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Medizinische Klinik mit Schwerpunkt Infektiologie und Pneumologie

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Habilitationsschrift

Epidemiologie und Behandlung der importierten Malaria tropica

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Abkürzungen

ACT	„Artemisinin combination therapy“
GMP	Good manufacturing practice
O-IE	„Once-infected erythrocytes“
PADH	„Post-artemisinin delayed haemolysis“
VFR	„Visiting friends and relatives“ – Reisende
WHO	Weltgesundheitsorganisation

1. Einleitung

1.1. Epidemiologie der importierten Malaria tropica

Die Malaria ist die weltweit bedeutendste parasitäre Infektionskrankheit und verursacht weltweit pro Jahr ca. 212 Millionen Neuinfektionen und ca. 429.000 Todesfälle(1). In Europa ist die Malaria seit den 1960er Jahren des letzten Jahrhunderts nicht mehr endemisch, allerdings treten in Europa jedes Jahr über 6000 Malaria-Infektionen auf, von denen 99,9% durch Reisende importiert werden(2). Nach Deutschland wurden im Jahr 2001 über 1000 Fälle importiert; in den Folgejahren kam es zu einem Abfall auf ca. 600 Fälle/Jahr. Seit dem Jahr 2014 ist wieder ein deutlicher Anstieg auf ca. 1000 Fälle pro Jahr zu verzeichnen(3). Ein ähnlicher Trend ist auf europäischer Ebene zu beobachten(2). Im Jahr 2015 wurden 72% aller Fälle durch *Plasmodium falciparum* – dem Erreger der Malaria tropica – hervorgerufen. Die Mortalität der importierten Malaria tropica wird in verschiedenen Publikationen zwischen 0,4% und 4,4% angegeben(4–6).

Von besonderer und zunehmender Bedeutung bei der importierten Malaria sind Reisende, welche als die Gruppe der „visiting friends and relatives“ (VFR) - Reisende bezeichnet werden. Diese Gruppe besteht aus Menschen, welche zuvor nach Europa immigriert sind und häufig zum Besuch von Familie und Freunden in ihre malaria-endemischen Herkunftsländer reisen. Diese Reisenden wenden häufig keine Maßnahmen zur Malaria-Prophylaxe an und haben ein deutlich erhöhtes Risiko für eine Malaria-Infektion(7), da sie sich des Risikos eines tödlichen Verlaufs durch Verlust ihrer in der Kindheit erworbenen Semi-Immunität häufig nicht bewusst sind.

Sollte die die Infektion mit *P. falciparum* nicht rechtzeitig erkannt und behandelt werden, entwickeln Patienten das Bild der komplizierten Malaria. Die schwere bzw. komplizierte Malaria tropica wird zum einen über eine hohe Parasitendichte sowie über klinische bzw. laborchemische Parameter, welche eine Organdysfunktion oder einen Organausfall anzeigen, definiert und von der unkomplizierten Malaria abgegrenzt(8). Die unbehandelte Malaria tropcia führt zur exponentiellen Vermehrung der Parasiten im Blut, zur Sequestration der Parasiten in den Kapillaren der Organe, zu Organversagen und schließlich zum Tod. Die Behandlung der komplizierten Malaria tropica wird aufgrund der

Schwere des Krankheitsbilds grundsätzlich intravenös eingeleitet und die Patienten – zumindest in industrialisierten Ländern – intensivmedizinisch betreut.

1.2. Medikamentöse Therapie der Malaria

Malaria-Medikamente auf Chinolin-Basis wie z.B. Chloroquin, Chinin und Mefloquin waren seit Jahrzehnten als Standard für die Therapie der Malaria tropica etabliert. Diese Substanzen sind effektiv, ihre Anwendung ist jedoch durch erhebliche unerwünschte Wirkungen wie z.B. Herzrhythmusstörungen oder neuropsychiatrische Effekte gekennzeichnet. Seit 1982 sind jedoch in Thailand bereits erste Resistenzen von *P. falciparum* gegen Mefloquin beobachtet worden(9). Ferner wurden auch für die meisten übrigen zur Verfügung stehenden Medikamente zur Behandlung der Malaria weltweit zunehmende Resistenzen beobachtet(10). Die klinische Entwicklung einer neuen Substanzklasse war daher eine absolute Priorität in den Anstrengungen zur Kontrolle und Elimination der Malaria.

Artemisin (chinesisch: qinghaosu) ist eine Substanz gewonnen aus der Pflanze *Artemisia annua*, die seit ca. 1500 Jahren in China als antipyretische Substanz verwendet wird. Vor ca. 40 Jahren wurden die Artemisine in China neu entdeckt und ihre Wirkung gegen Plasmodien bestätigt(11). Artemisin-Derivate wie Artesunat, Artemether, Artemotil und Dihydroartemisinin wirken im Vergleich zu bisher eingesetzten Substanzen deutlich schneller gegen *P. falciparum*; sie können die Parasitenlast pro Zyklus um den Faktor 10.000 senken (im Vergleich zu 100- bis 1000-facher Senkung bei bisher verwendeten Medikamenten)(12). Artemisine wirken ferner im Gegensatz zu bisher verwendeten Substanzen auf alle Stadien der Plasmodienentwicklung einschließlich junger Ringformen(12), sodass hierdurch auch die pathophysiologisch bedeutsame Sequestration von Parasiten in den Kapillaren effektiver verhindert wird. Da Artemisine nur eine sehr kurze Halbwertszeit von 15 (Ausgangssubstanz) bis 60 Minuten (Hauptmetabolite) haben(13), sollten sie immer mit einer zweiten Substanz mit längerer Halbwertszeit kombiniert werden, um Rekrudeszenzen zu vermindern und das Risiko zur Bildung von Resistenzen durch Verkürzung der Anwendungsdauer auf drei Tage zu senken(10). Seit dem Jahr 2006 werden artemisinbasierte Kombinationstherapien (ACT)

