

Experts Group Meeting on delayed haemolytic anaemia following treatment with injectable artesunate

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Meeting objectives

After an initial review of the available reported cases of delayed anaemia.

- Presentations and discussion on the possible mechanisms of action and the need for additional non-clinical/mechanistic studies.
- Next steps proposed by the pharmaceutical sponsors of injectable artesunate drugs.
- Recommendations.

Summary of recommendations

Background

Injectable artesunate is a life-saving therapy in severe *Plasmodium falciparum* malaria, providing a significant reduction of mortality vs quinine, reducing deaths by 34.7% in the Asian SEAQUAMAT trial and by 22.5% in the African AQUAMAT trial.^{1,2}

A number of cases of delayed haemolytic anaemia have been identified following treatment of severe malaria with injectable artesunate. Initially, this syndrome was identified in travellers returning to Europe, but there is also emerging evidence of delayed anaemia occurring in patients from malaria endemic regions of SE Asia and Africa. Delayed haemolytic anaemia does not appear to be specific to a particular injectable artesunate preparation. Reports are not restricted to one manufacturer or formulation, with delayed haemolytic anaemia described for injectable (i.v. and i.m.) artesunate, intra-rectal artesunate and oral artemether-lumefantrine.

Currently available data are mainly from retrospective studies. In particular, delayed anaemia has been defined differently across the available studies, making comparison of data complex. Severe malaria has been defined differently across studies and this also complicates analysis. Thus, the frequency of this syndrome cannot be determined and its specific causes are still unclear.

It should be noted that all reported cases of delayed haemolytic anaemia after injectable artesunate have been managed successfully with subsequent patient recovery. While a number of cases have required transfusions, there have been no reports of fatal outcome. However, this must be considered together with the limited duration of the controlled clinical trials and limited pharmacovigilance in Africa.

Conclusion

- Currently available information regarding haemolytic anaemia in patients receiving artesunate is limited, generally of low quality and therefore does not support a change to the current recommendation of injectable artesunate over quinine for severe malaria.
- However, healthcare professionals should be made aware of the potential for haemolytic anaemia and patients monitored for up to one month post treatment. Additional work to determine the frequency and severity of haemolytic anaemia in patients receiving injectable artesunate is warranted including assessment of the follow up oral regimen.
- The need for the continued use and further adoption of injectable artesunate as a life-saving treatment should be emphasised.

Action taken

Intensified pharmacovigilance is being conducted by artesunate drug manufacturers/ pharmaceutical sponsors. US agencies are disseminating information to practitioners to reflect the need for patient monitoring for up to one month after treatment start. Multiple initiatives in Europe and US/ Canada also involving manufacturers/ product sponsors/ license holders are promoting web-based spontaneous reporting systems.

Recommendations

- The report from this meeting should be shared for review by the WHO Technical Expert Group which is updating the Malaria Treatment Guidelines.
- Physicians need to be made aware of the possibility of delayed haemolysis after injectable artesunate for severe malaria, and the need for continued monitoring of patients, particularly those with high pre-treatment parasitaemia. However, the high clinical benefits of artesunate should be emphasised compared to quinine to ensure the continued use and further adoption of injectable artesunate as a life-saving treatment in severe malaria.
- For data to be comparable across clinical studies, a consistent definition of delayed haemolytic anaemia and of severe malaria is required.
- Mechanistic studies on the effect of artesunate on erythrocyte production, destruction and regeneration should aim to result in measures that improve clinical outcomes.
- Prospective clinical trials need to be conducted in different patient populations. The objective of clinical studies should be to define the frequency of delayed anaemia, identification of prognostic factors and to examine interventions that may reduce the frequency of delayed anaemia. This includes determining the appropriate ACT dose suitable as follow on therapy following multiple doses of injectable artesunate to assess if there is a dose–response relationship with post-treatment delayed anaemia.
- Standardisation of methods for safety reporting systems would enable/ improve data collection.

Summary of data presented:

Clinical investigators and other experts were invited to present summaries of their data and scientific hypotheses related to haemolytic anaemia in patients with severe malaria receiving artesunate. The following is a summary of the data and perspectives shared by these experts.

Clinical observations

Most malaria patients will have an initial decrease in haemoglobin (Hb) over the first few days of anti-malarial therapy, with Hb levels usually in recovery by day 7 after therapy start. Clinical symptoms of anaemia may not be evident. In the subset of patients who develop a late haemolytic reaction after artesunate treatment, the majority exhibits the following haemolysis pattern:

After recovery from initial haemolysis, there is a second decrease in Hb with a nadir at around day 15 with increased lactate dehydrogenase (LDH) and low haptoglobin indicating intense haemolysis. Patients may initially appear to have recovered from their severe malaria and been discharged from hospital, returning after 1–3 weeks with symptoms of anaemia (fatigue, shortness of breath, pale pallor, lower extremity oedema). The patient recovers spontaneously, but many patients require blood transfusions.

A few cases have also been reported with a different pattern of haemolytic anemia as described below:

Initial haemolysis is intense and persisting, requiring repeated transfusions, and Hb levels fail to recover despite parasite clearance; LDH remains elevated and haptoglobin low. Haemolysis continues for up to 30 days post first dose and in some cases is refractory to blood transfusion, though the patient eventually recovers. Such patients are unlikely to have been discharged from hospital following the treatment of their severe malaria.

Potential mechanisms of action

Anaemia can result from erythrocyte destruction (haemolysis) and/or inhibition of erythropoiesis (bone marrow suppression).

1) Haemolysis

a) Erythrocyte pitting: Preliminary evidence suggests that the parasitocidal mechanism of action of artesunate could lead to a delayed haemolysis. Uniquely, artesunate is able to target ring-stage parasites, preventing their maturation and inhibiting erythrocyte sequestration. This mechanism explains the rapid action of artesunate and its beneficial effect on mortality and other clinical outcomes. Most of the non-viable ring stage parasites are cleared by the spleen by 'pitting' of erythrocytes whereby the parasite is removed and the erythrocyte resealed. These erythrocytes are, therefore 'spared' from immediate destruction, but they have a reduced lifespan of about 7–15 days, at which point they are removed from the circulation by the spleen. This delayed destruction of 'once infected' erythrocytes corresponds with the time course of recurrent anaemia seen clinically.

- Patients with high pre-treatment parasitaemia appear to be at higher risk of delayed haemolysis linked to the erythrocyte pitting, but there is not an absolute correlation.

b) Immunopathologic: Although at present there is no clear evidence for an immunologic component to haemolysis following injectable artesunate, this possibility cannot be ruled out.

c) Other possible mechanisms of haemolysis require further investigation, e.g. direct toxic effects of the drug or drug metabolites with a longer half life.

2) Bone marrow suppression

a) Inhibition of erythropoiesis: In *in vitro* models, artemisinins inhibit growth and differentiation of human proerythroblasts and basophilic erythroblasts by targeting the pathway of Hb synthesis. Malaria can also inhibit erythropoiesis, and so the clinical picture may reflect drug- and disease-mediated processes.

- Patients receiving higher artesunate doses appear to be at higher risk of bone marrow suppression, though further data are required.

b) Other possible mechanisms of bone marrow suppression require further investigation. For example, there may be an effect of the drug on bone marrow macrophages, which regulate erythroid differentiation, and on leukocytes.

The search for a possible cause for the observed anaemia is further complicated by the possibility of the production of different artesunate/ artemisinin metabolites with different, and possibly longer half lives. Metabolisation may further depend on individual patterns of drug metabolism in different individuals.

Presentations and discussion

1. Review of the efficacy of injectable artesunate versus injectable quinine Tsiri Agbenyega

Two major studies resulted in the change from quinine to artesunate as standard of care in severe *P. falciparum* malaria:

- SEAQUAMAT: South East Asian Quinine Artesunate Malaria Trial.¹
 - Multi-centre, open-label, randomised controlled trial comparing parenteral artesunate and parenteral quinine in (mostly) adults and children from Bangladesh, Myanmar, India and Indonesia.
 - ▶ Absolute reduction in mortality with artesunate of 34.7% (95% CI 18.5–47.6; $P = 0.0002$).
- AQUAMAT: African Quinine Artesunate Malaria Trial.²
 - An open-label, randomised trial comparing parenteral artesunate and parenteral quinine in children (<15 years) from 11 centres in nine African countries.
 - ▶ Relative reduction in mortality with artesunate of 22.5% (95% CI 8.1–36.9; $P = 0.0022$).
 - ▶ Artesunate also reduced the incidence of convulsions and coma. These are often associated with subsequent neurological sequelae.

