Artesunate in Severe Malaria

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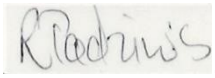
A research report submitted to the University of the Witwatersrand, Johannesburg in fulfilment for the requirements of the degree of Master of Medicine 2018.

DECLARATION

I, Ratidzo Tadzimirwa, 0602615R, declare that this research report is my own work which is being submitted for the degree of Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

……  …….

13th day of ……November…..2019

CO-AUTHOR DECLARATION

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Ratidzo Tadzimirwa, 0602615R declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

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Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

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LETTER OF CONTRIBUTION

To South African Journal of Infectious Disease (SAJID)

The MMed titled Artesunate in severe malaria, in submissible format (for the journal of Southern African Journal of Infectious diseases) is primarily my work.

I, Dr R Tadzimirwa, 0602615R developed the project, wrote the protocol and collected all the data personally. I collated the statistical information with the assistance of my statistician, Prof Omar and wrote the final paper with input from my supervisor Dr Kalla and Dr Brown as well as assistance from Professor Omar on the submissible article.

Dr Brown and Dr Kalla were supervisors in this project. Both of them provided guidance at various stages of development of the protocol as well as the writing of the final manuscript. Professor Omar taught me statistics and supervised statistical analysis as well as the write up of the final manuscript.

Regards,

Dr R Tadzimirwa

Professor Omar

Dr Brown

Dr Kalla

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ABSTRACT

BACKGROUND

In South Africa (SA), malaria is endemic in three provinces, Mpumalanga, KwaZulu-Natal and Limpopo and resulted in 87 deaths during 2011. The Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT) trial showed superiority of intravenous (IV) Artesunate compared to quinine, however its use in the intensive care (ICU) is not widely studied.

METHODS

We performed a retrospective review of artesunate treated malaria patients admitted to the 2 academic ICUS's (April 2010 to 2014).

RESULTS

A total of fifty-six patients were included. The mean age of patients was 40.3 years, standard deviation (SD) 11.6. There were 40 male patients (71.4%). The mean Apache II score was 19 (SD 5.4) with a predicted mortality of 32.2%. A total of 48% of patients had at least one co-morbidity with human immune deficiency virus (HIV) being the most prevalent (32%). 71.4% travelled to a malaria area, 26.8% had no travel history and 1.8% unknown travel. We observed a lower than predicted mortality rate of 21.4%, standardised mortality ratio of 0.67. Heart rate, respiratory rate and Glasgow Coma Scale (GCS) all improved significantly from admission to discharge, ($p \leq 0.01$). Markers of severe malaria: acidemia, bilirubin, urea and bleeding risk (platelets) also improved, ($p \leq 0.01$). Non-survivors had a lower GCS and worse acidemia on Day one, ($p \leq 0.01$). Mechanical ventilation was associated with an increased risk of death, Odds ratio (OR) 35, 95% confidence interval (CI) 7.0- 182, as was vasopressor use, OR 4.3, CI 1.7-11.4.

CONCLUSION

IV Artesunate was found to be efficacious and associated with a lower than predicted mortality in patients with severe malaria requiring ICU admission.

ABBREVIATIONS

CHBAH	Chris Hani Baragwanath Academic Hospital
CI	Confidence interval
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CR1	Complement Receptor 1
CSA	Chondroitin-4-Sulfate
CTB	CT Brain
Hb	Haemoglobin
HBA/S	Haemoglobin A/Haemoglobin S heterozygotes
HIV	Human Immunodeficiency Virus
ICAM-I	Intercellular Adhesion Molecule 1
ICU	Intensive Care Unit
IQR	Interquartile range
IRBC	Infected Red Blood Cells
IV	Intravenous
NICD	National institute for communicable diseases
OR	Odds ratio
PfEMP1	*P. Falciparum* Erythrocyte Membrane Protein
PfHRP	*P.Falciparum* Histidine –Rich Protein
RBCs	Red Blood Cells
RRT	Renal Replacement Therapy
SA	South Africa
SD	Standard Deviation

Var	Variant Gene
WHO	World Health Organisation

1. ARTICLE FOR SUBMISSION

The efficacy of intravenous artesunate in treatment of severe malaria

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1.1 BACKGROUND

According to the 2013 World Malaria Report, malaria is a major cause of mortality and morbidity in malaria endemic areas of the world. In 2010, there were an estimated 207 million cases of malaria reported worldwide, with 627 000 estimated deaths in 2012 and most of the estimated cases and fatalities reported in sub-Saharan Africa. (1) This makes malaria the second most common cause of infectious disease related deaths in the world, after tuberculosis. (2)

Malaria occurs throughout most of the tropical regions of the world, (3) *P.falciparum* predominates in Africa, New Guinea, the Dominican Republic and Haiti whilst *P. vivax* is more common in Central America and Asia. (4)

In SA, malaria is endemic in three provinces, Mpumalanga, KwaZulu-Natal and Limpopo. (5) The highest transmission occurs in areas bordering the Kruger National Park and Swaziland. In 2011 there were 7 626 cases of malaria reported throughout SA with 87 deaths reported for the same period.

Malaria cases and related deaths are also reported in Gauteng and show a peak during the rainy season. This seasonality is related to travellers returning from malaria-endemic areas during the summer holidays. (6)

Although quinine has been the mainstay of treatment for severe malaria, complications such as quinine induced hyperinsulinaemic hypoglycaemia, arrhythmias, sterile abscesses and painful intramuscular (IM) injection make it less desirable. Furthermore the South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) showed mortality benefit with the use of IV artesunate over quinine and these findings were replicated by the AQUAMAT trial in African children excluding SA. (7, 8)

Intravenous artesunate remains an unregistered drug for treating severe malaria in SA. Given this problem and the lack of local data regarding the treatment of severe malaria with artesunate, we decided to evaluate its efficacy.

1.2 METHODS

1.2.1 STUDY DESIGN AND SETTING

A retrospective descriptive study was done at two academic intensive care units (ICU's) in Johannesburg.

1.2.2 POPULATION AND SAMPLE

All patients included were admitted to the ICUs of Chris Hani Baragwanath Academic Hospital (CHBAH) and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with a diagnosis of severe malaria, treated with IV artesunate, between 1 April 2010 and 30 April 2014, who were part of the Parenteral Artesunate Access Programme. Pregnant patients and children were excluded.

1.2.3 ETHICS

Ethical approval for the study was obtained from the Human Research Ethics Committee, Medical (HREC) (approval number M141140), CHBAH and CMJAH. Given that artesunate is a section 21 drug, written informed consent was required by the treating physician before the Medicine Control Council (MCC) would approve the drug administration.

1.2.4 OUTCOMES

The primary outcome was to determine the observed mortality and relate it to the predicted mortality based on the APACHE II severity of illness score. Secondary outcomes included the clinical changes from admission to discharge, the incidence of documented hypoglycemia (complication), length of ICU stay and the markers of disease severity.

1.2.5 DATA COLLECTION

Collection of data from patients' ICU records was done using a standardized data collection sheet. This was then entered into an electronic spreadsheet (MS Excel). No identifying data was entered into the study collection tools.

1.2.6 STATISTICAL ANALYSIS

Data was analysed using Statistica version 13.3. All data was evaluated for normal distribution. Normally distributed data was described using mean and standard deviation (SD) while non parametric data using medians and interquartile ranges (IQR).

Continuous variables were compared using Wilcoxon's matched pairs test or T-test for dependent variables depending on distribution. Binary data was compared with the chi squared test.

1.3 RESULTS

A total of fifty-six patients were admitted with severe malaria to two academic ICU's during the 49 month study period. The mean age of patients was 40.3 years with a standard deviation (SD) of 11.6 years. The majority of the patients were men (71.4%). Table 1.

Nearly half of the patients had at least one co-morbidity (48.2%). The most common co-morbidity was HIV (32.1%).Table 2. Travel outside of Johannesburg, to a malaria endemic region within the past 30 days was recorded. Figure 1. Of note, 26.8% of patients had no history of travel to a malaria endemic region - this called Odessan malaria. The most commonly visited malaria region was Mozambique (33.9%). Figure
1.

