT study had \( Pf \)HRP2 concentrations >1000 ng/mL, we conducted the former study incorporating the asymptomatic carriers and uncomplicated malaria patients for a more accurate definition of the probability of non-malarial disease with low plasma \( Pf \)HRP2 concentrations (Chapter 6). It is reassuring that the identified \( Pf \)HRP2 thresholds denoting a high or low probability of alternative disease respectively were consistent between these two studies using different study populations and modelling techniques.

This work contributes to a growing body of evidence about the diagnostic utility of \( Pf \)HRP2 in African children. A recently published study from Malawi reported that a \( Pf \)HRP2 concentration >1700 ng/ml had sensitivity of 98% and a specificity of 94% for cerebral malaria with histological evidence of sequestration.\(^{29}\) Compared to malarial retinopathy, the sensitivity and specificity of this plasma \( Pf \)HRP2 threshold were 90% and 87%, respectively. Their chosen \( Pf \)HRP2 thresholds seem to be within the plausible ranges of our findings, although our studies included also non-cerebral malaria. We estimated that with plasma \( Pf \)HRP2 concentrations between 1000 to 3162 ng/mL, the probability that death was caused by severe malaria varied between 83% and 93% (Chapter 5). In this large study we described a U-shaped relation between \( Pf \)HRP2 and the probability of in-hospital death with a nadir in case fatality at a plasma \( Pf \)HRP2 concentration of 174 ng/mL. Below this threshold, we found an increasing risk of death with decreasing plasma \( Pf \)HRP2 concentrations, presumably representing deaths caused by non-malarial disease. This assumption is supported by increased frequency of positive blood cultures in patients with low \( Pf \)HRP2 concentrations (Chapter 6). On the other hand, positive blood cultures were also overrepresented in patients with very high \( Pf \)HRP2 concentrations (>5000 ng/mL) indicating concomitant sepsis in patients with severe malaria.