von der WHO als Mittel der ersten Wahl zur Therapie der Malaria empfohlen(12). Daten zur klinischen Entwicklung der Medikamente waren jedoch ausschließlich aus endemischen Regionen verfügbar (14–17). Artemisine waren für den Einsatz außerhalb endemischer Regionen bislang nicht systematisch untersucht worden.
Intravenöses Chinin war seit 1950 die Standardtherapie der komplizierten Malaria tropica. Im Jahr 2005 zeigte die SEQUAMAT Studie an Patienten in Südostasien erstmals einen Überlebensvorteil für Patienten, welche mit intravenösem Artesunat gegenüber intravenösem Chinin behandelt worden waren (Mortalität 15% unter Artesunat, 22% unter Behandlung mit Chinin, Risikoreduktion 34,7% (CI_{95} 18,5%-47,6%, p=0.0002)(18). Der Überlebensvorteil für Artesunat konnte daraufhin in der AQUAMAT-Studie im Jahr 2010 bei Kindern mit komplizierter Malaria in Afrika bestätigt werden(19).

1.3. Intravenöses Artesunat zur Behandlung der komplizierten Malaria tropica in nicht-endemischen Regionen

Intravenöses Artesunat hat durch die Europäische Arzneimittelbehörde den Status als „orphan drug“ erhalten(20). Bis heute ist die Substanz jedoch nicht als GMP-konform hergestelltes Medikament erhältlich; daher existieren auch keine prospektiven Studien zur Sicherheit und Effektivität des Medikaments in Europa oder den USA. In den USA wurde die Substanz im Rahmen eines Patientenregisters retrospektiv unter Verwendung einer nur für die US-Armee GMP-konform hergestellten Formulierung untersucht; hierbei wurde die klinische Effektivität und das gute Sicherheitsprofil bestätigt(21). Die einzige derzeit für die Therapie verfügbare Formulierung ist ein Präparat aus China, dessen Herstellung von der WHO zertifiziert worden ist(22). Es wird in Deutschland und in den meisten anderen Europäischen Ländern im Rahmen von individuellen Heilversuchen oder – soweit entsprechende nationale gesetzliche Rahmenbedingungen dies erlauben – im Rahmen von registrierten Einzelanwendungen („*named patient programme*“) angewandt und dessen Wirkung und Sicherheit beobachtet. Systematische nationale oder europäische Daten zu dieser Therapie, die das Überleben bei komplizierter Malaria verbessert, fehlen jedoch.

1.4. Ausgangspunkt der Arbeit

Aufgrund des Fehlens einheitlicher europäischer Behandlungsempfehlungen existierten eine Vielzahl an Behandlungsschemata für die importierte Malaria tropica sowohl für die Behandlung von nicht-immunen wie auch für semi-immune Patienten. Auch für das Auftreten und die Behandlung von Komplikationen der Malaria tropica wie z.B. auch die Anwendung von Austauschtransfusionen(23) oder der Erythrozytenapherese(24) in Europa lagen wenig systematische Daten vor, die aber für das mittel- bis langfristige Ziel der Vereinheitlichung von Europäischen Leitlinien zur Behandlung von importierten Infektionen von hoher Relevanz sind.

Sowohl für die unkomplizierte Malaria, aber auch insbesondere für die komplizierte Malaria war ungeklärt, ob sich die Ergebnisse der Studien in Malaria-endemischen Ländern auf die Situation und die Patienten in nicht-endemischen und häufig industrialisierten Ländern übertragen lassen: zum einen werden in nicht-endemischen Ländern weit überwiegend nicht-immune Patienten behandelt, wohingegen die meisten Patienten in endemischen Ländern eine Semi-Immunität entsprechend dem Grad der Malaria-Endemizität aufweisen. Insbesondere bei der komplizierten Malaria bestehen in industrialisierten Ländern zudem erhebliche Unterschiede im Standard und Möglichkeiten der intensivmedizinischen Therapie, um typische Komplikationen der Malaria tropica wie z.B. das Nieren- oder Lungenversagen zu behandeln. Aufgrund des dokumentierten Überlebensvorteils in endemischen Ländern sowie der geringeren Fallzahlen in Europa und den USA kann die Frage einer Übertragung der Ergebnisse auf europäische Patienten in einer randomisierten kontrollierten Studie aus ethischen und statistischen Gründen absehbar nicht mehr geklärt werden. Patienten in Europa könnten jedoch möglicherweise auch von der deutlich besseren Verträglichkeit im Vergleich zur Standardtherapie und von der schnelleren Parasitenelimination klinisch profitieren. Insbesondere könnten kardiologisch vorerkrankte Patienten von der Möglichkeit einer Behandlung ohne Medikamente, die in hohem Maße die QT-Zeit verlängern, profitieren. Über die Ergebnisse der Behandlung der importierten Malaria tropica von Patienten in Deutschland und Europa mit Artemisin-Kombinationstherapien und insbesondere auch mit intravenösem Artesunat lagen jedoch keine Daten in relevantem Umfang vor.