Conclusion: Artesunate results in a substantial reduction in mortality from severe *P. falciparum* malaria compared with quinine.

2. Reported cases of delayed anaemia

Data are summarised at the end of this section in Table 1.

2.1. Initial European cases Thomas Zoller

Retrospective case series of patients treated in Europe in TropNet centres 2006–2010.³

- Patients were returning travellers, treated with Guilin i.v. artesunate (non-GMP) either for 7 days, or for 3–4 days following a change in the recommendations. All 25 patients were treated successfully with rapid parasite elimination and no haemodynamic effects or allergic reactions.
- Treatment associated haemolysis was not pre-defined as these were the first reported cases of unusual haemolytic activity after anti-malarial therapy (as compared to treatment with quinine). Treatment-associated haemolysis with artesunate was noted to be a recurrence or persistence of haemolysis after clearance of parasitaemia and completion of anti-malarial treatment.
- The frequency of delayed haemolysis was 6/25 (24%) in this retrospective case series; 5/6 received blood transfusion. All patients recovered.
- Onset of haemolysis was on day 14–31 post first dose, with a duration of ± 10 days in most cases. There were two main patterns of haemolysis:
 - Recurring (n = 3): Initial decrease in Hb and increase in LDH, then stabilisation followed by decrease in Hb around day 15 with increased LDH and an intense haemolytic reaction. Recovers with blood transfusion.
 - Persisting (n = 3): Intense initial hemolysis, with LDH remaining elevated after acute phase; continuing haemolysis for around 30 days, with resistance to blood transfusion.
- Patients with haemolysis received a higher dose of artesunate (12.8 mg/kg \pm SD 2.89) vs those without haemolysis (7.6 mg/kg \pm SD 3.3; $P = 0.006$). Note that the difference in dose was driven by a change in treatment guidelines and does not reflect disease severity.
- The relationship to parasitemia has not been investigated formally, but the impression from the investigators was that there was no clear correlation. However, all patients had high initial parasitemia (range 5%–51% parasitized erythrocytes).

- *Investigations:* Coomb's test was negative. Reticulocytes were elevated at the time of detection of the recurrent or persistent haemolysis. There were no changes in erythrocyte morphology. There was no relationship to G6PD deficiency. The clinical presentation was not typical of black water fever.
- *Immunology:* Drug-induced immune-haemolytic anaemia via drug-dependent or drug-independent haemolysis was investigated. Plasma and serum samples from time of treatment, time of haemolysis and convalescence were challenged with urine and artesunate, but there was no antibody reactivity. These investigations were limited because they were performed on recovered frozen samples, they were not systematic serial samples and any metabolites occurring in serum rather than urine would remain undetected.
- *Note:* One case with complement-loaded erythrocytes after receiving artemether-lumefantrine is being investigated further.

Comments

- Analysis of neutrophil counts could indicate whether there was any direct artesunate toxicity.

Conclusion: An immunopathogenic mechanism appears possible. This has not been demonstrated, but cannot be ruled out. Such a mechanism might be generated by unknown metabolites or contaminants and possibly genetic differences in drug metabolism.

2.2. Dutch/ Belgium cases Peter J De Vries

Retrospective analysis of patients included in a patient registry treated with artesunate.⁴

- Patients were returning travellers.
- No GMP product was available in Europe, so an exemption was obtained to use Malacef[®] (artesunate from Guilin) imported by ACE Pharmaceuticals on a named patient basis.
- This was not a prospective trial and so there were no fixed criteria for indication or patient selection, however, haemolysis was generally defined as increased reticulocyte counts, unconjugated bilirubin and LDH and decreased haptoglobin and Hb values.
- 7/55 (13%) patients with severe malaria had delayed anaemia; 4/7 received blood transfusion. All patients recovered. Treatment with steroids (n = 2) was not shown to be effective.
- The Hb nadir occurred between days 8–30, but was usually around day 13. In six cases, haemolysis was late onset (recurrent). In the one case of persistent anaemia, the patient received seven blood transfusions (24 units of packed red cells).
- All patients who had delayed anaemia had baseline hyperparasitaemia (11–34%).
- *Investigations:* There were no relevant findings from Coombs test.

Conclusion: Delayed haemolysis does occur after treatment of severe malaria with i.v. artesunate. However, as the drug is rapidly cleared, the delayed haemolysis cannot strictly be termed an adverse event. It is possible that there is a long-lived artesunate metabolite, but there is no evidence for this at this time.

Action taken

- Standard of care has been amended to follow patients for at least one month.
- Pharmacovigilance is being initiated for the named patient programme using an internet-based data registry.
 - Expanding use of i.v. artesunate across Europe is challenging because of differences in the programmes that allow its use without an EMA authorisation.
 - Collection of safety data is obligatory, but other data may be lacking and the information collected cannot be presented to regulatory authorities as research.

- Data has been published and records are kept by ACE Pharmaceuticals. Data have been forwarded to the Netherlands health care inspectorate, but not to the Uppsala Monitoring Centre.

2.3. German cases Thierry Rolling/ Jakob P Cramer

Prospective study (started mid-2011) of all severe malaria cases treated with artesunate with follow up for at least 1 month.⁵

- Delayed haemolysis was not pre-defined, but generally described as re-occurrence of biochemical markers compatible with delayed haemolytic anaemia (increased LDH and total bilirubin, decrease in Hb, low/no haptoglobin) 1–2 weeks after starting parenteral artesunate.
- Since mid-2011, 3/3 (100%) cases of severe malaria had delayed haemolysis. The Hb nadir was 8.6, 5.7 and 6.6 g/dL. All cases had received 4 × 2.4 mg/kg i.v. artesunate followed by oral mefloquine.
- After early stabilisation of Hb, there was a decrease in Hb levels at around week 3, with increased LDH. Reticulocyte counts increased relatively late in the course of haemolysis. Under supervision patients normalised within 1 month.

Retrospective study of returning travellers with severe malaria treated since 2006 with either intravenous quinine or artesunate.

- Since 2006 16/36 patients had suitable data for analysis. Patients were treated with various regimens: quinine regimens (n = 8); quinine + i.r. artesunate regimens (n = 4); and i.v. artesunate regimens (n = 4).
- Delayed haemolysis was defined as: decrease in median Hb between week 2 and 3 plus an increase in median LDH between week 2 and 3.
- Frequency of delayed haemolysis was: quinine + i.r. artesunate 2/4 (50%); i.v. artesunate 3/4 (75%); artesunate overall 5/8 (63%). Blood transfusions were performed in 3/5 cases.
 - None of the 8 patients receiving quinine in the absence of artesunate had delayed haemolysis.
- The Hb nadir was at day 14–15 with recovery generally by 1 month. The relationship to drug/ parasite burden was not analysed.

Conclusion: All three of the cases prospectively followed had delayed haemolysis after i.v. artesunate. In the retrospective analysis, 5/8 patients receiving i.v. or i.r. artesunate and 0/8 receiving i.v. quinine had delayed haemolysis¹.

2.4. French cases Stéphane Jauréguiberry

- Quasi-prospective surveillance data collected as part of a named patient programme between in 2011–2012. Further data was obtained from the National Reference Centre for Malaria and through proactive follow up of individual cases. These data support the efficacy of injected artesunate in severe malaria but also suggest a significant incidence of anaemia, following one of two possible patterns, one persistent and the other delayed. At the request of the presenting investigator, detailed results presented at the meeting will be incorporated into this report only following peer reviewed publication of the data.