1.3.1 PRIMARY OUTCOME

The mean APACHE II score was 19 points SD 5.4 points. The predicted mortality for this group of patients was 32.2%. We observed a lower than predicted mortality rate of 21.4%. The standardized mortality rate (SMR) was 0.67. The SMR was obtained as: actual mortality divided by predicted mortality. There was no significant difference in the mortality rates of those with a travel history (9/40, 22.5%) vs. those with no travel history (2/15, 13%), p=0.365 Fisher exact test.

1.3.2 SECONDARY OUTCOMES

Heart rate (HR), respiratory rate (RR) and Glasglow Coma Scale (GCS) all improved from admission to discharge. These improvements were clinically and statistically significant. Table 3. Mean systolic blood pressure increased from 105mmHg at admission to 118mmHg at discharge but this was not statistically significant. There were statistically significant improvements in several indicators of severe malaria, between admission and discharge. Acidemia, total bilirubin, markers of acute kidney injury (AKI) and bleeding risk (platelets) all improved. Table 4

1.3.3 SURVIVORS VS. NON-SURVIVORS

Non-survivors had a lower GCS and more severe metabolic acidosis on admission (Day 1). There was a trend towards a lower parasitemia in the non-survivors. As expected, non-survivors had a higher Apache II score. Table 5. Patients who were mechanically ventilated had an increased risk of death, Odds ratio (OR) of 35, (Confidence Intervals (CI) 7.0- 182). Similarly, patients requiring vasopressors were also at increased risk of death (OR 4.3, CI 1.7-11.4)

The frequency of complications and indicators of severity are given in table 6. There were 7 patients with seizures during their ICU stay. Four patients had single seizures, 2 patients had 2 seizures and one had 4 seizures.

1.4 DISCUSSION

Our group consisted of an older cohort (mean age 40.3 years) with almost half having at least one co-morbidity (48.2%) and 32.2% being HIV positive. There was a greater level of acidemia (median TC02 was 17mmol/l on

admission) and a greater level of renal dysfunction (median urea 22.4mmol/l on admission). In addition, our group required significantly more organ support: 49% renal replacement therapy, 31% vasopressor support and 30% mechanical ventilation. Despite a more severely ill group of patients in our cohort, the mortality was similar (21.4%). This, together with a higher predicted mortality for our group (32.2%) provides support for the efficacy of IV artesunate, in severe falciparum malaria requiring ICU. Another reason for the similar level of mortality, despite our group having more disease severity, is that our group had better organ support by way of haemodialysis and were all cared for in ICU.

The largest adult randomized clinical trial comparing IV artersunate and quinine for severe falciparum malaria was the SEAQUAMAT trial which included 1461 patients, of which 86% (1259) were adults. The mean age in this study was 27.9 years. The acidemia and renal dysfunction were relatively mild, median TC02 of 22mmol/l in the total patient cohort and median urea of 9.2 and 10.4mmol/l for the 2 study groups. The utilization of organ support was also relatively low: 8% renal replacement therapy, 3% vasopressor support. Mechanical ventilation in the 2 groups was 4 and 5%. The overall mortality for the study was 19%. (7)

Odyssean malaria describes the occurrence of malaria in a person with no history of recent travel to a malaria endemic region. It is also colloquially known as airport, suitcase, minibus or taxi-rank malaria. (9) It is most likely that an anopheles malaria vector mosquito has been inadvertently transported from a malaria area to a non- malaria region and affects local residents. (9) 8 outbreaks with 14 laboratory proven cases and 8 probable cases were identified between 2007 and 2013 where the case fatality rate was 10 fold higher than the national case fatality rate. (9) This may in part be explained by a delay in the diagnosis, possibly due to the lack of a significant travel history.

This study confirms the significance of odyssean malaria in SA and particularly in Gauteng where our study was performed. We found that 26.8% of our malaria cases could in fact be attributed to odyssean malaria. Our data,

although small suggests a trend to a lower mortality (13%) for those with odyssean malaria vs 22.5% for those with a travel history. A possible explanation for this finding is that it may well be fortuitous that the patients with odyssean malaria in this cohort of patients had a relatively high parasitaemia (13%) making for an easier diagnosis and rapid initiation of treatment. However, with regards to the low parasitaemia in non-survivors, it's possible that non-survivors were non-immune to malaria, as opposed to patients living in malaria endemic areas.

Regarding the possible adverse effects of artesunate, there were no episodes of hypoglycemia (≤2.2mmol/l). However, there were 3 cases were glucose levels were <3.5mmol/l with no documented clinical sequalae. There were 7 patients with seizures during their ICU stay. We were unable to determine the cause of the seizures due to missing clinical information.

1.5 LIMITATIONS

This was a retrospective study. However, the efficacy of IV artesunate has already been shown in falciparum malaria. Patients may have received initial quinine prior to consent for artersunate therapy. A second limitation in this study was the small sample size, and incomplete patient records.

1.6 CONCLUSION

Our data serves to affirm the efficacy in a specific subgroup with severe falciparum malaria requiring intensive care.

1.7 DISCLOSURE STATEMENT

The authors have no conflict of interest to declare

1.8 TABLES

Table 1: Baseline Patients Description

Variable	Mean/Median	n
Age mean (SD)	40.3 y (11.6)	56
Gender male (%)	40 (71.4%)	40/56
Weight mean (SD)	73 kg (15.3)	44
%Parasitemia (Falciparum) median (IQR)	10.6 (13.2)	44
Apache II mean (SD)	19 (5.4)	35
Predicted mortality	32.2%	

Table 2: Past Medical History

	Description	n	Percentage
1	No known co-morbidities	29	51.8
2	HIV positive	18	32.1
3	Any Other co- morbidities	9	16.1

Table 3: Clinical Characteristics Compared From Admission (Day 1) To Discharge (DC) Using Wilcoxon Matched Pairs Test Or T Test For Dependent Samples

Parameter	Day 1	Discharge	n	P value
Heart rate/min- mean (SD)	108 (21.4)	98 (24.5)	55	0.001
Respiratory rate- mean (SD)	27 (9.4)	23 (7.2)	53	0.007
GCS below 15- median (IQR)	3 (6)	0 (2)	38	0.000
Systolic BP median (IQR)	105 (50)	118 (45)	54	0.55

Table 4: Changes In Indicators Of Severe Malaria From Admission (Day 1) To Discharge (DC) Using Wilcoxon Matched Pairs For Dependent Samples

Parameter	Day 1	Discharge	n	P value
TC02 meq/l- median (IQR)	17 (8)	23 (7)	50	0.000
Total Bilirubin- median (IQR)	63 (101)	53 (88)	34	0.013
Urea mmol/l- median (IQR)	22.4 (17.2)	11.5 (10.4)	52	0.000
Platelets 10^3/ul -median (IQR)	48 (56)	89.5 (92)	53	0.000

Table 5: Differences Between Survivors and Non-Survivors Using

The Mann

Whitney U Test for Independent Samples

Parameter	Non Survivors	Survivors	n	P value
GCS below 15 median (IQR)	7.5 (2.5)	2 (5)	44	0.000
TC02 meq/l median (IQR)	13 (8.5)	18.5 (6)	40	0.010
Parasitemia % median (IQR)	6.6 (10)	11.2 (14.3)	33	0.053
Apache II mean (SD)	22.5 (4.9)	18.2 (5.3)	35	*

***** not reported as the value is not clinically relevant

Table 6: Frequency of Complications/ Disease Severity of Malaria

Number	Complication	N	% Frequency
1	Lactate ≥ 4.0	26	50
2	Requiring RRT	28	49
3	pH ≤ 7.25	21	37.5
4	Prolonged ICU stay > 4d	19	34
5	Requiring inopressor	16	31
6	Requiring invasive MV	21	30
7	Hb ≤ 7.0	14	28.6
8	Na <130	12	21.4
9	K<3.5 or >6.0	11	19.6
10	Platelets ≤ 25	7	12.5
11	Seizures	7	12.5
12	HGT <3.5	3	5.5