1.5. Fragestellungen

Für die vorliegende Arbeit waren die folgenden Fragestellungen von Relevanz:

- Wie ist die Epidemiologie der nach Europa importierten komplizierten Malaria tropica ?
- Welche Therapieverfahren werden angewandt und welche Behandlungsergebnisse und Komplikationen können bei der importierten komplizierten Malaria tropica in Europa beobachtet werden ?
- Welche Vorteile bringt die Therapie der importierten Malaria tropica mit Artemisinen und welche möglichen klassenspezifischen unerwünschten Wirkungen treten bei der Artemisintherapie auf ?
- Welche Effektivität und Sicherheit in der Behandlung bieten Artemisin-basierte Therapien für die importierte unkomplizierte und die komplizierte Malaria tropica in Europa ?

2. Eigene Arbeiten

2.1. Epidemiologie und Behandlung der importierten komplizierten Malaria tropica in Europa

Kurth F, Develoux M, Mechain M, Malvy D, Clerinx J, Antinori S, Gjørup IE, Gascon J, Mørch K, Nicastri E, Ramharter M, Bartoloni A, Visser L, Rolling T, Zanger P, Calleri G, Salas-Coronas J, Nielsen H, Just-Nübling G, Neumayr A, Hachfeld A, Schmid ML, Antonini P, Lingscheid T, Kern P, Kapaun A, da Cunha JS, Pongratz P, Soriano-Arandes A, Schunk M, Suttorp N, Hatz C, **Zoller T**. Severe malaria in Europe: an 8-year multi-centre observational study. *Malaria Journal 2017 16:57. https://doi.org/10.1186/s12936-016-1673-z*

Epidemiologische und klinische Daten zur komplizierten Malaria tropica waren bislang nicht in relevantem Umfang vorhanden. Zudem existieren in verschiedenen Ländern Europas bis heute sehr unterschiedliche Behandlungsstandards.

Es wurden über 8 Jahre hinweg Daten von Patienten mit importierter komplizierter Malaria aus 28 Zentren aus 12 europäischen Ländern im Hinblick auf Demographie, klinische Präsentation, Behandlung, supportive Therapie und Arzneimittelsicherheit gesammelt und ausgewertet.

Die Analyse der Daten aus mehreren europäischen Zentren erlaubte die Erstellung von Risikokarten für Länder, in welchen die Infektion, die zur komplizierten Malaria geführt hatte, erworben wurde. Aus Westafrika kommen mit Abstand die meisten Patienten mit komplizierter Malaria – vor allem, wenn die Gruppe der sog. „VFR-Reisenden“ separat betrachtet wird. Für europäische Reisende mit komplizierter Malaria war Ostafrika ein weiteres relevantes Reisegebiet.

Diese Arbeit hatte ferner den Zweck, die sehr unterschiedlichen Standards in der Behandlung der importierten komplizierten Malaria tropica über verschiedene Länder Europas hinweg zu erfassen. In die 8-jährige Studienlaufzeit fiel zudem der Übergang der Behandlungsstandards von intravenösem Chinin auf intravenöses Artesunat. Zum Ende der Studienperiode wurden 60% der Patienten in den teilnehmenden Zentren mit diesem Medikament behandelt. Die „*post-artemisinin delayed haemolysis*“ (PADH, siehe unten)

wurde in 27% der mit intravenösem Artesunat behandelten Patienten beobachtet. Erneut bestätigte sich, dass das Alter von >60 Jahren ein wesentlicher Risikofaktor für die Entwicklung der häufigsten Komplikationen (cerebrale Malaria und akutes Nierenversagen) darstellt.

Die Daten erlauben eine gezielte Ansprache, Aufklärung und Prophylaxe der Gruppen an Reisenden mit dem höchsten Risiko für eine komplizierte Malaria. Zu intravenösem Artesunat konnten klinische Daten gesammelt werden, welche die sichere Anwendung des Medikaments verbessern. Wichtige epidemiologische Risikofaktoren konnten in dieser Studie nun auch in Daten aus verschiedenen Europäischen Zentren bestätigt werden.

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Severe malaria in Europe: an 8-year multi-centre observational study

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Abstract

Background: Malaria remains one of the most serious infections for travellers to tropical countries. Due to the lack of harmonized guidelines a large variety of treatment regimens is used in Europe to treat severe malaria.

Methods: The European Network for Tropical Medicine and Travel Health (TropNet) conducted an 8-year, multicentre, observational study to analyse epidemiology, treatment practices and outcomes of severe malaria in its member sites across Europe. Physicians at participating TropNet centres were asked to report pseudonymized retrospective data from all patients treated at their centre for microscopically confirmed severe *Plasmodium falciparum* malaria according to the 2006 WHO criteria.

Results: From 2006 to 2014 a total of 185 patients with severe malaria treated in 12 European countries were included. Three patients died, resulting in a 28-day survival rate of 98.4%. The majority of infections were acquired in West Africa (109/185, 59%). The proportion of patients treated with intravenous artesunate increased from 27% in 2006 to 60% in 2013. Altogether, 56 different combinations of intravenous and oral drugs were used across 28 study centres. The risk of acute renal failure (36 vs 17% $p = 0.04$) or cerebral malaria (54 vs 20%, $p = 0.001$) was significantly higher in patients ≥60 years than in younger patients. Respiratory distress with the need for mechanical ventilation was significantly associated with the risk of death in the study population (13 vs 0%, $p = 0.001$). Post-artemisinin delayed haemolysis was reported in 19/70 (27%) patients treated with intravenous artesunate.