**Prognosis of severe malaria**

In line with studies in Asian adults,\(^{28,30}\) we reported that plasma \( Pf \)HRP2 has strong prognostic significance in African children (Chapter 5). We showed a 20% increase in risk of death per unit increase in \( \log_{10} Pf \)HRP2 above 174 ng/mL. These studies and the Malawian study taken together, confirm the central pathophysiological role of the total
parasite burden including the sequestered parasite burden.\textsuperscript{28-30} This severe malaria in Asian adults as well as African children, whereas potential differences in pathophysiology of severe malaria between these groups have been much debated, related to differences in clinical presentation and findings of autopsy studies.\textsuperscript{3,23,31-33} Autopsy studies have demonstrated the sequestration of infected red blood cells in the vital organs and particularly the brain.\textsuperscript{34} However, assessment of the sequestered parasite burden in the alive patient was only possible by detection of malarial retinopathy in patients with cerebral malaria.\textsuperscript{23,35,36} Ophthalmoscopy requires equipment and considerable expertise and will therefore not be practicable in most clinical settings in malaria-endemic areas. Plasma \textit{Pf}HRP2 reflects the total parasite burden including the sequestered parasites in the entire clinical spectrum of severe malaria and is closely correlated with outcome. This can be a useful tool for the case definition of severe malaria in studies addressing the pathophysiology in severe malaria, as well as for epidemiological and intervention studies, and also assist the clinician in diagnosing and treating individual patients. Our data call for the development of a semi-quantitative \textit{Pf}HRP2 rapid diagnostic test, with suitable plasma \textit{Pf}HRP2 thresholds for the case definition of severe malaria. We would propose plasma \textit{Pf}HRP2 thresholds of 200 and 1000 ng/ml, indicating high (>10\%) and low probability (1\%) of alternative disease, respectively. Particularly the lower threshold may depend on the level of malaria-specific immunity and the prevalence of alternative disease in the population. Extending to low transmission areas, these effects may be balanced out by the strong association of malaria and bacteraemia, where the burden of bacterial disease has reported to be lower.\textsuperscript{37} In addition, the prognostic value of \textit{Pf}HRP2 was similar in low versus high transmission settings (Chapter 5). It will be impractical to define different thresholds for every transmission, clinical care or research setting; therefore our proposed thresholds will be a compromise depending on the planned use of the test. In addition to plasma \textit{Pf}HRP2, the large dataset of the AQUAMAT study was used to identify other clinical and laboratory indicators that could predict the outcome of severe malaria (Chapter 3). We identified 5 highly significant independent predictors of mortality, including acidosis indicated by a large base deficit, cerebral manifestations, elevated blood urea nitrogen and signs of chronic illness at admission. The overall mortality of severe malaria was 9.8\% which increased up to 43\% in presence of the 3 most frequent predictors cerebral malaria (coma and/or convulsions), acidosis (BE<-8 mmol/L) and elevated blood urea concentrations (BUN≥20 mg/dL). Numerous other studies have identified cerebral manifestations and acidosis as important prognostic factors, which have been associated with sequestration of infected red blood cells leading to impairment
of microcirculatory flow, causing tissue hypoxia and anaerobic metabolism.\textsuperscript{4,35,38,39} The association of an elevated BUN concentration with poor outcome has scarcely been reported in children.\textsuperscript{26,40} We also confirmed the prognostic value of elevated BUN concentrations in the substudy in Mozambique, wherein the logistic regression model was adjusted for age and a high proportion of patients was HIV-coinfected (Chapter 7). The origin and significance of the elevated BUN levels remains unclear. Renal failure is a common complication in adults with severe malaria,\textsuperscript{41,42} but this is uncommon in African children.\textsuperscript{3} Protein break down might contribute to elevated BUN concentrations and hence be more prominent in children with malnutrition and HIV. For many years, hypovolaemia has been postulated to contribute to the metabolic acidosis and be of relevance in the pathophysiology of severe malaria.\textsuperscript{43-45} Hypovolaemia could also attribute to elevated BUN concentrations. However, a recently completed large randomized trial on the treatment of children with impaired perfusion and shock showed that a fluid bolus with either normal saline or human albumin increased mortality in children with severe malaria or other severe infection compared to no fluid bolus.\textsuperscript{46} Prognostic indicators can be of practical relevance to the clinician or clinical researcher and plea for the use of point-of-care test, but may be aspecific clues to the underlying pathophysiology of severe malaria.\textsuperscript{47} More research into the pathophysiology of malaria is urgently needed in order to understand the specific pathways leading to death and identify new targets for treatment. To date, apart from supportive care that includes glucose for hypoglycaemia and blood transfusion for anaemia, there are no adjunct therapies that reduce mortality from malaria.\textsuperscript{48} Treatment with an effective antimalarial remains the pillar in the management of the severe malaria.

\section*{Treatment of severe malaria}

The AQUAMAT study was the first large randomized controlled trial demonstrating a life-saving treatment for severe malaria in African children (Chapter 2). This trial included 5425 patients in 11 sites in 9 African countries across various transmission settings and was the largest trial in the treatment of severe malaria ever. In African children, we found that parenteral artesunate reduced the mortality by 22.5\% compared to parenteral quinine. In addition to the SEAQUAMAT trial,\textsuperscript{49} and various earlier small comparative studies comparing artesunate versus quinine for the treatment of severe malaria,\textsuperscript{50-53} the evidence is conclusive that artesunate is a better treatment than quinine.\textsuperscript{54}
Artesunate has a greater parasitocidal effect than quinine, which is related to the broader stage specificity of action.\textsuperscript{55} The artemisinins kill the young stage parasites, before they mature, and hereby prevent the sequestration of the infected red cells in the vital organs leading to potentially fatal microvascular obstruction. This is supported by the AQUAMAT subgroup analysis, showing that the benefit of artesunate was greater in patients with hyperparasitaemia on the peripheral blood film. Notably, children with the highest total body parasite burden (PfHRP2 values >2300 ng/ml) showed the greatest benefit of artesunate (Chapter 5). In this subgroup, the treatment effect was similar to that observed in Asian adults,\textsuperscript{49} whereas the overall treatment benefit in the AQUAMAT trial was lower than that observed in the SEAQUAMAT trial due to the dilution of the study population with non-malarial disease. The life-saving benefit of artesunate was not at the expense of more children with neurological sequelae as assessed at 28 days post-discharge (Chapter 2), although a recent study challenges the appropriate length of follow up for the detection of malaria-related neurological sequelae.\textsuperscript{56}