1.9 FIGURES

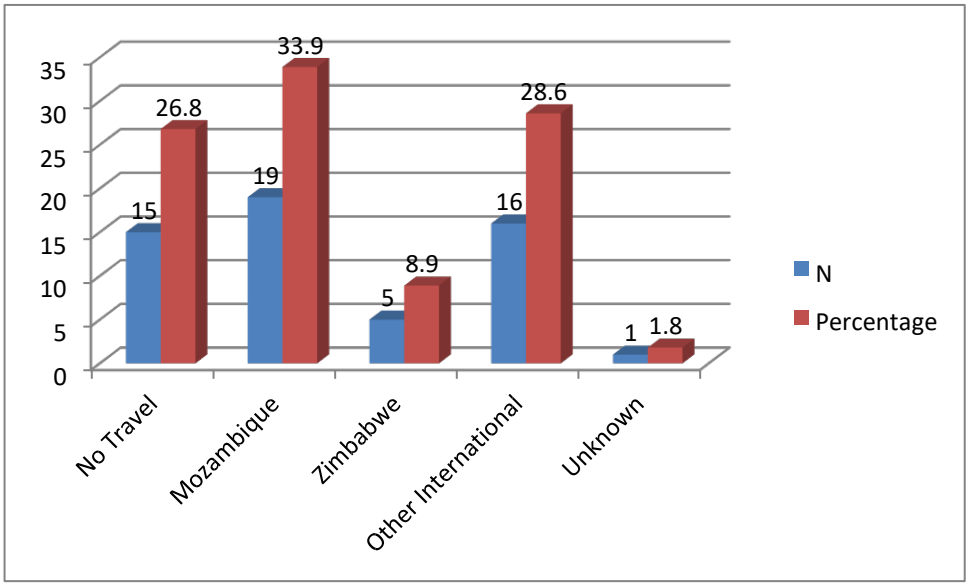

Figure 1: Travel history

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2. APPENDIX A: EXTENDED LITERATURE REVIEW AND APPROVED PROTOCOL

2.1 INTRODUCTION

Malaria is a disease caused by the protozoa, *Plasmodium*. It is transmitted to humans by the bite of an infected female anopheles mosquito. There are five species of the genus *Plasmodium* and they all have the potential to cause disease in humans; *Plasmodium (P) falciparum, P.vivax, P.ovale, P.malariae* and *P.knowlesi. P.knowlesi* is a monkey malarial parasite which is found only in South East Asia and can cause disease in humans. (3) The life cycle of the *Plasmodium* parasite takes place in both the human body and in the mosquito.

2.2 EPIDEMIOLOGY

According to the 2013 World Malaria Report, malaria is still a major cause of mortality and morbidity in malaria endemic areas of the world. In 2010, there were an estimated 207 million cases of malaria reported worldwide, with 627 000 estimated deaths in 2012 and most of the estimated cases and fatalities reported in sub-Saharan Africa. (1)This makes malaria the second most common cause of infectious disease related deaths in the world, after tuberculosis. (2)

Malaria occurs throughout most of the tropical regions of the world. (3) *P.falciparum* predominates in Africa, New Guinea, the Dominican Republic and Haiti. *P. Vivax* is more common in Central America and Asia. (4) The prevalence of both *P.falciparum* and *P. vivax* is approximately equal in South America, the Indian subcontinent, eastern Asia and Oceania. *P.malariae* is found in most endemic areas especially throughout sub-Saharan African, but is much less common. *P.ovale* is relatively unusual outside of Africa and, where it is found, comprises less than 1% of isolates. Patients infected with *P. knowlesi* have been identified on the island of Borneo and to a lesser extent elsewhere in South East Asia where the main hosts, long-tailed and pig-tailed macaques, are found. (3, 10)

Endemicity of malaria traditionally has been defined in terms of parasitaemia rates or palpable-spleen rates in children between the ages of 2-9 years old.

It's hypoendemic if rates are less than 10%, mesoendemic if 11 - 50%, hyperendemic is 51 - 75% and holoendemic if greater than 75%. In holoendemic areas and hyperendemic areas, where there is intense P falciparum transmission, people may sustain more than one infectious mosquito bites per day and are infected repeatedly throughout their lives. (6) In such settings, rates of morbidity and mortality due to malaria are considerably higher during early childhood. Immunity against disease is hard won in these areas, and the burden of disease in young children is high. By adulthood, most malaria infections are asymptomatic. (3)

Constant, frequent, year-round infection is termed *stable transmission*. In areas where transmission is low, erratic or focal, full protective immunity is not acquired, and symptomatic disease may occur at all ages. This situation usually exists in hypoendemic areas and is termed *unstable transmission*. (3, 11) Malaria can behave like an epidemic disease in some areas particularly those with unstable transmission such as Northern India, Sri Lanka, Iraq, Turkey, the horn of Africa, Rwanda,
Madagascar and Central Asia. (3)

In SA, malaria is known to be endemic in three provinces; Mpumalanga, KwaZulu-Natal and Limpopo. (5) The highest transmission occurs in areas bordering the Kruger National Park and Swaziland. In 2011, 7 626 cases of malaria were reported throughout SA resulting in 87 deaths. Despite Gauteng being a nonmalaria area, malaria and related deaths are also reported in this province, peaking during the rainy season. This seasonality is related to travellers returning from malariaendemic areas, particularly Mozambique. (6) The National and Provincial malaria control programmes have been very successful in reducing the malaria risk in SA. Notified malaria cases in SA have dropped by 80% over the last decade due to key interventions on preventive measures. (12)

2.3 PATHOGENESIS

Human infection occurs after a female anopheles mosquito bite which releases sporozoites into the body. Sporozoites reach the liver via the bloodstream where they invade hepatocytes.

In the liver reproduction is asexual and results in formation of daughter merozoites. The hepatocytes become swollen and burst to release merozoites into the bloodstream. Merozoites invade red blood cells (RBCs) and multiply over and over again into trophozoites. (3) In *P.vivax* and *P.ovale* infections, dormant forms called hypnozoites remain in hepatocytes and may cause disease within three weeks but may remain dormant for up to a year. (13)

After forty-eight hours, trophozoites have used up most of the host haemoglobin and have matured into schizonts. When each infected RBC ruptures, it releases six to thirty daughter merozoites. Each of these merozoites can invade a new RBC and repeat the cycle. In the case of *P.falciparum*, after a number of asexual cycles, some of the parasites develop into male and female gametocytes. With the rest of the *Plasmodium* species, male and female gametocytes develop immediately after release from the liver. When the female anopheles mosquito bites an infected human it ingests the male and female gametocytes which then form a zygote in the mosquito's midgut. The zygote matures into an ookinete then into an oocyst in the gut wall. The oocyst expands and bursts to release sporozoites which migrate to the salivary glands of the mosquito to be released into a human with the next bite. (3, 14)

The following processes in the pathogenesis of Falciparum malaria are important in disease causation and subsequent progression of disease to severe malaria. Adhesion of infected RBCs to human endothelium is considered a key event in the pathogenesis of severe malaria. (15) RBCs infected with *P. falciparum* exhibit:

1. cytoadherence: attachment to venular and capillary endothelium
2. rossetting: adherence to uninfected RBCs
3. agglutination: adherence to other infected RBCs.