Conclusion: The majority of patients with severe malaria in this study were tourists or migrants acquiring the infection in West Africa. Intravenous artesunate is increasingly used for treatment of severe malaria in many European treatment centres and can be given safely to European patients with severe malaria. Patients treated with intravenous artesunate should be followed up to detect and manage late haemolytic events.

Keywords: Malaria, Falciparum, Severe malaria, Artesunate, Quinine, *Plasmodium*, Europe, Clinical study

Background

Around 5200 cases of malaria are imported to EU countries per year, of which up to 10% progress to severe malaria [1]. Because most patients with imported malaria are not semi-immune, progression to severe malaria is considerably more frequent in non-endemic than in endemic countries. Non-immune patients carry a substantial risk of suffering from complications of the infection itself or from complications associated with intensive care treatment.

The epidemiology of imported severe malaria is changing. In addition to tourist or business travellers to tropical

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regions, migrants visiting friends and relatives (VFR) in their previous home country are increasingly affected [2, 3]. This population is less likely to seek pre-travel advice and to take anti-malarial prophylaxis [4, 5]. Most migrants are not aware of the waning of semi-immunity against malaria when they travel to their home countries.

Guidelines to define and to treat severe malaria have undergone major changes in the past decade at international and national levels. Criteria for the definition of severe malaria were amended by the World Health Organization (WHO) in 2006, 2010 and 2015, particularly with regard to the definition of hyperparasitaemia [6], and numerous classification and treatment recommendations still exist across European countries. Another challenge is the transition from intravenous quinine to intravenous artesunate as first-line treatment for severe malaria. Despite its superior potential to save lives and shorten duration of hospital and intensive care unit (ICU) treatment [7–10], many difficulties with regard to registration, availability and quality of artesunate have to be overcome before it will be easily available and widely used outside of specialist referral centres for tropical medicine in Europe. The pathophysiology of late haemolytic reactions occurring 2–6 weeks after treatment is not fully understood and harmonized guidelines for follow-up care of patients receiving this drug in Europe need to be developed [11, 12].

Data on epidemiology and treatment of imported severe malaria across Europe, together with a 'road map' towards drug approval of intravenous artesunate for the treatment of severe malaria in non-endemic countries are needed to eventually improve and harmonize treatment recommendations. Data are however available only from national cohorts and case registries. The European Network for Tropical Medicine and Travel Health (TropNet) [13] conducted an 8-year, multi-centre, observational study to analyse epidemiology, treatment practices and outcome of severe malaria in its member sites across 12 European countries.

Methods

This multicentre observational study was performed among member sites of TropNet. All patients with microscopically confirmed severe falciparum malaria, according to the 2006 WHO criteria treated at one of the participating TropNet centres between 2006 and 2014, were eligible. Physicians at participating TropNet centres were asked to report pseudonymized retrospective data on demographic characteristics, medical and travel history, clinical presentation, anti-malarial drug regimen, supportive treatment, parasitaemia before and under treatment, complications under treatment, adverse drug reactions, outcome and follow-up during 28 days from all patients treated for severe malaria at their centre. The treatment remained the responsibility of the treating physician. Parasite clearance time was defined as time to the first thick blood smear without evidence of asexual parasites after initiation of anti-malarial treatment. For the analysis of this dataset, post-artemisinin delayed haemolysis (PADH) was defined as a decrease in haemoglobin together with signs of haemolysis (elevated LDH) after completion of anti-malarial treatment and complete parasite clearance. Electronic case report forms were used for data entry and transmission to the coordinating centre at Charité University Hospital, Berlin, where data were transferred into a database and checked manually for plausibility.

The primary objective of this study was to assess clinical presentation, treatment and outcome in patients with imported severe malaria. Descriptive statistics was performed on sociodemographic, medical, treatment, and outcome data. Mann–Whitney U test (two groups, continuous data), Fisher exact test (two groups, categorical data), or Kruskal–Wallis test (>two groups continuous data) at a two-sided significance level of $\alpha = 0.05$ were used for comparative analysis. Analysis of variance (ANOVA) for continuous data and Pearson Chi squared test for categorical data were used to test the distribution of demographical characteristics of patients over time. Data are displayed as median (±interquartile range). Statistical analysis was performed using JMP (JMP 7.0, SAS Institute Inc, NC, USA).

The study was approved by the Ethics Committee of Charité University Hospital, Berlin. Ethical clearance for transfer of retrospective pseudonymized patient data was sought at participating Tropnet centres according to local regulations.

Results

From 2006 to 2014, 190 patients with severe falciparum malaria were reported by the participating 28 TropNet centres from 12 European countries. After excluding three cases without documented criteria for severe malaria and two cases with double reporting, 185 datasets were available for analysis. The number of patients per centre ranged from one to 31 patients. The number of reported patients per country is shown in Table 1.