Parenteral artesunate has several other advantages compared to parenteral quinine. It is safe and easy to administer and can be given by i.v. or i.m. route. Artesunate is also a safe drug, since no serious adverse effects were identified in this study or previous studies with similar dosing.\textsuperscript{49} The only known side-effect is neutropenia when artesunate is given at high dose (6 mg/kg) during 7 days,\textsuperscript{57} which dose will not be reached at the current recommended dosing regimen. The current dosing regimen has been derived from adult studies and our population pharmacokinetic (POPPK) assessment of intramuscular artesunate was the first to examine the covariates on the pharmacokinetic (PK) profiles of artesunate and its active metabolite dihydroartemisinin in African children (Chapter 9).

Following an intramuscular injection of artesunate, artesunate and dihydroartemisinin exposure were lower in small children. Increased clearance of antimalarial drugs resulting in lower exposure has been observed in other antimalarials,\textsuperscript{58,59} and may contribute to the development of antimalarial drug resistance.\textsuperscript{60,61} Independently, the presence of anaemia may aggravate lower exposure in young children (Chapter 9). This has not yet been observed in other studies and may be a drug-specific phenomenon due to iron-mediated degradation of the artemisinin peroxide bridge.\textsuperscript{62}

We proposed a body weight-adjusted and practical dosing regimen for intramuscular artesunate in children from 6 to 25 kg. Based on our experience from the AQUAMAT trial, we considered various practical dosing issues like the vial size and the minimum volume of prepared drug solution that can accurately be administered. This dosing scheme may assist in the implementation of artesunate in malaria-endemic countries.
Until parenteral artesunate becomes widely available, quinine remains commonly used and is a suitable alternative for the treatment of severe malaria. Despite longstanding and wide-spread use of quinine, there is no evidence of the development of high-grade resistance. Since the available body of evidence is small for treatment of one of the most life-threatening diseases in sub-Saharan Africa, we re-addressed the PK co variates and dosing regimens of quinine with POPPK modelling methods assessing a wide range of co variates. A loading dose of 20 mg/kg has been recommended for over a decade, yet has not been implemented everywhere due to toxicity concerns. More than 85% of patients reached therapeutic quinine blood concentrations (above 8 mg/L) after an initial loading dose, whereas only 30% did so if a routine dose (10 mg/kg) of quinine was given (Chapter 8). Intramuscular quinine was rapidly and reliably absorbed and none of the studied covariates affected the PK parameters. Also for quinine, body weight was identified as a co variate; however this did not affect therapeutic drug levels. Quinine has reliable pharmacokinetics at the current recommended dosing regimen. If artesunate is unavailable and quinine is used, then a loading dose should be given unless a loading dose was already given within 24 hours before admission or a routine dose was given within 12 hours prior to admission.

Conclusions and recommendations for clinical practice and research

PfHRP2-based RDTs are an acceptable alternative to routine microscopy for the diagnosis of severe malaria in African children. In settings where reliable microscopy is available, negative RDT results should be confirmed by microscopy and effectuate the search for an alternative diagnosis.

Plasma PfHRP2 concentrations have prognostic significance and can distinguish severe malaria from alternative diseases with coincidental peripheral blood parasitaemia in moderate to high malaria-endemic areas. In parasitaemic children with clinically suspected severe malaria and a plasma PfHRP2 concentration $<200$ ng/mL, the probability of non-malarial illness is greater than 10% and warrants additional diagnostic tests and treatment with effective antibiotics. A plasma PfHRP2 concentration $>1000$ ng/mL indicates a very high probability of severe malaria ($>99%$), although concomitant sepsis may occur.