Furthermore, the three processes above result in the sequestration of RBCs containing mature forms of the *Plasmodium falciparum* in vital organs,

particularly in the brain, lungs and kidney. There the parasites interfere with microcirculatory flow and metabolism. Severe malaria is associated with reduced deformability of uninfected RBCs. This compromises their passage through the partially obstructed capillaries and venules and shortens RBC survival. (3)

2.4 DETAILED PATHOGENETIC CHARACTERISTICS OF SEVERE MALARIA

2.4.1 CYTOADHERENCE

According to Aution *et al*., mature forms of parasites, can adhere to the vascular endothelium of several organs, including lung, heart, brain, liver, kidney, subcutaneous adipose tissues and the placenta. This feature of the disease, has been related exclusively to *P. falciparum*. (16) Parasite sequestration is thought to be the pathological basis of the severe manifestations of malaria, including cerebral malaria. It causes blood flow impairment leading to local hypoxia. It enhances parasite replication and resetting (the sticking of infected red blood cells (IRBC) to non-infected RBC's). (16) Sequestration is mostly mediated by mature parasite forms, approximately twenty hours after RBC invasion. The parasites produce new proteins that are exported to the IRBC surface and increase the adhesiveness of IRBC to the endothelium. (15, 16) Some of these proteins include the *P falciparum* erythrocyte membrane protein 1 (PfEMP1) and the *P.Falciparum* Histidine – Rich Protein (PfHRP). (3, 15, 16) These ligands are responsible for the establishment of knobs, which are symmetric membrane arrangements that appear on the surface of infected RBC. PfEMPI, a multimetric protein encoded by the *var* (variant) gene protrudes from the knobs and plays a major role in sequestration and thus virulence. (16)

To adhere to the endothelium, the parasites first adhere, roll and then become firmly attached to the endothelium adhesion molecules. These molecules include Intercellular Adhesion Molecule 1 (ICAM-I) and CD 36. ICAM 1 is a major sequestration receptor and mediates cerebral sequestration by serving as a rolling receptor. (13, 16) On the other hand, CD 36 gives stationary and staple adherence under flow. (16)

Sequestration is also seen in pregnancy, when parasites adhere to the placenta. PfEMPI is again the main adhesion receptor which adheres to the trophoblastic villous endothelium mainly through chondroitin – 4 - sulfate (CSA) and other sugars. As such malaria in pregnancy can be severe for mothers and can lead to fetal mortality. (16)

2.4.2 ROSETTING

Rosetting is one of the forms of cytoadherence of late stages of IRBC, to nonparasitized red blood cells and/or platelets. (15, 16) The IRBC ligand involved in rosette formation is PFEMPI, and there are three receptors associated with rosetting. These are complement receptor 1, (CRI), Heparan sulfate (HS) and the ABO blood group. (16) Although *P. falciparum , P.vivax* and *P.ovale* are all able to form rosettes, only those caused by *P. falciparum* have been associated with severe malaria and may enhance microcirculatory obstruction. (16)

2.4.3 HOST RESPONSE TO PLASMODIAL INFECTION

Initially, the host responds to plasmodial infection by activating non-specific defence mechanisms. (3) Both splenic immunologic and infiltrative clearance functions are augmented, and the removal of both parasitized and uninfected RBCs is accelerated. (3)

The parasitized cells that escape splenic removal are destroyed when the schizont ruptures. The material released on rupture induces the activation of macrophages and the release of pro-inflammatory cytokines. These cytokines cause fever and exert other pathologic effects. Temperatures of ≥ 40°C damage parasites in untreated infections. The effect of such temperatures is to further synchronise the parasitic cycle, with eventual production of the regular fever spikes and rigors. (3)

The geographic distribution of sickle cell disease, haemoglobin C and E, hereditary ovalocytosis, the thalassaemias, and glucose-6-phosphate dehydrogenase (G6PD) closely resemble that of malaria before the introduction of control measures. This similarity suggests that these genetic disorders confer protection against death from *P.falciparum* infection, (3, 17)

for example, Haemoglobin A/Haemoglobin S (HBA/S) heterozygotes (sickle cell trait), have a six fold reduction in the risk of dying from severe malaria. This decrease in risk appears to be related to impaired parasite growth at low oxygen tensions and reduced parasitized red cell cytoadherence. (3, 17)

Nonspecific host defence mechanisms stop the infection's expansion, and the subsequent strain-specific immune response that controls the infection. Eventually, exposure to sufficient strains confers protection from high-level parasitaemia and disease but not from infection. (3, 14) As a result of this state of infection without illness (premonition) asymptomatic parasitaemia is common among adults and older children living in regions with stable and intense transmission (holo and hyperendemic areas). Both humoral and cellular immunity are necessary for protection. (3)

2.5 CLINICAL MANIFESTATIONS OF MALARIA

In non-immune individuals infected with *P.falciparum* , the incubation period is 10 days with a range of 5 to 10 days. For non- falciparum malaria, the incubation period may be 15 to 16 days and up to 21 days. (3, 18) Uncomplicated malaria is characterised by fever, headache, sweating, myalgia/arthralgia, abdominal discomfort, nausea and vomiting. Paroxysms in which fever spikes, chills, and rigors occur at regular intervals are relatively unusual and suggest infection with *P.vivax* or *P.ovale*. The fever of *P.falciparum* infections is usually irregular. The usual examination findings in uncomplicated malaria include elevated temperature, anaemia, and an enlarged spleen. Slight enlargement of the liver is also common particularly among young children. Mild jaundice is also common and may be seen even in patients with uncomplicated malaria. (3, 19) The jaundice is due to increased haemolysis.

2.6 SEVERE FALCIPARUM MALARIA

Severe malaria is defined by clinical and laboratory evidence of organ dysfunction and includes:

- impaired consciousness including unarousable coma

- prostration
- multiple convulsions
- acidotic breathing
- acute pulmonary oedema
- acute respiratory distress syndrome (ARDS)
- acute kidney injury
- coagulopathy
- circulatory collapse or shock (systolic blood pressure <80mmHg in adults and

  <50mmHg in children
- clinical jaundice plus
- evidence of other vital organ dysfunction. (20)

Furthermore, the following laboratory findings in malaria patients are evidence of severe malaria:

- hypoglycaemia of <2,2mmol/l or <40mg/dl
- metabolic acidosis (plasma bicarbonate <15mmol/l)
- severe normocytic anaemia (haemoglobin <5g/dl in adults or <7g/dl in children),
- thrombocytopaenia
- haemoglobinuria
- hyperlactataemia (lactate >5mmol/l),
- renal impairment (serum creatinine >265micromoles per litre) and

- radiological findings in keeping with pulmonary oedema and ARDS. (20)

2.7 CLINICAL MANIFESTATIONS OF SEVERE MALARIA

Most of the major manifestations of severe malaria are described below.

2.7.1 CEREBRAL MALARIA

The onset of cerebral malaria may be dramatic with a generalised seizure or gradual with initial drowsiness and confusion. (18) Once coma ensues, it is associated with death rates of approximately 20% in adults and 15% in children. Whereas adults rarely suffer neurological sequelae (<3 % of cases), approximately 5% of children surviving cerebral malaria have residual neurologic deficits when they regain consciousness. These may include serious sequelae such as hemiplegia, cerebral palsy, cortical blindness, deafness and impaired cognition and learning. Fortunately, these deficits improve or resolve completely within 6 months. (18)

2.7.2 HYPOGLYCAEMIA

This is a common complication of severe malaria and is an independent risk factor for death. (21) Hypoglycaemia in malaria results from a failure of hepatic gluconeogenesis and an increase in the consumption of glucose by both host and parasites. (22) In the case of patients treated with quinine, quinine is a powerful stimulant of pancreatic insulin secretion. Hypoglycaemia is a significant risk factor for an adverse outcome in pregnant women receiving quinine. (22)

2.7.3 ACIDOSIS

Acidosis results from accumulation of organic acids. In adults co-existing renal dysfunction worsens the acidosis. (3) Increased lactate production and impaired metabolism, as well as impaired metabolism of ketoacids and other organic acids have been implicated in the development of metabolic acidosis. (22)

2.7.4 NON-CARDIOGENIC PULMONARY OEDEMA

The pathogenesis of non-cardiogenic pulmonary oedema in malaria is not clear. However, the mortality rate is >80%. Non cardiogenic pulmonary

oedema can be aggravated by overly vigorous administration of intravenous (IV) fluids. (23)

2.7.5 RENAL IMPAIRMENT

This is one of the common manifestations of severe malaria. The pathogenesis of renal involvement is unclear but may be related to proliferation of glomerular cells, protein deposition in the glomerular space, and decrease in the glomerular area. However, it has also been postulated that renal impairment results from erythrocyte sequestration and agglutination interfering with renal microcirculatory flow and metabolism, leading to acute tubular necrosis. (3, 23) Renal replacement therapy is often necessary when acute indications for dialysis are noted.