Demography, place of infection and anti-malarial prophylaxis

Demographic data are shown in Table 2. The proportion of female patients was comparatively small (29%). The majority of patients (106/185, 57%) were of European origin without history of migration. Tourism was the main purpose of travel in Europeans (54/106, 51%), whereas VFRs were the predominant purposes of travel

Table 1 Number of study centres and reported cases per country (n = 185)

Country	Number of centres	Number of reported cases	% of total number of cases
Austria	2	7	4
Belgium	1	19	10
Denmark	2	14	7
France	2	52	28
Germany	7	15	8
Italy	5	50	27
Netherlands	1	4	2
Norway	1	7	4
Portugal	1	1	1
Spain	2	12	6
Switzerland	3	3	2
UK	1	1	1

in patients with history of migration (55/68, 81%). European patients were on average older than patients with history of migration [median age 47 (IQR 33–57) vs 36 (IQR 27–45) years, p < 0.0001]. All malaria infections were acquired in Africa with the exception of two cases from Central America. By far the largest proportion of infections came from West Africa (109/185, 59%), followed by Central Africa (40/185, 22%), where Cameroon was the country with the highest number of imported cases (21/185, 11%, Fig. 1). VFR patients acquired malaria infections almost exclusively in West Africa, whereas European tourists acquired infections also in the tourist destinations of East Africa (Fig. 2). There was no change in age (F = 0.84, p = 0.5), gender (p = 0.11), origin of patients (p = 0.54) or purpose of travel (p = 0.10) during the 8-year course of the study. Almost 9 out of 10 patients (162/185, 88%) had not taken any anti-malarial chemoprophylaxis. Among 23 patients who took anti-malarial chemoprophylaxis, only six fully adhered to the prescribed regimen.

Clinical presentation

Clinical manifestations and laboratory findings leading to classification as severe malaria are shown in Table 2. Median baseline parasitaemia was 6.5% (IQR 4–11) and hyperparasitaemia (≥5%) was the most common criterion of severe disease, followed by jaundice, which was a criterion for severe malaria in this study according to WHO guidelines as of 2006. Eight patients with jaundice had no other vital organ dysfunction and would not have been classified as severe disease according to WHO guidelines as of 2010 [14].

Underlying chronic conditions were found in 43% of patients (63/185), of which hypertension was the most frequent (9%, 16/185). Seven per cent of patients (13/185) were HIV positive. The majority of patients (119/185, 64%) met one or two criteria of severe malaria, whereas 8% (17/185) met more than four criteria. Patients ≥60 years presented, on average, with more criteria for severe malaria than younger patients (median 3 vs 2, $p = 0.02$). Table 3 shows the risk of presenting with a particular criterion of severe malaria according to age (≥60 years vs younger patients). There was no difference in type and number of criteria for severe malaria among patients of European versus non-European origin with the exception of a lower median baseline parasitaemia (7 vs 5%, respectively, $p = 0.04$).

Anti-malarial treatment

Intravenous quinine was the main first-line treatment in 93/185 patients (50%) whereas intravenous artesunate was used in 63/185 patients (35%). Seven patients (4%) received intravenous quinine and artesunate in combination. Table 4 gives an overview of the drugs and drug combinations used as follow-on treatment after intravenous therapy. Altogether 56 different combinations of intravenous and oral drugs were used across the different centres.

The proportion of patients treated with intravenous artesunate increased steadily during the course of the study from 27% (8/30) in 2006 to 60% (18/30) in 2013. In 22/185 patients (12%) only oral first-line treatments were given such as oral quinine (n = 16, exclusively in Italy), oral atovaquone-proguanil (n = 1, in a patient with HIV), and oral artemether–lumefantrine (n = 5). Patients treated with oral anti-malarials presented exclusively with hyperparasitaemia (n = 19, median parasitaemia 7%, range 5–8%) and/or jaundice (n = 6) as criteria for severe malaria and had no co-morbidities (except the one patient with HIV).

Choice of the first-line treatment was very heterogeneous across different sites and was mainly dependent on the country where the patient was treated. Centres in Norway, The Netherlands and Belgium reported treatment almost exclusively with intravenous artesunate, whereas the participating treatment centres in Spain and France used intravenous quinine in the majority of reported cases (9/12, 75% and 44/50, 88%, respectively).

Concomitant and supportive treatment

An overview of supportive treatments is given in Table 5. Antibiotic therapy (44% of patients) and erythrocyte transfusion (21% of patients) were the most common. Erythrocyte exchange transfusion was performed in 8/185 (4%) patients at seven centres in Italy, Spain,

Table 2 Characteristics of patients with severe malaria (n = 185 patients in all categories)

Characteristics	n (median)	% [IQR]
Gender		
Male	132	71
Age		
Age in years	(42)	[31–52]
≥60 years	22	12
≤18 years	10	5
Origin of patients		
European, no history of migration	106	57
Immigrant/history of migration	68	37
Visitor from endemic country	11	6
Anti-malarial chemoprophylaxis		
None	162	88
Non-adherence to prescribed regimen	17	9
Doxycycline[a]	2	1
Chloroquine-proguanil[a,b]	2	1
Mefloquine[a]	1	1
Atovaquone-proguanil[a]	1	1
Criteria leading to classification as severe malaria		
Hyperparasitaemia >5%	132	71
Hyperparasitaemia >10%[c]	55	30
Hyperparasitaemia >2%[c]	154	83
Jaundice[d]	81	44
Impaired consciousness/coma	46	25
Acute renal failure	36	19
Liver function test >3 times upper normal	36	19
Circulatory collapse/shock	27	15
Anaemia <8 g/dl	27	15
Respiratory failure	22	12
Spontaneous/abnormal bleeding	13	7
Acidosis	9	5
Hypoglycaemia <40 mg/dl	6	3
Multiple convulsions	3	2
Number of criteria for severe malaria met by individual patients		
1	59	32
2	60	32
3	30	16
4	19	10
5	10	5
>5	7	3
Underlying co-morbidities		
Any	63	43
Hypertension	16	9
HIV	13	7
Diabetes	10	5
Chronic heart disease	8	4
Hepatitis	3	2
COPD	2	1
Other chronic conditions	11	6