In malaria-endemic areas with a high HIV prevalence, HIV testing is recommended in the diagnostic work-up of clinically suspected malaria. It will assist in the contemplation of alternative HIV-related diagnoses. In addition, severe malaria in HIV-coinfected
patients presents with a higher parasite burden, more severe disease and carries a higher case fatality rate.

In African children with severe malaria, cerebral malaria (coma and/or convulsions), acidosis (base excess<-8 mmol/L), elevated BUN concentrations (≥20 mg/dL) and signs of chronic disease (wasting or oedematous malnutrition, generalized lymphadenopathy, oral candidiasis) are independent predictors of a poor outcome.

Artesunate reduces the mortality of severe malaria compared to quinine with 22.5%. Parenteral artesunate is now the antimalarial treatment of choice for children as well as adults with severe malaria. It is safe and easy to administer and has no serious side effects. The recommended dose is 2.4 mg/kg at admission, after 12 hours and then once daily until the patient is able to take oral antimalarial treatment. Body weight and Hb affected the clearance of dihydroartemisinin (the active metabolite of artesunate), resulting in a lower exposure in young children (<15 kg). We propose a body weight-adjusted practical dosing regimen for children. Where parenteral artesunate is not available, parenteral quinine including a loading dose is a suitable alternative with reliable pharmacokinetics. In all children with severe malaria, routine administration of broad spectrum antibiotics is recommended. Prompt and appropriate treatment of sepsis could further reduce the mortality of severely ill children with malaria.

Future perspectives and implications for health policies and research

Over the past few years, an increasing number of reports have shown declining malaria transmission in several parts of Africa. There are concerns that international efforts to fight malaria will lose interest now mayor improvements have been made to control malaria and economic recession affects donor funds. With declining malaria transmission, the acquisition of malaria-specific immunity is delayed and severe malaria with high case fatality will continue to be a problem in all age groups, with an extended risk to older children and adults. To further reduce malaria mortality, ongoing research is needed to improve our understanding of the pathophysiology and ameliorate the diagnosis and treatment of severe malaria. Since most patients suffering of severe malaria live in sub-Saharan Africa, strategies should be tailored to these settings.

Plasma PfHRP2 is an ideal biomarker for the development of a (semi-) quantitative RDT for severe malaria, because of its predictive value for outcome and its ability to distinguish severe malaria from non-malarial disease in parasitaemic children in moderate to high transmission areas. Currently existing whole blood PfHRP2-based
RDTs show substantial differences in performance,\textsuperscript{14} which might be related to the test-specific \textit{Pf}HRP2 antibodies or the presence of \textit{Pf}HRP2 deletions or polymorphisms in the parasite population, amongst other factors.\textsuperscript{71-73} We have assessed the possibility of \textit{Pf}HRP2 (and \textit{Pf}HRP3) deletions and the relationship of \textit{Pf}HRP2 polymorphisms with plasma \textit{Pf}HRP2 concentrations in a subset of AQUAMAT patients with low \textit{Pf}HRP2 concentrations and high peripheral blood slide counts.\textsuperscript{74} In agreement with findings from Baker et al.,\textsuperscript{75} we did not find evidence for \textit{Pf}HRP2 deletions in field isolates causing severe malaria in African children. In addition, sequence polymorphisms were not a significant cause of variation in plasma \textit{Pf}HRP2 concentration. This is promising for the utility of (semi-) quantitative plasma \textit{Pf}HRP2-based RDTs. An evaluation of various \textit{Pf}HRP2-based rapid diagnostic tests for the semi-quantitative measurement of plasma \textit{Pf}HRP2 is currently underway. In line with WHO methods for RDT testing, we recommend the use of patient-derived plasma \textit{Pf}HRP2 standard curves over test calibration with recombinant \textit{Pf}HRP2 standards.\textsuperscript{76}