2.7.6 HAEMATOLOGIC ABNORMALITIES

Haematologic abnormalities include anaemia, thrombocytopaenia and leukopaenia. (24) Although not completely understood, it is thought that anaemia results from a combination of haemolysis of parasitized and unparasitized red blood cells, depressed as well as ineffective erythropoiesis. (24) Other contributing factors may include decreased red blood cell deformability and splenic phagocytosis. (24) Leukopaenia is common, though leucocytosis may occur.

2.7.7 LIVER DYSFUNCTION

Deranged liver functions are commonly seen as a complication of severe malaria. Presence of raised hepatic enzymes with near normal coagulation parameters suggests malarial hepatopathy. (25) Hepatic dysfunction contributes to hypoglycaemia, lactic acidosis and impaired drug metabolism. (25)

2.8 DIAGNOSIS OF MALARIA

The diagnosis of malaria rests on the demonstration of asexual forms of the parasites in stained peripheral blood smears. If negative, repeat smears should be done if there is a high index of suspicion. Both thick and thin smears should be routinely requested. (3, 26) The gold standard for diagnosis of malaria remains the examination of thick and thin films for parasites, with thick films being more sensitive at identifying parasites and thin films being

important for identification of malaria species. Alternative diagnostic modalities include fluorescence microscopy of parasite nuclei stained with acridine orange and polymerase chain reaction assays. Bedside antigen testing is commonly employed which detects the presence of histidine-rich protein-2 of *P. falciparum* (PfHRP) or a parasite-specific lactate dehydrogenase. (18, 27)

2.9 TREATMENT OF SEVERE MALARIA

Malaria represents a medical emergency because it may rapidly progress to severe complications and death without prompt and appropriate treatment. (18) Although IV quinine has been the mainstay of treatment for severe malaria, complications such as quinine induced hyperinsulinaemic hypoglycaemia, arrhythmias, sterile abscesses and painful intramuscular (IM) injection make it less desirable. Furthermore, hypoglycaemia is an independent risk factor for death in severe malaria, and even dosage adjustments of quinine did not reduce the frequency or severity of hypoglycaemia. (21)

2.9.1 ARTESUNATE BACKGROUND

Artemesinin derivatives are manufactured from the Chinese wormwood plant, artemesinin annua and have been used in the treatment of malaria for over 1000 years in this region. They were developed to improve solubility and bioavailability for oral use which has been incorporated into the management of malaria in various combinations from the 1970s. (28) IV artesunate is rapidly metabolised via esterase hydrolysis to its active metabolite dihydroartemisinin (DHA) in less than 25 minutes. The active metabolite undergoes metabolism via UDP-glucuronosyltransferase system and its half-life after IV administration ranges from 18 minutes to 2.14 hours. This implies that the pharmacokinetics of the drug should not be significantly altered by other drugs. (29)

2.9.2 ARTESUNATE AS FIRST LINE THERAPY

In 2011, the World Health Organisation guidelines recommended IV artesunate as first line therapy for severe malaria after artesunate was shown to be far superior to quinine in South East Asian adults. There, its use resulted in a 38.6 % reduction in mortality, as well as African children where a 22%

reduction in mortality was achieved in two landmark randomised controlled trials. (7, 8)

In the Democratic Republic of Congo, these findings were reflected with artesunate and other artemisinin derivatives, achieving reduction in parasitaemia and clinical resolution faster than quinine (23 versus 24 hours, $p<0.001$). (30)

In a Cochrane review by Jones et al (2010), six trials that were carried out in Asia using artesunate and quinine were analysed and the findings provided further evidence that treatment with artesunate resulted in a significant reduction in mortality, parasite clearance time, and hypoglycaemia in people with severe malaria in Asia. (31)

Furthermore, the preparation and administration of IV artesunate as boluses is easier and its use eliminates the need for cardiac monitoring, rate-controlled infusions or the hypoglycaemia associated with quinine infusion. (32)

In addition, time to drug administration and parasite clearance from the time of admission is shorter than with quinine and artesunate costs less per person when compared to quinine. (30) However, the artemisinin derivatives have relatively short half-lives which necessitates use of oral antimalarials after the initial IV infusions to prevent relapse.

2.9.3 ADVERSE REACTIONS

Artesunate was well tolerated with no serious drug related side effects (including serious cardiac or neurological side effects). Post treatment hypoglycaemia was less frequent in patients assigned to artesunate than to quinine. (8) Adverse reactions to artesunate include Type 3 hypersensitivity reactions occurring at a rate of 1:3000 people treated with artesunate.

Post-artesunate haemolytic anaemia affects 20% of European travellers with severe imported malaria, 60% of whom required blood transfusion in initial retrospective reports, however, a larger prospective French study, showed less than 5% of their cohort required blood transfusion. It occurs 2 to 3 weeks after initial treatment with artesunate and clearance of parasite, with

resolution during weeks 3 to 6. It is characterised by raised Lactate Dehydrogenase and supressed haptoglobin in the absence of conventional causes of haemolysis. It has not been shown to cause death. (33 - 35)

2.9.4 ARTEMISNIN RESISTANCE

Another prominent concern is the emergence of artesunate resistance as evidenced by prolonged time to parasite clearance and relapse after oral treatment of patients with uncomplicated malaria in Cambodia and Thailand, where interventions are urgently required to minimise spread of resistant malaria. (36, 37)

2.9.5 ARTESUNATE USE IN SA

Prior to its registration in SA, provision had been made for compassionate use. There has been an access project since April 2010, where selected patients with severe malaria were treated with artesunate through the Parenteral Artesunate Access Programme managed by the University of Cape Town (UCT) which was available only at certain hospitals called sentinel hospital sites. The supplier of artesunate to the sentinel sites is Guillian Pharmaceuticals (China) via a local distributor, Equity Pharmaceuticals. Countries that have already approved the use of artesunate, include United Kingdom, People's Republic of China, Nigeria, Ghana, Kenya, Tanzania, Zambia, Thailand, Cameroon, Sierra Leone. (20) With the widespread approval of IV artesunate as well as that World Health Organisation WHO, has replaced IV quinine with artesunate, it becomes important in SA to perform research and publish information on use of artesunate use in South African patients with severe malaria. To date no local or international literature is available to show that any research has been done and published on use of artesunate on South African patients. We are hoping that this study will add to the body of knowledge on the use of artesunate in severe malaria in SA.

Therefore, the aim of this study is to do a retrospective description of a cohort of patients that were treated with intravenous artesunate for severe malaria in the Intensive Care Unit (ICU) at Chalortte Maxeke Johannesburg Academic Hospital (CMJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH)

from the inception of the Parenteral Artesunate Access Programme in April 2010 at these hospitals.

2.10 THE PROTOCOL

2.10.1 RESEARCH TOPIC

Descriptive analysis of a cohort of patients admitted and treated with intravenous artesunate at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Chris Hani Baragwanath Academic Hospital (CHBAH) from April 2010 to April 2014.

2.10.2 HYPOTHESIS

Treatment of patients with severe malaria with IV artesunate significantly reduces risk of death and improves other outcomes.

2.10.3 STUDY OBJECTIVES

1. To describe the demographics of patients with severe malaria who were treated at

   Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital from April 2010 to April 2014.
2. To describe the clinical conditions of these patients on admission at or before initiation of intravenous artesunate therapy.
3. To describe the course taken by the illness while on intravenous artesunate.
4. To describe the complications that occurred in any of the patients.
5. To describe the outcome of the treatment of these patients with intravenous artesunate:
   1. Length of stay in ICU
   2. Morbidity
   3. Mortality
   4. Adverse events
   5. Hypoglycaemia

6. Convulsions
7. Disease severity markers including need for inotropic support, ventilation, dialysis

2.10.4 METHODOLOGY

2.10.4.1 Study Design

The study is a retrospective, descriptive study.

2.10.4.2 Study Population and Sample

The collection of data from patient's records took place in the Intensive Care Units of Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital.