Data are number of patients, unless indicated otherwise

COPD chronic obstructive pulmonary disease, *HIV* Human immunodeficiency virus

[a] Patients with reported adherence to chemoprophylaxis only

[b] Chloroquine-proguanil was taken for travel to Togo in 2007 and Burkina Faso in 2010

[c] Hyperparasitaemia >2 and >10% were not used as criteria for severe malaria in this study according to WHO guidelines 2006 and are shown for informational purposes only

[d] Clinical jaundice was used as criterion for severe malaria in this study according to WHO guidelines 2006

Belgium, and The Netherlands. Seven of these patients were treated with intravenous quinine and one with intravenous artesunate. Baseline parasitaemia was >10% in all these patients (median 18%, IQR 10–27). Erythrocyte apheresis was performed in 5/185 patients (3%) at two centres (Vienna, Austria and Leiden, The Netherlands). Parasitaemia was >15% in all these patients (median 19%, IQR 18–28) and all five patients were treated with intravenous artesunate.

Adverse drug reactions

Adverse drug reactions were reported in 27/100 patients (27%) treated with intravenous quinine, and in 21/70 (30%) patients treated with intravenous artesunate. None of them was fatal. Cinchonism was the most common adverse drug reaction in patients treated with intravenous quinine (19/100, 19%). It was rated as mild in 17/19 cases and moderate in 2/19 cases by the treating physician. Hypoglycaemia occurred in 4/100 patients (4%) and cardiac arrhythmias in 1/100 patient (1%) treated with intravenous quinine.

In patients treated with intravenous artesunate, PADH was reported in 19/70 patients (27%), a finding which first became known during the study period in the year 2011. Onset of PADH was reported during days 10 to 14 (median 14) and median duration of haemolysis was reported to be 14 (IQR 8–18) days. Three patients (15%) with PADH received blood transfusions, with 2 patients (10%) re-hospitalized (for 3–5 days, respectively). In 1 patient, PADH was reported after therapy with only oral artemether–lumefantrine. This patient and some of the other patients with delayed PADH have already been reported elsewhere [15–17].