With declining malaria transmission and an increasing number of patients living with HIV/AIDS in sub-Saharan Africa, distinguishing malaria from other causes of severe febrile illness is increasingly important. A \textit{Pf}HRP2-based (semi-) quantitative rapid diagnostic test would therefore be a first useful test. Since bacteraemia has been associated with a higher case fatality rate than severe malaria alone,\textsuperscript{19,77} studies investigating alternative causes of severe febrile disease, including in HIV-positive patients, are urgently needed. Such studies are likely to be challenged by the low sensitivity of blood cultures, which is the gold standard for the detection of bacterial disease. Studies identifying and including biomarkers of bacterial disease are warranted, of which procalcitonin might be one of the most promising.\textsuperscript{78,79}

Despite the challenges to diagnose bacterial disease, our findings suggest that bacteraemia and malaria parasitaemia commonly co-exist in severely ill African children. Positive blood cultures in severely ill children with a low parasite burden likely represent alternative disease in children with asymptomatic parasitaemia, whereas bacteraemia in children with a high parasite burden represents concomitant illness. Routine administration of broad spectrum antibiotics is therefore recommended in the treatment of severe malaria in African children. Prompt and appropriate treatment of sepsis could further reduce the mortality of severely ill children with malaria parasitaemia, which was still 8.5% in children treated with parenteral artesunate. Further trials are needed to assess which antibiotic and regime would be most suitable for that.

Our studies support the central role of red blood cell sequestration in the pathophysiology of severe malaria. Improvement of the microcirculation may be an important target.
for adjunctive malaria treatment. A potential adjunct treatment under study is a depolymerized heparin with strongly reduced anticoagulant properties that has been shown to inhibit merozoite invasion of the erythrocytes and the microvascular cytoadherance of *P. falciparum* infected erythrocytes.\textsuperscript{80}

The recently confirmed artemisinin resistance in Western Cambodia poses a threat to the treatment of severe malaria, and efforts to reduce malaria mortality and the burden of malaria worldwide.\textsuperscript{81} Hyperparasitaemia and reduced ARS/DHA exposure in young children are potential population risk factors that may contribute to development of artemisinin drug resistance.\textsuperscript{61} The findings of our POPPK study suggest that the current dose of artesunate should definitely not be reduced.\textsuperscript{82,83} Additional POPPK studies are urgently needed to confirm our findings, identify other potentially relevant co variates and optimize the artesunate dosing regimen for African children.

Lastly, the translation of research findings demonstrating life-saving interventions in the treatment of severe malaria has been very slow. Although usually a body of evidence consisting of many studies and expert opinion changes medical practice, rather than a single large randomized controlled trial like the AQUAMAT study. Not only drug efficacy, but also costs will convince policy makers to adopt parenteral artesunate for the treatment of severe malaria. An economic analysis conducted alongside the AQUAMAT showed that the cost of averting malaria-related deaths in sub-Saharan Africa by switching from quinine to artesunate was extremely low, with a mean value of US$ 123 per death averted.\textsuperscript{84} This is very cost-effective compared to other interventions aimed to reduce malaria mortality, and in view of the high malaria burden in sub-Saharan Africa, also affordable.\textsuperscript{84}

Another hurdle in the implementation of artesunate has been overcome since the Chinese factory that developed artesunate (Guilin Pharma) has improved the manufacturing process and the consequent quality of the formulation which has led to WHO GMP prequalification. The factory claims that it can produce enough artesunate to fulfil the demand and has promised to keep costs at or below current prices. Meanwhile the US Army project has also developed a GMP formulation. Medicines for Malaria Venture (MMV) has liaised with WHO, Guilin Pharma and Mahidol-Oxford Tropical Medicine Research Unit in order to facilitate the implementation of artesunate in the field, including the drug registration issues and training materials for local health care staff. It is expected that within the next few years, artesunate will replace quinine for the treatment of severe falciparum malaria everywhere in the world.
General discussion and conclusions

References