¹ Artesunate versus quinine in the treatment of severe imported malaria: comparative analysis of adverse events focussing on delayed haemolysis. Thierry Rolling^{1,2}, Dominic Wichmann³, Stefan Schmiedel¹, Gerd D Burchard^{1,2}, Stefan Kluge³ and Jakob P Cramer^{1,2} Malaria Journal 2013, 12:241 doi:10.1186/1475-2875-12-241

2.5. USA/Canada cases Bryan Smith, Paul Arguin, USA/ Anne McCarthy (Canada)

A retrospective follow up analysis of patients that received i.v. artesunate produced by USAMMDA for compassionate use on a named patient basis between 2007 and March 2013. Data are preliminary.

- 186 patients in the USA received i.v. artesunate through the programme in the US and 101 in Canada through the Canadian Malaria Network.
- There have been no official reports of haemolytic anaemia after i.v. artesunate, but review of patient records identified 1 case in the US and 2 cases in Canada.
- A specific definition of delayed anaemia is not yet available as analysis is ongoing.
- In the US case, the Hb nadir was at day 15 (5.7 g/dL), LDH and reticulocytes were elevated, and the patient received a blood transfusion.
- In the Canadian cases, one patient had a Hb of 6.8 g/dL on day 9 with haemoglobinuria and was transfused with unremarkable recovery. The second patient had delayed haemolytic anaemia occurring over >4 weeks, and received 11 units of packed red cells with recovery starting at 5 weeks.
- All of these patients had high pre-treatment parasitaemia.

Action taken

- Since the European cases were reported, the treatment protocol for i.v. artesunate in severe malaria has been revised by the CDC in October 2012 to include 4-week follow up and an evaluation for haemolytic anaemia.

2.6. Chinese cases Linchun Fu

Analysis of open label, multicentre, randomised controlled clinical trials conducted from 1983–2006 in 2975 patients with severe malaria.

- A total of 1083 patients were followed up to at least day 28 with no reported haemolytic disorders; 708 of these received i.v. artesunate.
 - Delayed haemolysis was evaluated based on adverse event recording/ clinical observation; no haematological laboratory testing was conducted past day 7, at which point patients were discharged from hospital. Although it is possible that delayed haemolysis did occur, the symptoms were very mild and not recognised by the investigators.
 - In one study, 33 cases of cerebral malaria were treated with i.v. artesunate, and although Hb was very low in some patients, there were no reports of delayed haemolysis.

Note: Severe malaria is treated with parenteral artemisinins (artesunate/ artemether) or parenteral pyronaridine. Quinine is not recommended in China.

Clinical report of a single case treated with i.m. artemether.

- A returning traveller (Africa) with severe *P. falciparum* malaria experienced delayed haemolytic anaemia with onset at day 11, observed clinically as haematuria, and received a blood transfusion. There was no further haemolysis despite receiving oral artesunate (100 mg o.d.) for a further 6 days.

Conclusion: The benefit of artesunate in severe malaria is such that it should still be regarded as the treatment of choice despite the suspected haemolytic effect. Severe malaria patients treated with i.v. artesunate should be followed up for 2–4 weeks post-treatment.

2.7. Mahidol–Oxford Research Unit data Arjen Dondorp

SEAQUAMAT trial of 1461 Asian adults and children (n = 202 children).¹ AQUAMAT trial of 5425 African children.²

- In both trials, artesunate had a significant mortality benefit over quinine, which was particularly pronounced in patients with high baseline parasitaemia (>200,000 μL^{-1}).
- This clinical benefit is thought to be mediated by the rapid action of artesunate vs quinine and its broad specificity against blood stages. Artesunate is active against ring-form parasites, preventing their sequestration in the microcirculation, as well being active against mature parasites.
 - Around 80% of the non-viable ring-form parasites are cleared from erythrocytes in the spleen by 'pitting', whereby the parasite is removed and the erythrocyte resealed. The pitted erythrocytes remain RESA antigen-positive, and return to the circulation, but have a life span of only about 180 hours (7.5 days).
 - In contrast, quinine has activity mainly against mature parasites and erythrocyte pitting does not occur to any great extent.

Haematological outcomes in AQUAMAT:

- There were no differences in the rate of severe anaemia between artesunate (5.7%) and quinine (4.6%) ($P = 0.18$), or for black water fever (0.7% and 1.2%, respectively [$P = 0.076$])
- The incidence of blood transfusion was the same (55%) in the quinine and artesunate groups.
- There were no clinical assessments of severe anaemia reported in 102 children with neurological sequelae (there was no haematological laboratory follow up).

Conclusion: Artesunate saves lives by killing ring form parasites before they mature and sequester. These dead parasites are mainly removed in the spleen by erythrocyte pitting and the damaged erythrocytes have shortened survival. An increased incidence of severe delayed anaemia is thus, in part, an *expected consequence* of saving lives.

Retrospective data from adults in Thailand and Bangladesh (not SEAQUAMAT data) comparing artesunate (N = 41) and quinine (N = 38): preliminary results.

- The initial decrease in haematocrit is very similar for artesunate and quinine up to about day 5–6, with recovery until about day 7 then there is a secondary decrease in haematocrit with a nadir at day 10 and recovery starting at about day 14 with continuing steady improvement through day 28.
- The proportion of patients with delayed anaemia, defined as >10% decrease in haematocrit after day 7, was not significantly different overall ($P = 0.52$), though there was a trend for an increased incidence of delayed anaemia with artesunate (13/41 [32%]) vs quinine (8/38 [21%]).
- More severe anaemia (>20% decrease in haematocrit after day 7), occurred in 9/41 (22%) of patients in the artesunate group and 2/38 (5%) in the quinine group ($P = 0.13$).
- In the artesunate group, there was a trend for an increased incidence of delayed anaemia in patients with parasitemia >200,000 μL^{-1} compared with those with lower parasitaemia ($P = 0.36$).
- There did appear to be some bone marrow suppression at artesunate doses >20 mg/kg.
- Two patients in the artesunate group required blood transfusion, but there was significant co-morbidity.

Conclusion: Delayed falls in haematocrit were more prominent following artesunate than quinine, and in hyperparasitaemic patients.

2.8. SMAC study cases Peter Kremsner/ Jakob P Cramer

First SMAC study (Severe Malaria in African Children), comparing i.v. artesunate 12 mg/kg (WRAIR) split over three doses or five doses (N = 197).⁶

- Both regimens were highly effective and there were no remarkable issues with anaemia.

Follow-up study to SMAC (above) with 3 doses of i.v. and i.m. artesunate or 5 doses of i.m. artesunate (Guilin) (N = 1050) as well as a prospective sub-study of the SMAC study was conducted at two sites in Gabon and Ghana, with blood monitoring at days 0, 7, 14 and 28 (n = 102). In these unpublished studies anaemia starting at day 7 after treatment was a major finding. At the request of the presenting investigator, detailed results presented at the meeting will be incorporated into this report only following peer reviewed publication of the data

2.9. Discussion

- Reticulocyte data have not been analysed in all cases and no serial measurements of reticulocyte counts were made. There appears to be a reticulocyte response, but this does not rule out some degree of bone marrow suppression.
- In patients with uncomplicated *P. falciparum* malaria, there is also an initial decrease in Hb, even with very low parasitaemia. Thus, the initial decrease in Hb appears to be unrelated to parasite burden or disease severity and Hb levels are recovering by around day 5–7. The clinical course of delayed haemolysis diverges from this expected course at about day 5 with a Hb nadir at around day 10–14 and recovery within 1 month.
- It is possible that delayed haemolysis may be a class effect related to the mechanism of action of artemisinins. Most reported cases occurred after injectable artesunate, but there is a single case from China with i.m. artemether.
- Giving oral ACT after injectable artesunate in severe malaria increases the total cumulative dose of artemisinin and this could possibly increase the risk of prolonged anaemia. It may be possible to use alternative follow on therapy that does not contain an artemisinin.

2.10. Summary

- The value of injectable artesunate in reducing mortality from severe malaria was re-iterated. Artesunate should remain the drug of choice in severe malaria. However, better follow up of patients is required to identify and manage delayed haemolytic anaemia.
- Although delayed haemolytic anaemia has been identified in returning travellers and patients from malaria endemic countries in Africa and South East Asia, it can be managed clinically and there have been no reported deaths.
- Most data available are retrospective and prospective studies are required.
- The mechanism(s) of the delayed haemolytic anaemia need to be further elucidated.