The study population was all patients that were admitted to the ICUs at CMJAH and CHBAH with severe malaria and were treated with intravenous artesunate. The study sample covered all patients who were treated on the Parenteral Artesunate Access Programme from April 2010 to April 2014.

Inclusion Criteria: All adult patients (>18yrs) that had severe malaria and were treated with intravenous Artesunate at CMJAH and CHBAC. That means that these were the same patients that met the eligibility criteria for the Parenteral Artesunate Access Programme. The Parenteral Artessunate Access Program did allow the treatment of paediatric patients, but for the purpose of this study only adults >18yrs were included in this analysis.

Exclusion Criteria: Pregnancy (1st trimester of pregnancy)

Consent: Because Artesunate is a section 21 drug, all patients that received it would have given consent for treatment. If the patients were too ill to do so, their family or supervisor would have given consent on their behalf.

Confidentiality: Strict confidentiality was maintained with handling of patients' records.

Only authorised persons could handle the patients' records. All information obtained during the course of this research, including: hospital records, personal data and research data was kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies individual patients.

2.10.4.3 Detailed Description of methods and techniques to be used

Collection of data from patients' records was done using data collection sheets. The data collection sheets required the same information from each patient's record. For each patient, there was a data collection sheet at the time of admission. There were subsequent data collection sheets for each patient showing subsequent monitoring of patients.

All clinical records of each patient (manual and/or electronic) were used in order to gather all information required to complete the data capturing sheets. The ward admission book was also used for data collection.

1. Variables: 1=continuous, 2=categorical The following variables were used for the study

Variable	**Continuous**	**Categorical**
Age	1	
Gender		2
Blood Pressure	1	
Pulse	1	
Respiratory rate	1	
Temperature	1	
Variable	**Continuous**	**Categorical**
Haemoglobin	1	
Blood sugar	1	

Ph	1	
Pregnancy test		2
Country visited in last 4 weeks		2
Lactate	1	
Parasite percentage	1	
Urea	1	
Creatinine	1	
Bicarbonate	1	
Jaundice		2
Abnormal bleeding		2
Glasgow coma scale	1	
Platelets	1	
Potassium	1	
Sodium	1	
Total bilirubin	1	
Direct bilirubin	1	
AST	1	
ALT	1	
Albumin	1	

2.10.5 STATISTICAL ANALYSIS

We will use Statistica version 13.3 for analysis. All data will be evaluated for normal distribution. Normally distributed data will be described using mean and standard deviation (SD) while non parametric data using medians and interquartile ranges (IQR).

Continuous variables will be compared using Wilcoxon's matched pairs test or T-test for dependent variables depending on distribution. Binary data will be compared with the chi squared test.

2.10.6 TIMING AND ETHICS

The protocol was submitted to the Ethics Committee in November 2014 and to the

Protocol Assessment Committee in February 2015. Data collection started in March

2015 pending Protocol Assessment Committee approval. (see Gantt chart below) Unconditional ethics approval has been granted.

	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14	Oct-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15	Apr-15	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15
Literature Review																				
Preparing Protocol																				
Ethics Application																				
Protocol Assessment																				
Collecting Data																				
Data Analysis																				
Writing up - Thesis																				
Writing up - Paper																				

2.10.7 FUNDING

I do not require funding for this study. As the principal investigator I will do my own photocopying as the need arises.

2.10.8 PROBLEMS

Since it's a retrospective study the biggest problems were missing information as well as missing records. Illegible handwriting by colleagues may also be a problem.

2.10.9 DEPARTMENTS INVOLVED

The general intensive care units of CHBAH and CMJAH. CHBAH is a 34 bed ICU unit with 9 paediatric beds, 9 trauma, 9 general and 9 high care beds. Patients from my study occupied the 9 general ICU beds. There is 24 hour medical cover. CMJAH is a

20 bed general medical ICU which manages medical and surgical patients. It includes 12 main ICU beds, 7 beds high care and 1 isolation bed.

2.10.10 PUBLISHING INTENT

- The research was being done for MMed purposes but also with an intention to publish in a journal.
- Each hospital reserves the right to retain the intellectual property rights of data collected at their individual hospitals.

2.10.11 REFERENCES (ENDNOTE X8) AS PER APPENDIX E-SUBMISSIBLE ARTICLE STYLE GUIDELINE ON PAGE 53

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APPENDIX B: DATA COLLECTION FORM

Appendix 1: Admission data Capturing Sheet

1 Database No.	
2 Date of Admission to Hospital	
3 Date of Admission to ICU	
4 Date of Discharge from ICU	
5 Date of Discharge from Hospital	
6 Date of Death	

Demographic Data

3 Age ☐

4 Gender:
- Male ☐
- Female ☐

Travel History

5 Country of origin
- Africa ☐
- RSA ☐
- Asia ☐
- Other ☐
- No data available ☐

6 Countries visited in last 4 weeks
- Asia ☐
- Other African country ☐
- Other ☐
- No data available ☐

7 RSA province visited in last 4 weeks
- Mpumalanga ☐
- KwaZulu-Natal ☐
- Limpopo ☐
- No data available ☐

8 Malaria prophylaxis taken
- Yes ☐
- No ☐
- No data available ☐

9 Malaria preventive measures instituted during visit to malaria area

Yes ☐
No ☐
No data available ☐

Admission Detail

10 Past medical history and co-morbilities ☐

11 Admission data:

Temperature ☐
Heart Rate (Pulse) ☐
Blood Pressure (BP) ☐
Respiratory Rate (RR) ☐
Glasgow Coma Scale (GCS) ☐
Weight ☐
Blood Sugar (HGT) ☐

On Examination

12 Convulsions

Yes ☐
No ☐

13 Systolic BP < 80mmHg

Yes ☐
No ☐

14 Altered Consciousness

Yes ☐
No ☐

15 Severe Weakness

Yes ☐
No ☐

16 Respiratory Distress: RR > 30/min (or Kussmaul Respirations)

Yes ☐
No ☐

17 Visible Jaundice

Yes	
No	

18 Macroscopic Haematuria

Yes	
No	

19 Abnormal bleeding (eg Retinal Haemorrhages)

Yes	
No	

Laboratory findings of severe malaria		**Details**
20 severe malaria Haemoglobin (Hb) < 5g/dl		
Yes		
No		
21 Hypoglycaemia (HGT < 2,2 mmol/L)		
Yes		
No		
22 Acidosis (Ph < 7,25 or plama bicarbonate < 15mmol)		
Yes		
No		
23 Hyperlactataemia (Venous lactate > 4)		
Yes		
No		
24 Hyperparasitaemia		
Yes		
No		
25 Elevated bilirubin (> 50 µmol/L)		
Yes		
No		
26 Urea > 10		
Yes		
No		

27	Creatinine > 265	
	Yes	
	No	

Other laboratory results for the purpose of this research		**Details**
28	Platelets	
29	Potassium (K+)	
30	Sodium (Na+)	
31	Total CO2	
32	Total bilirubin	
33	Direct bilirubin	
34	Albumin	
35	AST	
36	ALT	
37	GGT	
38	ALP	

Method of diagnosis of malaria used		**Details**
39	Rapid diagnostic test	
	Yes	
	No	
40	Blood smear	
	Yes	
	No	
41	Date of diagnosis of malaria	
42	Antimalarial drugs used	
	i.	
	ii.	
	iii.	
43	Other drugs used	
	i.	
	ii.	
	iii.	
	iv.	
	v.	