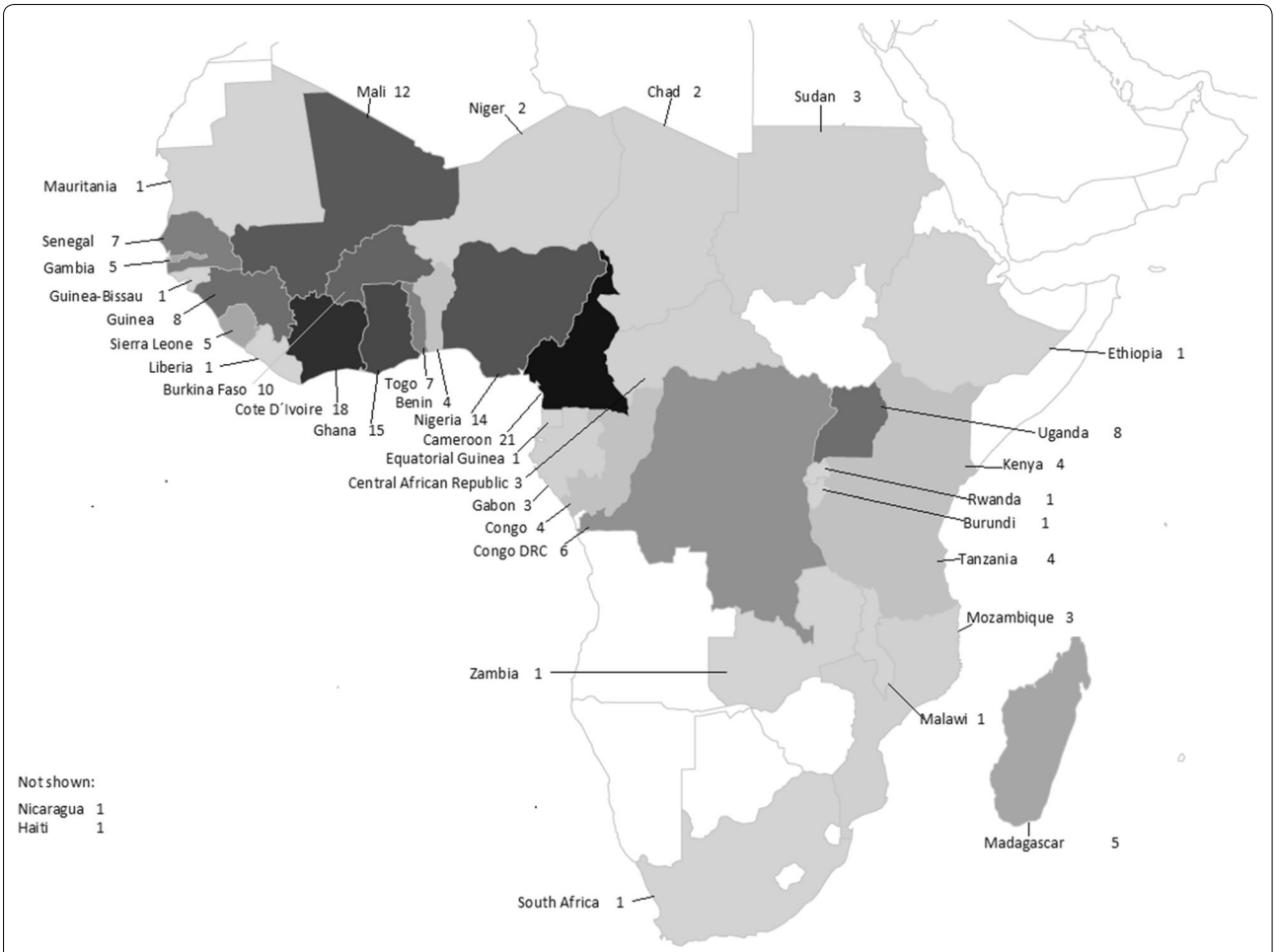

Fig. 1 Countries where malaria infections where acquired (n = 185). Countries and number of cases per country are shown. *Gray-scale colour* corresponds to the proportion of cases acquired in the respective country

In one patient, an acute cerebellar syndrome (ataxia, dysarthria, dysmetria, adiadochokinesis) was described beginning 3 days after the end of anti-malarial treatment (day 10). The patient had not shown any neurological symptoms during the acute phase of malaria and had been treated with a loading dose of intravenous quinine on day 1, intravenous artesunate from day 1 to 4 and oral artemether–lumefantrine from day 5 to 7. An MRI scan and lumbar puncture showed no abnormalities. Due to persistence of symptoms, the patient received physiotherapy until ten weeks after anti-malarial treatment, where neurological symptoms steadily improved. Complete recovery was reported 7 months after the malaria episode.

Outcome

Three patients died, two of European origin and one with history of migration, resulting in a 28-day survival rate of 98.4%. All three patients had been treated with intravenous quinine and one of them also with intravenous artesunate simultaneously. All deaths occurred within the first 3 days after admission. All three patients had presented with hyperparasitaemia (9, 10 and 40%) and respiratory distress requiring mechanical ventilation. Respiratory distress with the need for mechanical ventilation was significantly associated with the risk of death in the study population (13 vs 0%, $p = 0.001$). Of note, two of the patients who died were 22–34 years of age, respectively, and had no underlying co-morbidities. One of them had a history of migration. The third patient was 70 years of age, suffered from a pre-existing chronic cardiomyopathy and died from therapy-refractory shock.

In 76% of patients (117 of 153 patients with available data) treatment took place in an ICU, where the median length of stay was three (IQR 2–5) days. The median length of inpatient treatment was 7 days (IQR 5–9). Median time to 99% parasite clearance was 48 h (IQR 24–72, n = 126) and median time to complete parasite