Table 1. Summary of data on delayed anaemia after treatment of severe malaria with injectable artesunate.

| Presenter/Country/ Data source ^{reference} | Patients | Treatment | Criteria for delayed anaemia/ haemolysis | Frequency of delayed anaemia | Frequency of transfusion | Time course | Relationship to dose/ parasite burden |
|--|--|---|--|---|--|---|---|
| T Zoller Germany, Denmark, Copenhagen, Norway & Sweden ³ | N = 25 returning travellers | i.v. artesunate | Treatment-associated haemolysis with artesunate was noted to be a recurrence or persistence of haemolysis after clearance of parasitaemia and completion of anti-malarial treatment | 6/25 (24%) | 5/25 (20%) | Diagnosis at day 14–31 Active haemolysis \pm 10 days | Dose: Patients with haemolysis received higher artesunate doses than patients without hemolysis ($P = 0.006$) Parasite: Not investigated |
| P De Vries The Netherlands & Belgium ⁴ | N = 55 returning travellers | i.v. artesunate | Generally defined as increased reticulocyte counts, unconjugated bilirubin and LDH and decreased haptoglobin and Hb values | 7/55 (13%) | 4/55 (7%) | Hb nadir between days 8–30 | Dose: Not investigated Parasite: All patients hyperparasitaemic |
| T Rolling & JP Cramer Germany ⁵ | N = 3 returning travellers | i.v. artesunate | Generally defined as re-occurrence of biochemical markers compatible with delayed haemolytic anaemia (increased LDH and total bilirubin, decrease in Hb, low/no haptoglobin) 1–2 weeks after starting parenteral artesunate | 3/3 (100%) | 1/3 (33%) | Decrease in Hb levels at around week 3 | Dose: Not investigated Parasite: All patients hyperparasitaemic |
| T Rolling & JP Cramer Germany | N = 16 returning travellers | quinine + i.r. artesunate (n = 4) i.v. artesunate (n = 4) quinine (n = 8) | Decrease in median Hb between week 2 and 3 PLUS increase in median LDH between week 2 and 3 | i.r. artesunate 2/4 (50%) i.v. artesunate 3/4 (75%) Quinine 0/8 | 3/8 (38%) | Hb nadir at day 14–15 with recovery generally by 1 month | Dose: Not investigated Parasite: All patients hyperparasitaemic |
| S Jauréguiberry France | N = 70 returning travellers | i.v. artesunate | Anaemia defined as Hb <13g/dL for males, <12g/dL for females and categorised as: Rising pattern: Hb nadir and peak hemolysis before day 8 with no positive marker of hemolysis after day 8. Persistent pattern: Anaemia or hemolysis observed before and after day 8 and profile does not fit with rising or delayed haemolysis. Delayed pattern: Hb level or hemolysis markers are more markedly altered (>10% variation) after day 8 than before. Complex pattern: Anaemia not included in previous categories | Persistent: 13/70 (18%) Delayed: 17/70 (24%) | 2/68 (3%) late transfusion in persistent/delayed | Hb nadir around day 15 | Dose: No relationship Parasite: No relationship with initial parasitaemia |
| B Smith & P Arguin USA A McCarthy Canada | N = 287 returning travellers & visitors from endemic countries | i.v. artesunate | Not available | 3/39 (8%) | 3/39 (8%) | Hb nadir day 9–15 | Dose: All patients received 4×2.4 mg/kg, so could not be investigated Parasite: Parasite data has been collected |



MMV

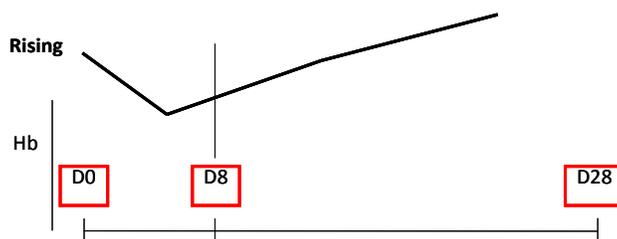
 Medicines for Malaria Venture

| Presenter/Country/ Data source ^{reference} | Patients | Treatment | Criteria for delayed anaemia/ haemolysis | Frequency of delayed anaemia | Frequency of transfusion | Time course | Relationship to dose/ parasite burden |
|--|------------------------------|---|---|---|---|---|---|
| L Fu China | N = 2975 endemic malaria | various artemisinin preparations | Delayed haemolysis was evaluated based on adverse event recording/ clinical observation | 0/1083 followed to day 28 | NA | NA | NA |
| L Fu China | N = 1 returning traveller | i.m. artemether | Observed clinically as haematuria, onset at day 11 | 1/unknown | 1/unknown | Onset day 11 with haematuria | NA |
| A Dondorp Bangladesh/ Thailand | N = 79 endemic malaria | i.v. artesunate (n = 41) quinine (n = 38) | >10% decrease in haematocrit after day 7 | >10% ↓ haematocrit 13/41 (32%) artesunate 8/38 (21%) quinine >20% ↓ haematocrit 9/41 (22%) artesunate 2/38 (5%) quinine | 2/41 (5%) artesunate 0/38 quinine | Initial decrease in haematocrit until day 6, with recovery, then a secondary decrease in haematocrit with a nadir at day 10, recovery starting at about day 14, steady improvement through day 28 | Dose: Not investigated Parasite: Trend for an increased incidence of delayed anaemia in patients with parasitemia >200,000 μL^{-1} compared to patients with lower parasitaemia |
| P Kremsner & JP Cramer SMAC (first study) ⁶ | N = 197 endemic malaria | i.v. artesunate 12 mg/kg split over three doses or five doses | Investigator reporting of adverse events | None reported | | | |
| P Kremsner & JP Cramer SMAC (follow up study) | N = 1050 | 3 doses of i.v. and i.m. artesunate or 5 doses of i.m. artesunate | Investigator reporting of adverse events. Haematology was not routinely performed after day 7 | 773 adverse events, 155/773 (20%) anaemia 88/773 (11%) anaemia at or beyond day 7, 70/773 (9%) on day 7 (scheduled visit) 18/773 (2%) after day 7 | | | |
| P Kremsner & JP Cramer Africa (SMAC) sub-study | N = 72 endemic malaria | i.v. artesunate | Decrease in Hb plus an increase in LDH between days 7–14 and low haptoglobin on day 14 | 5/72 (7%) | 1/72 (1%) | Hb nadir around day 14, elevated LDH on day 14 | Patients with delayed haemolysis tended to be younger and have higher parasitaemia that those without delayed haemolysis |

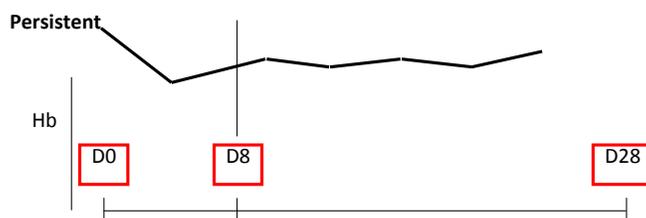
3. Mechanisms of action and plan for future studies

3.1. Peripheral destruction of parasitised cells Pierre Buffet

- The mechanism of action of artemisinins against ring-form parasites is unique; the parasite is killed, but the host cell remains in circulation after parasite removal in the spleen by pitting.⁷
- Erythrocytes can pass through the spleen by a closed and fast microcirculation, or by a slow and open microcirculation. In the latter case, erythrocytes must squeeze between endothelial cells and this is where dead parasites are removed from the erythrocyte and erythrocyte pitting occurs.⁷
- In uncomplicated malaria, a malaria-induced decline in haematocrit occurs before any anti-malarial therapy. After therapy is initiated, there is an initial decline in haematocrit of about 2%, with a nadir between day 3 and day 7. This is a consequence of parasite killing and parasite-related erythrocyte loss rather than a direct effect of the drug. There is then a rapid recovery in Hb from day 7–14, generated by a 5–15% increase in reticulocyte production. Hb recovers over the next month to normal pre-malaria Hb levels.⁸
- Many potential causes of anaemia in malaria exist, but their relative contributions to anaemia is unknown difficult to quantify.^{9,10}
- The issue of timing of erythrocyte clearance is important as the parasitised erythrocytes will need to be removed from circulation at some point and when this happens may depend on the mechanism of action of the drug. After treatment with injectable artesunate, there appears to be some general patterns to the time course of changes in Hb:^{3,4,8}



- Rising (normal recovery): Nadir of haemoglobin before day 8 and no positive marker of haemolysis after day 8.