Appendix 2: Data Capturing Sheet at subsequent examinations

1 Database No.	
2 Date of Admission	
3 Subsequent examnation date	

4 **Follow up vital parameters**									
Temperature									
Heart Rate (Pulse)									
Blood Pressure (BP)									
Respiratory Rate (RR)									
Glasgow Coma Scale (GCS)									
Weight									
Blood Sugar (HGT)									
On Examination									
5 Convulsions									
Yes									
No									
6 Systolic BP < 80mmHg									
Yes									
No									
7 Altered Consciousness									
Yes									
No									
8 Severe Weakness									
Yes									
No									
9 Respiratory Distress: RR > 30/min (or Kussmaul Respirations)									
Yes									
No									
10 Visible Jaundice									
Yes									
No									

11 Macroscopic Haematuria
Yes
No

12 Abnormal bleeding (eg Retinal Haemorrhages)
Yes
No

Laboratory findings of severe malaria **Details**

13 severe malaria Haemoglobin (Hb) < 5g/dl
Yes
No

14 Hypoglycaemia (HGT < 2,2 mmol/L)
Yes
No

15 Acidosis (Ph < 7,25 or plama bicarbonate < 15mmol)
Yes
No

16 Hyperlactataemia (Venous lactate > 4)
Yes
No

17 Hyperparasitaemia
Yes
No

18 Elevated bilirubin (> 50 µmol/L)
Yes
No

19 Urea > 10
Yes
No

20 Creatinine > 265

Yes										
No										

Other laboratory results for the purpose of this research	**Details**									
21 Platelets										
22 Potassium (K+)										
23 Sodium (Na+)										
24 Total CO2										
25 Total bilirubin										
26 Direct bilirubin										
27 Albumin										
28 AST										
29 ALT										
30 GGT										
31 ALP										

Disease Severity plus Intervention									

32 Dialysis

Yes										
No										

33 Ventilation Required

Yes										
No										

34 Inotropes Required

Yes										
No										

Drug side effects	**Details**

35 Any antimalarial drugs' side effects that developed

Yes	
No	

36 Any other drugs' side effects that developed

Yes	
No	

APPENDIX C: ETHICS CLEARANCE

R14/49 Dr Ratidzo Tadzimirwa

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141140

NAME: (Principal Investigator)	Dr Ratidzo Tadzimirwa
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Efficacy of Intravenous Artesunate in Treatment of Severe Malaria in South Africa
DATE CONSIDERED:	28/11/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr IS Kalla and Dr J Brown
APPROVED BY:	Professor Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/12/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

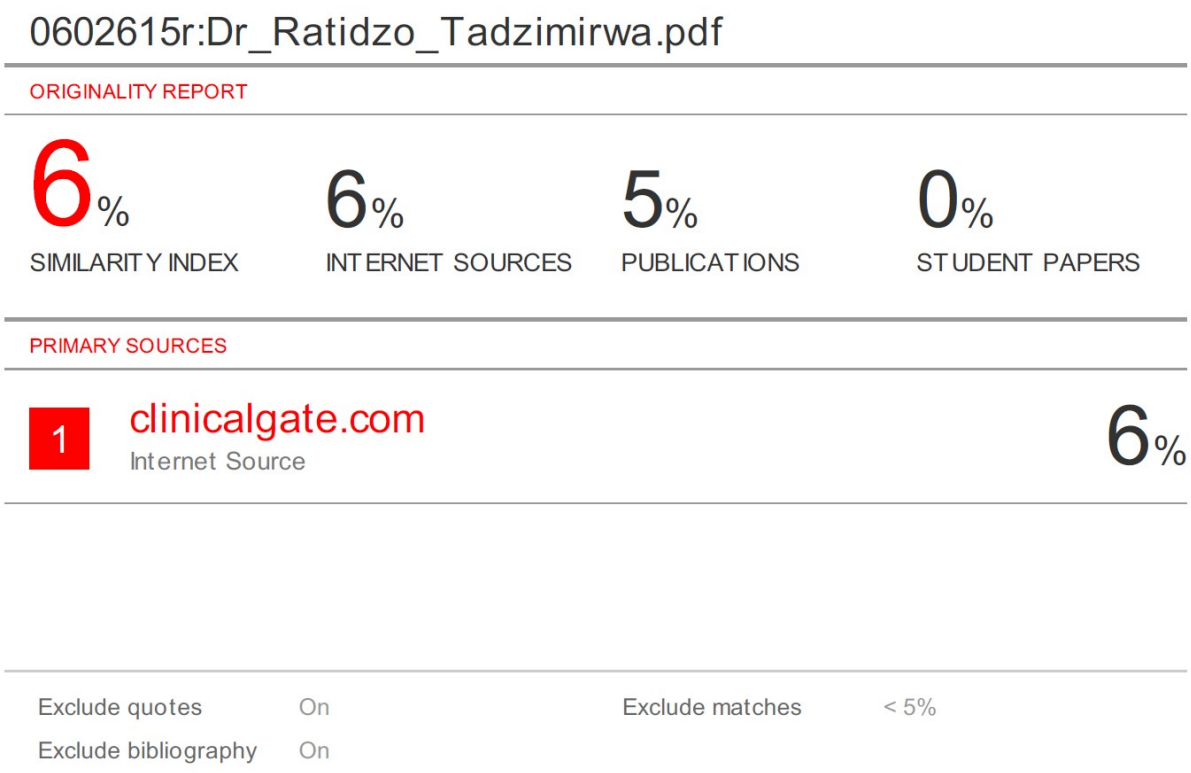
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D: PLAGIARISM REPORT



0602615r:Dr_Ratidzo_Tadzimirwa.pdf

ORIGINALITY REPORT

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APPENDIX E: SOUTH AFRICAN JOURNAL OF INFECTIOUS DISEASES AUTHOR GUIDELINES

Author Guidelines

Manuscripts submitted to the SAJID must be in the form of *Research Articles, Brief Reports, Clinical Case Studies, Correspondence, Reviews, State-of-the-Art Articles, Commentaries and Opinion Papers, Editorials* or *Supplement Articles.* The Journal welcomes the publication of *Guidelines, Conference Proceedings Newsletters or Press Releases,* and *Book Reviews.* Articles, Brief reports and Reviews are peer reviewed; other categories are reviewed by the Editors. Commentaries and Editorials are generally invited contributions, indicating the authors' identity, while manuscripts in the form of Reviews, and State-of-the-Art Articles may also be requested by the Editors.

All manuscripts must have conflict of interest and funding statements. When authors submit a manuscript, whether an article or a letter, they are responsible for disclosing all financial and personal relationships that might bias their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist. Authors should do so in the manuscript on a conflict-of-interest notification page that follows the title page.

Manuscripts describing research in human subjects or animals must indicate ethics clearance from appropriate research review committees. When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Articles describe original investigations at an acceptable degree of completion, constituting an advance in the field. Articles must not exceed 3500 words of text, without counting the abstract, references or legends, and illustrations and tables must be limited to the minimum necessary for clear and concise presentation. The abstract must either be structured, using *Background, Methods, Results,* and *Conclusions* as headings and comprising no more than 250 words, or unstructured with a 200 word limit. Articles are limited to a maximum of 7 insets (tables and figures combined) and 50 references.

Brief Reports present complete studies that are narrower in scope than those described in Articles or that present new developments. Manuscripts that are descriptive or primarily methodological in nature, or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to a total of no more than 2000 words of text, a total of 2 inserts (tables or figures), and 15 references.

Correspondence (letters) must be submitted in reference to a previous publication in SAJID (within the previous 12 months), or relate to a topical matter in line with the interests of FIDSSA, PHASA or their affiliated societies. Please prepare the letter in manuscript format, including a title page. The letter must not exceed 750 words of text, 1 insert (table or figure) and 10 references.

Commentaries and Editorials are generally invited by the Editor and are overviews of articles in SAJID, or of other research in epidemiology or infectious diseases, or matters relating to public health and other issues of special interest to FIDSSA, PHASA or their associated societies. Unsolicited commentaries are also considered.

Reviews and *State-of-the-Art Articles* that are research oriented or fall within the fields of interests of FIDSSA, PHASA or any of their affiliated societies will be considered for publication by SAJID. Prospective authors of such manuscripts are advised to communicate with the Editor in advance to ensure that a specific contribution is deemed appropriate and timely. Manuscripts of Reviews and State-of-the-Art Articles will be peer-reviewed.

Reviewers

The Journal would encourage authors to supply the names of at least 2 potential reviewers for their manuscript, as well as to indicate any reviewers they would feel may have a potential conflict of interest with regard to their submission.