- Persistent: Similar to the rising pattern until day 7, then Hb fails to recover, with levels remaining similar for up to 1 month, and haemolysis markers after day 8.



- Delayed (also termed recurrent): Similar to the rising pattern until day 7, then a more intense drop in Hb sometime after day 8, with positive haemolysis markers after day 8.

- Complex pattern: A pattern of Hb changes with characteristics of more than one of the above.

- Flow cytometry was used to determine the rate of pitting using the RESA antigen. The decrease in parasitised RESA+ erythrocytes corresponded with an increase in parasite-negative RESA+ erythrocytes as pitting proceeded. The maximum rate of pitting occurred between days 2–7.
- In French patients, around 80% of the parasitised erythrocytes were converted to pitted cells by around day 5. A decline in RESA+ erythrocytes was evident by the second week after therapy start. This corresponds to the timing of delayed haemolysis. Data presented during the meeting are being further analysed and will be made available only following its peer-reviewed publication.
- Persisting haemolysis is perhaps a different issue as patients appear to be refractory to blood transfusion, suggesting that the transfused erythrocytes are also being destroyed.
- There are a lot of pitted erythrocytes after artesunate treatment, but few after quinine.
 - Artesunate rapidly kills ring-form parasites, preventing sequestration. Thus, erythrocytes containing dead ring-form parasites are still in the circulation and every 2–3 h will pass through the spleen open circulation microfiltration system where pitting occurs.¹¹ Pitting is an erythrocyte sparing mechanism. The delay in erythrocyte death by about 7–15 days results in a delayed haemolysis.
 - Quinine does not impede parasite maturation or sequestration very efficiently. Quinine is active essentially against mature parasites in sequestered erythrocytes. These erythrocytes do not pass through the spleen and so pitting occurs only at a low level. The parasitised erythrocytes are lysed rapidly and so no delayed haemolysis occurs.
- Some patients with high parasitemia at admission and treated with i.v. artesunate have low levels of pitting and no delayed onset anaemia. This may be because of synchronicity and time when the treatment with artesunate is given.
 - In synchronous infections there can be spontaneous changes in parasitemia of 2–3 log.¹²
 - If artesunate is started 2–3 h before sequestration, then the drug effect will be similar to that of quinine as there will be few ring-form parasites, not much pitting and no delay in erythrocyte haemolysis.
 - Experiments using microfiltration to induce erythrocyte pitting artificially found that the degree of pitting is influenced by the exposure of the parasite to artesunate; it requires about a 5-h exposure to induce pitting.

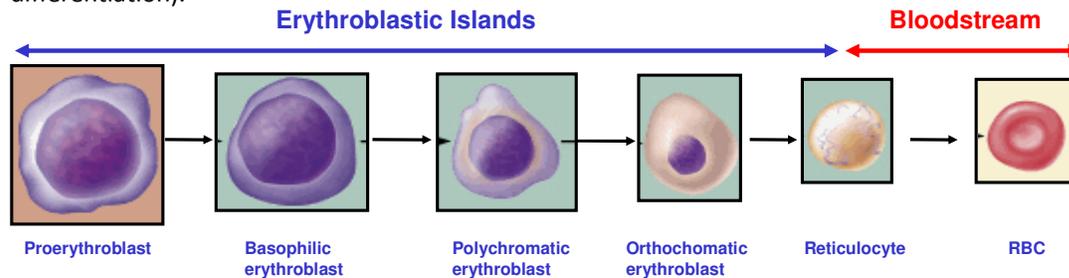
Research questions

- Is the delayed/persistent anaemia framework solid enough?
- Confirmation is required through a relatively large prospective study to overcome the heterogeneity linked to synchronicity and immunity.
- Could we validate a prognosis marker based on the proportion of pitted erythrocytes at day 3?
- Is pitting truly artesunate specific?
- What is/are the mechanisms of the persistent anaemia pattern?
- What is the contribution of impaired bone marrow regeneration to persistent anaemia (quantification, mechanisms)?

Conclusion: A delayed onset haemolysis pattern was observed in 20–25% of patients. The proportion of the delayed erythrocyte loss that can be attributed to the loss of pitted erythrocytes is correlated with initial parasitemia. It is likely that the delayed haemolysis is caused by an artesunate-specific mechanism linked to drug efficacy rather than a conventional toxic effect. If this is the case then delayed onset haemolysis should be observed with all artesunate sources/lots and in most patients that have high parasitaemia. Impaired erythrocyte regeneration is probably not a major contributor to the recurring haemolysis pattern, though the regeneration potential is probably inadequate to address the level of anaemia. Other/ additional persistent mechanisms of erythrocyte loss occur in patients with persistent haemolysis. These mechanisms may not be artesunate specific.

3.2. Insights into human erythropoiesis Mohandas Narla

An adult bone marrow needs to produce 2 million erythrocytes per second to maintain steady state circulating haemoglobin levels in normal adults. The production can be increased up to 20 fold under demands of erythropoietic stress to compensate for severe anaemia. The process is shown below and takes about 7 days from proerythroblast to mature erythrocyte (terminal erythroid differentiation).



- All erythrocyte production occurs in association with macrophages. Macrophages are involved in signal transduction and they ingest ejected nuclei.
 - Cytokine-mediated suppression of effective erythropoiesis can occur via macrophages, which can produce a number of inhibitory factors.
- Haematopoietic cells from bone marrow can be expanded *in vitro* and purified into the distinct stages of erythroblast based on the expression of just three surface antigens.
 - This technique has been used to determine effects on erythropoiesis by quantification of erythroblasts at distinct stages of terminal erythroid differentiation.
- Artesunate could potentially affect erythropoiesis by impairment of:
 - a) Terminal erythroid differentiation via a direct effect on erythroblasts.
 - b) Terminal erythroid differentiation indirectly via macrophages.
 - c) Reticulocyte maturation.
- While bone marrow analysis is the best way to study these effects it will be difficult to justify repeated sampling. Although not optimal, an *in vitro* culture system can provide useful insights.

3.3. Human *in vitro* models to evaluate the toxicity of artemisinins on erythroid cell differentiation Donatella Taramelli

The *in vitro* models to evaluate the toxicity of artemisinins on human erythropoiesis were developed in 2009 following the general concern that artemisinin derivatives could be embryotoxic in the first trimester of human pregnancies, as shown *in vitro* and *in vivo* in pregnant rodents. The artemisinin drugs and dihydroartemisinin (DHA) in particular, target the primitive embryonic erythropoiesis causing embryonic erythrocyte depletion when the treatment is performed during a critical period of time.^{13,14} The study presented was planned to investigate the effect of DHA on human developmental erythropoiesis in order to characterise the target erythroid stage and to predict the window of susceptibility in human pregnancy. These data may help to partially explain the delayed anaemia of artesunate-treated patients.

- As a model for human developmental erythropoiesis, peripheral blood purified huCD34+ cells were committed towards erythrocytes. DHA (0.5 or 2 μ M) was added at time points corresponding to the different stages of erythrocyte differentiation for a total of 14 days.¹⁵ Erythroid growth and differentiation were investigated by cytofluorimetric analysis of glycophorin A expression, by morphological analysis and erythroid globin gene expression analysis with real-time PCR.
- Day 0, stem cells and proerythroblasts.

- There was dose-dependent inhibition of cell growth up to day 7, but no effects on day 11 or day 14 ($P < 0.05$).
- There was no effect on glycophorin-A (GpA). NB: GpA has an important role in regulating erythrocyte mechanical properties and in maintaining cell shape.
- Cell differentiation was completed in DHA-treated cells.
- Globin expression was normal.
- Day 4–7, proerythroblasts and basophilic erythroblasts.
 - There was dose-dependent inhibition of cell growth up to day 14 ($P < 0.05$).
 - There was a reduction in GpA⁺ cells from day 7 to day 14 ($P < 0.05$).
 - Cell differentiation was delayed.
 - Globin expression was abnormal, with an excess of γ -globin.
- There was no effect of DHA on polychromatic erythroblasts.

Conclusion: DHA inhibits human erythroid cell growth and differentiation in a dose-dependent manner and is stage specific with a transient effect on stem cells and progenitors, but specific toxicity for proerythroblasts and basophilic erythroblasts and no effect on mature stages.

Using the K562 erythroid cell line, further experiments were conducted on the effect of artemisinin derivatives on erythroid differentiation up to the basophilic erythroblast stage.¹⁶

- In terms of potency, the greatest effect was with DHA > artesunate > artemisone > artemisinin.
- The endoperoxide bridge was necessary for toxicity as deoxyartemisinin had no effect on erythroid differentiation.
- A cell signalling hypothesis was proposed whereby DHA induces cytochrome C release, triggering a cascade which changes the balance between the key haematopoietic transcription factor GATA-1 (decreased) and GATA-2 (increased), thus impairing cell growth and cellular differentiation, inducing apoptosis and switching off the γ -globin production leading to decreased haemoglobin synthesis.
- In addition, in malaria patients, phagocytosis of haemozoin by bone marrow macrophages on erythroblastic islands leads to the production of soluble toxic products, for example, 4-hydroxynonenal (4-HNE), that may affect erythroid and thrombocyte growth.^{17,18} Thus, there may be combined inhibitory effects on erythropoiesis from the infection and drug treatment.

Conclusion: At therapeutic doses (0.5–2 μ M), artemisinins inhibit growth and differentiation of human proerythroblasts and basophilic erythroblasts by targeting the pathway of Hb synthesis.

3.4. Discussion

Pattern of haemolysis/ prognostic factors

- Can the pattern of anaemia described for uncomplicated malaria⁸ be applied to severe malaria with hyperparasitaemia?
 - The main point is that anaemia can occur before treatment, early during/after treatment and late after treatment in both cases, not that they are the same.
- What prognostic factors could be used for delayed anaemia? Prognostic factors are particularly important in patient populations that are difficult to follow up (children).
 - A point of care test for determining the proportion of pitted cells is technically possible, but would require significant development.
 - In the short-term, patients with high initial parasitaemia (>5–10%) appear to be more at risk from delayed anaemia; such patients should be followed more carefully.

Erythrocytes, pitting and the spleen

- Is pitting dependent on reduced erythrocyte deformability?

- Early studies indicated that ring-form infected erythrocytes treated with artesunate had normal deformability. However, experimentally, erythrocytes infected with live ring-form parasites seem to cross narrow slits more slowly. This might cause retention of these erythrocytes in the spleen, though the relevance of this is not clear.
- Although erythrocyte surface changes may play a role in splenic destruction of erythrocytes, the spleen has multiple ways of sensing cells that need to be removed from circulation.
- If there are more parasites, then there should be more pitting and therefore more haemolysis? Removal of pitted erythrocytes explains only about 50% of the haemolysis. What about the other 50%?
 - The relationship between pitting and haemolysis does not completely match possibly because measuring peripheral parasitemia does not truly reflect total parasite burden. Before treatment part of the ring-stage biomass may be out of the circulation, for example, in the spleen.
 - Histidine-rich protein2 (HRP2) could provide a more accurate measure of parasite biomass.
 - Pitting is part of the story, but there may be other mechanisms of haemolysis that need to be investigated.

Bone marrow suppression

- In the case reports, the main issue seems to be erythrocyte destruction, what role for bone marrow suppression?
 - The very low Hb levels should elicit a very strong reticulocyte response, and this seems to be somewhat impaired.
 - At high doses (>20 mg/kg) bone marrow suppression is evident with a depressed reticulocyte response. Granulocytopenia is also described at cumulative doses of artesunate.
 - There is evidence of bone marrow suppression from some of the studies being conducted on the use of artemisinins in cancer.
 - Bone marrow suppression is also seen with anaemia of inflammation, so this is a confounding factor.
 - The most likely explanation is a mixed effects model of haemolysis and bone marrow suppression.
- To adequately determine reticulocyte response in clinical trials, samples need to be taken very frequently as reticulocytes only last 24 h in the circulation. These samples can be taken by finger prick, so are not too invasive.
- Note that in the *in vitro* models of bone marrow suppression, although the doses used are in the therapeutic range, drug challenge does not mimic the pharmacokinetic profile in patients.
- In areas of seasonal malaria, the relative contributions of bone marrow suppression and haemolysis to anaemia could vary throughout the season; greater haemolysis early in the season, greater bone marrow suppression later in the season depending on the interaction of immunity and repeated malarial anaemia.
- There was some neutrophil suppression in the WRAIR regulatory studies. The patients treated in Germany appeared more susceptible to other infections, suggesting neutropaenia.

3.5. Recommendations/ actions

- Physicians treating severe malaria with injectable artesunate need to be made aware of the need to monitor patients for delayed anaemia which should be regarded as an expected potential clinical course of the disease.
 - Patients with parasitaemia >5–10% may be more at risk of delayed anaemia and should be advised of the relevant symptoms on hospital discharge and followed more closely.

- To reduce the risk of prolonged anaemia at high artesunate doses, particularly the contribution of bone marrow suppression, one option might be to not use artemisinin follow-on therapy after injectable artesunate.
 - Further data are needed on the risk and dose–response of artesunate and delayed anaemia.
 - Recommendations need to consider the availability, efficacy and safety of other follow on therapies. Cost is also an issue, but there is the possibility to influence costs if there is a medical need.
 - It is important that follow on therapy is not discouraged as monotherapy may not be sufficiently effective in all patients, particularly those with high parasitemia.
- For WHO guidelines, defining the optimum total cumulative dose of artemisinin would be necessary.
 - The data to make such recommendations are currently lacking.

Further studies

- Prospective investigations of the use of ACT after i.v. artesunate vs alternative follow on or adjunctive therapies.
- Prospective investigations to define the dose–response for injectable artesunate and delayed anaemia.
 - NB: For comparison across studies, definitions of severe malaria and delayed anaemia should be consistent.
- Investigations of other aspects of haemolysis should be incorporated into prospective clinical studies. For example, Heinz bodies, erythrocyte surface area, haemoglobinuria, and band 3 aggregation.
 - ▶ ACTION: Pierre Buffet to compile a list of parameters relevant to haemolysis.
- The effect of artesunate on neutrophils and other white blood cells requires investigation.
- Non-clinical studies on the effect of artesunate on erythrocyte destruction and regeneration should aim to result in measures that improve clinical outcomes, such as the development of prognostic factors.
 - ▶ ACTION: Pierre Buffet to compile a list of possible studies.

3.6. Summary

Erythrocyte pitting and subsequent delayed haemolysis appears to be a possible mechanism of action to explain delayed anaemia after the treatment of severe malaria with injectable artesunate. There is also some inhibition of erythropoiesis with artesunate. Physicians need to be made aware of the possibility of delayed haemolysis after injectable artesunate for severe malaria, and the need for continued monitoring of patients, particularly those with high pre-treatment parasitaemia. However, the continued use and further adoption of this life-saving treatment should not be impaired. Further prospective studies are required to best optimise the use of injectable artesunate to maintain the mortality benefit of the drug, while minimising the risk of delayed anaemia.

4. Next steps

4.1. USAMMDA/Sigma-Tau perspectives Bryan Smith

USAMMDA/ Sigma-Tau are in the final stages of obtaining FDA registration of their GMP injectable artesunate product; filing is expected Q2 2014.