Supplements

Requirements for supplement manuscripts generally follow those for SAJID manuscripts, including conflict of interest and funding statements. Inquiries relating to suitability of topic, programme organisation, production and costs should be made to the Editor.

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Review procedure. The Editor-in-Chief and Emeritus Editor screen all unsolicited manuscript submissions and some of these are rejected without further review. All other manuscripts are sent to a minimum of two outside experts for

review. After receipt of the reviewers' reports, the Editor-in-Chief and the Emeritus Editor with administrative assistance of the Journal Secretary discuss the merits of the manuscripts and the Editor-in-Chief makes the final decision to accept, reject, or request revision of the manuscript. A request for revision does not guarantee ultimate acceptance of the revised manuscript

Related manuscripts. If there appears to be significant overlap between a manuscript submitted to SAJID and another submitted manuscript by the same authors to SAJID or another journal, the editors will take the matter up with the corresponding author, and based on the response, take appropriate action (ask for modification, or reject with detailed explanation). Further action may include informing the appropriate authority in the authors' resident institution and if overlapping is discovered after publication in SAJID, publishing an appropriate announcement to that effect in the journal.

DOCUMENT REQUIREMENTS

Checklist

The following are required for your manuscript to be processed:

Covering letter

Word count limits

Conflict of interest statement

Funding statement

List of potential reviewers

Covering Letter

All manuscripts submitted to SAJID must be accompanied by a letter declaring that the manuscript has not been submitted or accepted for publication elsewhere. This letter must confirm and declare that all authors have seen and approved the content and have contributed significantly to the work. Authors should suggest potential unbiased reviewers who are qualified to review their manuscript. A covering letter must also accompany a revised submission and must address issues raised in the review process.

Manuscript Preparation

The SAJID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (*Ann Intern Med* 2000; 133:229-231 [editorial]; **http://www.icmje.org**, full text). Text, tables, references, and legends must be double- spaced. Italics should be used for genus and species names and for genes but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions. For layout of manuscript and appropriate style see a recent issue of SAJID.

Title page. On the title page, please supply a running head of not more than 40 characters and spaces, a title of not more than 160 characters and spaces, the names and affiliations of all the authors, and word counts of the abstract and text. Each author's first name, subsequent initials and surname must be used.

Footnote page. Footnotes must include:

- Statement that authors either have or have not a commercial or other association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding)

- Statement naming sources of financial support (including grant numbers)

- Name, date (month and year), and location (city, and country if not South Africa) of a meeting at which all or part of the information has been presented (include an abstract number, if available)

- Name, address, telephone and fax numbers, and e-mail address of the person to whom correspondence should be addressed

- Current affiliations and addresses for authors whose affiliations have changed since completion of the study

Abstract. The abstract for an Article may be structured with the headings Background, Methods, Results, and Conclusions (250-word limit) or unstructured (200-word limit). Abstracts of Brief Reports should be no more than 100 words. Whether structured or unstructured, the abstract must state the purpose of the research, the methods used, the results, and the conclusions. Do not cite references in the abstract. Include up to 10 key words, separate from the abstract. Please remember that the abstract is particularly useful for literature retrieval purposes.

Text. The text of Articles must be no longer than 3500 words, and that of Brief Reports no longer than 2000 words. The Methods section must include a statement that informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the National Department of Health (http://www.doh.gov.za) or the South African Medical Research Council (MRC; http://www.sahealthinfo.org/ethics/index.htm) and /or those of the authors' institution(s) were followed in the conduct of clinical research or that animal experimentation guidelines (see MRC website above) were followed in animal studies.

References. Articles are generally limited to 50 references, Brief Reports to 15 references. Only works that have been published or accepted for publication can be included in the reference list. Unpublished observations by the authors (authors' unpublished data) personal communications (SP Stanley, personal communication), and manuscripts submitted for publication (J Odendaal, S Coovadia and J Radebe, submitted) should be mentioned parenthetically in the text Please number references in order of appearance; those cited only or first in tables or figures are numbered according to the order in which the table or figure is cited in the text. Example: If table 3 is cited in the text after reference 20, a new reference cited in table 3 will be reference 21.

References must follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org, full text). Provide all authors' (or editors') names when there are fewer than 7; for 7 or more, list the first 3 and add "et al." Titles of journals not listed in *Index Medicus* should be spelt out in full. Reference to a doctoral thesis or Master's dissertation should include the author, title, institution, location, year and publication information, if published. For online resources, include a URL and date accessed. Accuracy of references is the responsibility of the authors.

Examples of the proper format are as follows:

Sonnenberg P, Glyn Thomas R, Glynn JR, Shearer S, Godfrey-Faussett, Murray J. Clinical and radiological features of pulmonary disease due to culture-positive Mycobacterium tuberculosis or non-tuberculous mycobacteria in South African gold miners. South Afr J Epidemiol Infect 2005; 20: 130-135

Marin M, Nguyen HQ, Langidrik JR, et al. Measles transmission and vaccine effectiveness during a large outbreak on a densely populated island: Implications for vaccination policy. Clin Infect Dis 2006; 42: 315-319

Strebel PM, Papania MJ, Halsey NA. Measles vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. 4th ed. Philadelphia: WB Saunders, 2004: 389-440.

Mothibeli KM, McGee L, Smith AM, Klugman KP. Molecular epidemiology of pneumococcal serotype 3 isolates.[abstract ID P56]. In: Programme and Abstract Book of the 1st Joint Congress of the Federation of Infectious Diseases Societies of Southern Africa (Sun City, North-West Province). Johannesburg: Presentations Graphics, 2005: 42.

World Health Organization. Initiative for vaccine research. Available at: http://www.who.int/vaccine_research/diseases/measles/en/. Accessed 1 February 2005.

Acknowledgment(s). The page preceding the references may include a statement thanking those who assisted substantially with work relevant to the study.

Statistical analysis. The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of measure. All Data should be expressed in metric units; use of SI units is encouraged. Use ºC for temperature.

Tables and figures. Articles are limited to a maximum of seven inserts (tables and figures combined), Brief Reports to a maximum of two inserts. Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, even if already defined in the text.

Tables should be numbered in the order of mention in the text. Tables should be typed double-spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory material should be kept to a minimum. Footnotes should be placed below the table and designated by superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word , consult the Special Instructions for Tables.

Figures should be also numbered in the order of mention in the text and should appear at the end of the manuscript and references. Your figures should be prepared in accordance with the Guidelines for Submission of Artwork. Letters, numbers, and symbols should be clear and of sufficient size to be legible when the figures are reduced. Photomicrographs should have internal scale markers. Figures reproduced from other publications must be accompanied by permission from the copyright holder. If the manuscript is accepted, the author will be required to send one complete set of glossy, hard-copy figures.

Figure legends should be double-spaced and appear on a separate page preceding the figures. Any abbreviations or symbols used but not defined in the figure itself must be defined in the legend.

Style. Authors are referred to the *American Medical Association Manual of style: A Guide for Authors and Editors* (9th ed., Williams& Wilkins, 1997) and the *Chicago Manual of Style* (15th ed., University of Chicago Press, 2003).

For commercially obtained products mentioned in the text, list the full names of manufacturers. Generic names of drugs and other chemical compounds should be used.

Nomenclature. SAJID recommends the latest widely accepted nomenclature, as set out in documents prepared by recognised international agencies e.g. the *International Journal of Systematic and Evolutionary Microbiology, Bergey's Manual of Determinative Bacteriology* (9th ed., revised, Williams& Wilkins, 1993), *Virus Taxonomy – The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses* (Springer-Verlag, 1995). The latter document also supplies standard abbreviations for virus species.

Clinical trials registration. All clinical trials must be registered in a registry that is electronically accessible to the public, free of charge. Registration should occur before patient enrolment and the registry's URL and the trial's registration number must be supplied at the end of the manuscript's abstract. For information on acceptable registries, consult the ICMJE Web site, http://www.icmje.org . The National Library of Medicine's registry which is free and open to all investigators, generally meets with the requirements of journals for the publication of clinical trials.

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Procedure

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