- In response to the reports of delayed anaemia, the following actions have been already taken.
 - A ‘Dear Investigator’ letter has been sent to all the physicians that have used artesunate requesting information on delayed anaemia; no responses have been received.
 - The treatment investigational new drugs (IND) and consent form have been modified to recommend a 4-week follow up of patients.
 - The database of all patients treated with artesunate between 2007–2010 is now in data clean up and a retrospective chart review beyond the existing 7 days is being conducted for all treated patients. Hospital records are being sought and some individual cases are being followed up to obtain further data.
- Next steps.
 - A formal literature search will be undertaken to generate a white paper for submission to the regulatory authorities.
 - There will be a voluntary prospective patient registry for the US and Canada.
 - The investigator brochure and label will be updated with the following statement: “There have been rare instances of delayed late stage hemolysis reported in patients who have been treated with artesunate for severe malaria. It is unlikely that these episodes of delayed haemolytic anemia are solely due to artesunate treatment but the possibility cannot be ruled out. As a result patients who have had severe malaria treated with artesunate should be monitored for 28 days for evidence of haemolytic anemia.”
- Other actions are also being evaluated.
 - It is not clear whether there a need for a formal risk management plan. There is no question regarding the efficacy of injectable artesunate in saving lives and overcoming drug resistance. Anaemia is a severe adverse event, but patients have presented when they have experienced symptoms, it has been managed successfully with transfusion and no patient has died.
 - The most important message in any warning label is that the disease complex of ‘severe malaria’ does not resolve after parasites have been cleared, or even after drug therapy has been completed, but may continue for several weeks, including the risk of delayed anaemia.
 - Prospective trials may be conducted. If these do not delay registration and are to occur in a Phase IV context, then USAMMDA is supportive. However, trials that delay registration will delay patient access to potentially life-saving treatment and would be discouraged by USAMMDA.
- The impression received by USAMMDA from the FDA is that USAMMDA is taking the appropriate steps.

4.2. Guilin perspectives and post-marketing studies Jean-Marc Bouchez

Guilin Pharmaceutical Co. Ltd obtained WHO GMP for injectable artesunate (*Artesun*) in August 2010 and it was WHO prequalified in November 2010. It has also obtained a WHO supplier qualification in November 2011. The drug is currently approved and marketed in 22 countries in Africa, five in Asia and one in South America.

- The European Medicines Agency (EMA) and the State Food and Drug Administration (SFDA), P.R. China are working to harmonise standards of GMP between the two agencies.
 - A new GMP certificate will be obtained in accordance with new SFDA guidelines.
 - There is the possibility to submit a dossier to the EMA to obtain a European marketing authorisation as an orphan drug.

- *Artesun* distribution has been strictly controlled since 2007. Export drug liability insurance was obtained in 2008. All local agents have generalised system of preferences (GSP) certification.
 - Subsidiaries and branch offices cover around 80% of malaria endemic areas and a Client Relation Management (CRM) system is already in place. All drug lots can be tracked.
- A safety report on injectable artesunate has been commissioned from a pharmacovigilance perspective and was provided to attendees of the meeting. It identified 22 cases of delayed haemolytic anaemia from 209 cases obtained from database and literature searches. Twelve cases were Guilin artesunate, seven cases the ACE import and two cases were of non-severe malaria treated with oral artesunate. All cases concerned returning travellers.
 - No conclusions could be drawn on the formulation to be suspected.
 - No dose-dependent effect was observed.
- The company developed a standard operating procedure (SOP) for pharmacovigilance in December 2012. All medical representatives have a pharmacovigilance contact in their country and also a dedicated safety e-mail for Guilin in China.
 - The *Artesun* website is being finalised and includes all safety information on the drug and on-line forms for adverse reaction reports.
 - Quintiles have been contracted to perform the pharmacovigilance role in regard to data compiling and processing. Guilin has responsibility for collecting adverse event reports for forwarding to Quintiles, following up on adverse event reports and for regulatory reporting. The system should be operational by May 2013.
- A risk management plan is being discussed with Medicines for Malaria Venture (MMV).
 - One study is ongoing in the Democratic Republic of Congo to assist in the introduction of injectable artesunate to replace quinine in different settings. The baseline assessment of quinine treatment has been completed and the study is about to progress into implementation and break in of injectable artesunate treatment (i.v. and i.m.). The final phase will be enrolment and evaluation of injectable artesunate treatment. A protocol amendment for a weekly haematological follow-up until day 28 has just received ethical clearance from Kinshasa and Basel.
 - Two post-marketing initiatives are proposed. A cohort event monitoring study in Central Africa and a severe malaria registry in Western Africa. In both studies follow up will be for 28 days, with collection of haematological data on days 0, 3, 7, 14, 21, and 28. Suggested data for capture include: Hb, LDH, haptoglobin, bilirubin, soluble transferrin receptor and erythropoietin.

Comments

- In the two proposed studies, it may be possible to look at pitted erythrocytes using flow cytometry at one or two sites.

4.3. Discussion

- There are now several web-based reporting systems in use or proposed for capturing data on delayed anaemia, and it would be useful if these could be standardised so that the information can be more easily collated across the databases.
- The frequency of delayed haemolytic anaemia is highly variable between the reported studies and confounded by different definitions and terminology. Thus, in documentation we should avoid making statements on the frequency of this event, or similar statements, such as 'rare'.
 - Even if the frequency of delayed anaemia is low after injectable artesunate, large numbers of patients are treated for severe malaria.
- The Guilin severe malaria patient registry in West Africa will allow reporting of delayed anaemia with quinine as well as artesunate as both are used in the study areas.

- Delayed anaemia does not seem to be an issue of drug formulation or manufacture; it seems to be a possibility with any artesunate-based treatment.
- How delayed haemolytic anaemia is defined in clinical trials will determine product labelling.

Named patient registries in Europe

- There was a suggestion that a letter be sent from this group to EMA outlining the importance of artesunate in reducing mortality from severe malaria, but highlighting the need to follow all patients and report.
 - ▶ Action: MMV to consider following up with EMA by teleconference.
- In France, clinicians treating malaria made a request to conduct pro-active surveillance to the Agence National de Sécurité du Médicament et des produits de santé (ANSM), but it was not clear where funding for this would be obtained and so it has not progressed.
- In Germany, the legal basis for the import and use of Guilin artesunate is based on its registration in another country and that severe malaria an immediately life-threatening disease. There is no legal basis at all for a named patient programme for a drug which is not already under at least phase III study elsewhere (in the latter case, an early access programme might be possible).

Design of clinical trials

- The quality of evidence is currently low, and the consensus was that prospective trials are needed.
- There was an unresolved discussion regarding a prospective trial of injectable artesunate versus quinine in Africa with endpoints that would define the relative risk of anaemia and time course (early or delayed).
 - A mortality comparison at day 28 is not relevant because in the SMAC study, for example, there were no deaths after day 7.
 - Given the available evidence, it would probably no longer be ethically justifiable to conduct a study in severe malaria with quinine because of the mortality benefit with artesunate.
 - If in order to gain ethical approval, the study included only patients that are at low risk of mortality, then the consequences may be that either the mortality benefit of artesunate is underestimated or the risk of delayed anaemia is underestimated as the patients with high parasitemia are those that are at greatest risk for both of these outcomes.
 - Within the SMAC consortium, it might be possible to conduct a comparative quinine/ artesunate trial in moderately ill patients, but with high parasitaemia and close clinical monitoring.
 - In Germany, most patients receive quinine and if parasitaemia exceeds 10% then the patient receives artesunate so both drugs are used. However, the patient population is not then comparable.
 - A key objective is to define the frequency of delayed anaemia in different populations; a quinine comparator is not required to achieve this objective.
- There was also a strong argument that clinical trials should try to evaluate solutions to optimise the use of artesunate, for example by evaluation of different follow up therapies.
 - The research question and endpoints may be different for different populations so several trials may be required. For example, travellers, Asian adults, African children.
 - A comparison is required of artesunate + ACT follow up vs artesunate + non ACT follow up (atovaquone/proguanil or sulfadoxine/pyrimethamine + amodiaquine [SPAQ]). ACT alone might also be considered in some patient populations. NB: Such a study will help optimise treatment only if there is a real dose–response effect.
- For any trial, patient sub-groups may need to be prospectively defined. For example, patients who present with severe malaria and primary anaemia.
- Returning travellers may provide a good model for study as they are the very sensitive to drug/ disease effects because of their low immunity. Extensive monitoring of patients is also possible.

- It would be useful to define when the endpoint of severe malaria is. It is a serious disease and management should not end once drug treatment is completed. In clinical practice, patients should be followed up for as long as possible.
 - In AQUAMAT, the same proportion of patients received blood transfusion with quinine and with artesunate, so it may be the timing of the anaemia rather than the frequency that is the issue.

Other clinical studies

- Quinine and artesunate are both being used in different regions at present and some areas are in transition. Outside of a clinical trial setting, it may be useful to follow up patients receiving both therapies, for example, within a severe malaria patient registry. However, there would probably need to be some measures to try and extend follow up data.

4.4. Holding statement/ recommendations Andrea Bosman

The discussions held in this meeting are important for the Malaria Treatment Guidelines. This issue needs to be urgently resolved in order to ensure that funding for artesunate i.v. remains available and procurement is not interrupted. WHO is committed to moving forward on this issue through a review of the report of this meeting and issuing a statement from the Technical Expert Group on Malaria Chemotherapy.

- ▶ Action: The report of this meeting should be made available to the WHO Expert Group on Malaria Chemotherapy.
- There is a strong recommendation to use injectable artesunate in the treatment of severe malaria. Delayed anaemia does occur, but there have been no reported deaths.
 - There is the suggestion that the time course of malarial anaemia can follow patterns of rising, recurrent and persistent anaemia.
 - The different study designs, mainly retrospective, presented and discussed at the meeting mean that the frequency of delayed anaemia cannot be estimated.
- The definition of severe malaria and the definition of delayed anaemia in further studies need to be standardised.
- Within an individual clinical trial, the criteria for blood transfusion need to be prospectively defined if this is to be used as a study outcome.
- Many different mechanisms potentially contribute to delayed anaemia.
 - Around 20–50% of delayed haemolysis can be attributed to erythrocyte pitting. Thus, this can be considered part of the expected consequences of life-saving artesunate treatment.
- Persistent anaemia is difficult to manage; multiple blood transfusions are required. It may be less common than recurrent anaemia and may require more investigations.
- New clinical comparative studies are required, though there was no consensus on the comparator and the specific design.
 - The objective of clinical studies should be to define the frequency of delayed anaemia and to examine interventions that may reduce this frequency. This includes determining the correct dose of ACT as follow on therapy in relation to the dose/duration of injectable artesunate for severe malaria treatment and if there is a dose–response relationship with post-treatment delayed anaemia. Early predictors/ prognostic factors should also be examined.
- Harmonisation of Phase IV studies is recommended, particularly those proposing web-based spontaneous reporting systems by pharmaceutical companies.
- There needs to be better links with national pharmacovigilance centres and perhaps the international drug safety monitoring system managed by Uppsala.
- Patient registries are an additional approach which may need further discussions in a separate meeting.

The process at WHO for dissemination of findings:

- 1) Receive the full report of the MMV meeting and submit for review by the WHO Expert Group on Malaria Chemotherapy.
- 2) Develop on this basis a WHO statement/ summary page.
- 3) Submit to funding agencies which are putting on hold procurement of injectable artesunate pending the outcome of this review.

4.5. Conclusion David Reddy

- In general, delayed haemolytic anaemia after injectable artesunate appears to be both drug and disease related. It may not be restricted to i.v. preparations, but also i.m. and i.r. as well as possibly oral, though data are scarce.
- Delayed haemolytic anaemia has been observed in European travellers, African children and Asian adult patients, though the frequency of its occurrence is unknown in any population.
 - The potential for delayed haemolytic anaemia needs to be first understood and then effectively communicated. Spontaneous reporting needs to be triggered, pharmacovigilance initiated/ maintained.
 - WHO may wish to consider including information regarding this issue into treatment guidelines.
- Prospective collection of information would be beneficial, but first there must be a clear definition of delayed haemolytic anaemia.
- Clinical and mechanistic studies should aim to identify prognostic factors and clinical management approaches, e.g. most appropriate oral therapy(ies) for use following injected artesunate, to assist with updating guidelines.

4.6. Actions

- ▶ WHO to seek expert advice for submission of information to funding agencies.
- ▶ MMV to work with manufacturers and relevant experts to develop pharmacovigilance studies.
- ▶ MMV to follow up with EMEA on whether a centralised approach to artesunate use in Europe on a named patient basis is possible.
- ▶ WHO to follow up with MMV regarding patient registries.
- ▶ USAMMDA to maintain dialogue with MMV regarding the FDA position.

Attendees

| Name | Organisation |
|-----------------------------|---|
| Dr Peter J de Vries | Academic Medical Center, Amsterdam |
| Mr Cees KW van Veldhuizen | ACE Pharmaceuticals, Zeewolde |
| Dr Alan MaGill | Bill & Melinda Gates Foundation, Seattle |
| Dr Jakob Cramer | Bernhard Nocht Institute for Tropical Medicine, Hamburg |
| Dr Thierry Rolling | Bernhard Nocht Institute for Tropical Medicine, Hamburg |
| Dr Paul Arguin | Centers for Disease Control and Prevention (CDC), Atlanta, GA |
| Dr Hao Cui | State Food and Drug Administration, PR China |
| Dr Cuili Liu | State Food and Drug Administration, PR China |
| Dr Akim Adegnika | CERMEL, Lambaréné |
| Dr Thomas Zoller | Charité – University of Berlin, Berlin |
| Mr Ding Tao | Chinese Embassy, Vienna |
| Dr Bruno Moonen | Clinton Health Access Initiative, Nairobi |
| Dr Eric Pelfrene* | European Medicines Agency, London |
| Dr Eileen Navarro* | US Food and Drug Administration (FDA), Silver Spring |
| Dr Elizabeth O'Shaughnessy* | FDA, Silver Spring |
| Dr Hala Shamsuddin* | FDA, Silver Spring |
| Dr Jean-Marc Bouchez | Guilin Pharma, Ivory Coast |
| Dr Linchun Fu | Guilin Pharma, PR China |
| Ms Lily Su | Guilin Pharma, PR China |
| Ms Michelle Xiong | Guilin Pharma, PR China |
| Dr Pierre Buffet | Hôpital de la Pitié-Salpêtrière, Paris |
| Dr Stéphane Jauréguiberry | Hôpital de la Pitié-Salpêtrière, Paris |
| Dr Benjamin Mordmüller | Institut für Tropenmedizin, Tübingen |
| Dr Carsten Köhler | Institut für Tropenmedizin, Tübingen |
| Prof Arjen Dondorp | Mahidol–Oxford Tropical Medicine Research, Bangkok |
| Prof Tsiri Agbenyega | School of Medical Sciences, Kumasi |
| Prof Ogobara Doumbo | Malaria Research and Training Centre, Bamako |
| Dr Martin De Smet | Médecins Sans Frontières |
| Dr Mohandas Narla | New York Blood Center, New York |
| Dr Antonella Bacchieri | Sigma-Tau Pharmaceuticals, Rome |
| Dr Marco Corsi | Sigma-Tau Pharmaceuticals, Rome |
| Prof Sanjeev Krishna | St George's University, London |
| Prof Christoph Hatz | Swiss Tropical and Public Health Institute, Basel |
| Dr John Cutler | UNITAID, Geneva |
| Dr Ambachew Yohannes | UNITAID, Geneva |
| Prof Donatella Taramelli | Università di Milano/WRAIR, Milan |
| Prof Anne McCarthy | University of Ottawa |
| Prof Peter Kremsner | University of Tübingen |
| Dr. Stefanie Bolte | University of Tübingen |
| Dr Andrea Bosman | WHO-GMP, Geneva |
| Dr Peter Olumese | WHO-GMP, Geneva |
| Dr Shanti Pal* | WHO-Pharmacovigilance, Geneva |
| Dr Matthias Stahl* | WHO-Prequalification, Geneva |
| Dr Cathy McDermott | USAMMDA, US Army |

Col Bryan Smith USAMMDA, US Army

MMV

Dr David Reddy

Dr Stephan Duparc

Ms Heidi Divecha

Mr Pierre Hugo

Dr Wiweka Kaszubska

Dr Jörg Möhrle

* By teleconference

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