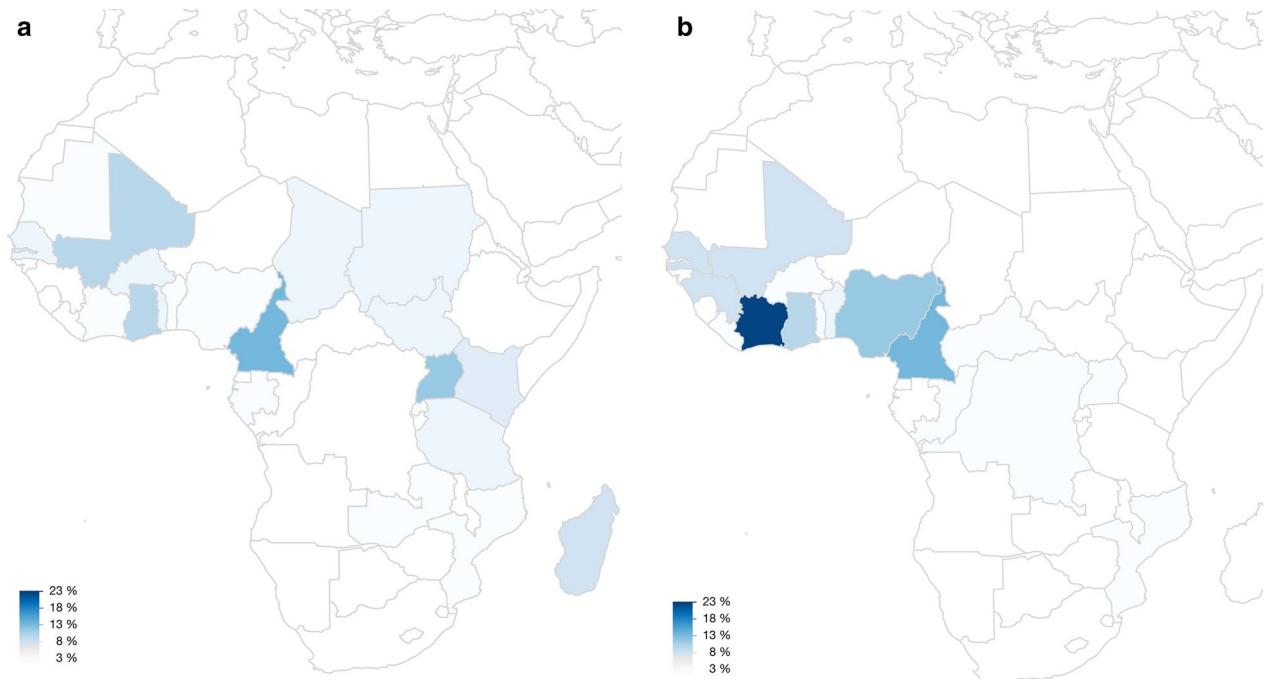

Fig. 2 **a** Countries where European tourist travellers acquired malaria (n = 54). Colour intensity corresponds to the proportion of cases acquired in the respective country. **b** Countries where visiting friends and relatives travellers acquired malaria (n = 55). Colour intensity corresponds to the proportion of cases acquired in the respective country

Table 3 Risk of presenting with particular criteria of severe malaria according to age ≥60 versus <60 years

	Patients ≥60 years n = 22	Patients <60 years n = 163	p value
Cerebral malaria	12 (54)	34 (21)	0.001
Acute renal failure	8 (36)	28 (17)	0.04
Hyperparasitaemia	17 (77)	120 (73)	0.8
Jaundice	8 (36)	73 (44)	0.5
Liver function test >3 times upper normal	7 (31)	29 (17)	0.15
Shock	6 (27)	21 (13)	0.1
Anaemia	3 (14)	24 (15)	1.0
Respiratory failure	3 (14)	19 (12)	0.7
Acidosis	2 (9)	7 (4)	0.29
Spontaneous bleeding	3 (13)	10 (6)	0.19
Hypoglycaemia	1 (5)	5 (3)	0.53
Multiple convulsions	0 (0)	3(2)	1.0

Data are number of patients (%)

clearance, was 72 h (IQR 60–120, n = 104). Data showing shorter parasite clearance time and shorter ICU and inpatient treatment in patients treated with intravenous artesunate compared to intravenous quinine were reported elsewhere [8]. The 22 patients who received oral anti-malarial treatment had comparatively long median 99% parasite clearance and complete parasite clearance times (72 h, IQR 48–72, and 120 h, IQR 84–144, respectively). There were no documented early or late parasitological failures.

Among 46 patients with cerebral malaria, six had neurological sequelae at discharge such as confusion, dysphasia, ataxia, and imbalance. In four patients (2/25 treated with artesunate vs 2/21 treated with quinine) confusion and ataxia persisted at day 28. Among 36 patients with acute renal failure at presentation, 13 (36%) patients

Table 4 Initial drug combinations and follow-on treatment in patients treated with intravenous quinine or intravenous artesunate for severe malaria

Patients	n	%
Treated with intravenous quinine	93	
Initial therapy		
Monotherapy	54	58
Combination with doxycycline	33	36
Combination with clindamycin	5	5
Combination with mefloquine	1	1
Follow-on treatment		
Oral quinine	29	31
Oral ACT	19	20
Oral AP	11	12
None[a]	34	37
Treated with intravenous artesunate	63	
Initial therapy		
Monotherapy	42	67
Combination with doxycycline	13	20
Combination with clindamycin	6	10
Combination with mefloquine	2/	3
Follow-on treatment		
Oral ACT	38	60
Oral AP	17	27
Oral quinine	2	3
Mefloquine	3	5
None[b]	3	5

Data are number of patients and %

ACT artemisinin-based combination therapy, *AP* atovaquone-proguanil

[a] Quinine, doxycycline or clindamycin were given for at least 7 days, n = 2 patients died before initiation of subsequent therapy

[b] Artesunate, doxycycline, clindamycin were given for at least 7 days

Table 5 Supportive treatments used in European patients with severe malaria (n = 185)

Supportive treatments	n	%
Antibiotic therapy	82	44
Erythrocyte transfusion	38	21
Vasopressor therapy	24	13
Mechanical ventilation	24	13
Invasive ventilation	18	
Non-invasive ventilation	6	
Haemodialysis and haemofiltration	20	11
Erythrocyte exchange transfusion	8	4
Erythrocyte apheresis	5	3

had elevated creatinine levels at discharge, which had not been reported by the patient or documented in medical charts before malarial infection. In nine of these patients (4/17 treated with artesunate vs 5/16 treated with quinine) elevated creatinine levels persisted after day 28. Two patients suffered from necroses of fingers and toes as sequelae of vasopressor therapy during malaria.

Among eight patients with jaundice as only criterion for severe malaria, who would not have been classified as severe according to WHO 2010 criteria, none experienced documented complications of treatment or sequelae, seven were not treated at an ICU and patients had a comparably short median length of stay in hospital of 4 days (IQR 4–6).

Discussion

Imported malaria remains a relevant clinical problem due to the rapid potential progression to severe and life-threatening disease in non-immune patients. This study presents clinical and treatment data from 28 centres of the TropNet from 12 countries, one of the largest databases collected on this patient population to date. Previous studies on severe malaria in Europe are only available either at centre [18–20] or country [3, 21–23] level.

Anti-malarial treatment—intravenous artesunate

Intravenous artesunate has been shown to improve survival in patients with severe malaria in endemic areas, with particular benefit for patients with high parasitaemia [>10% infected red blood cells (RBCs)] [7, 9, 10]. A randomized, controlled trial to confirm superiority of artesunate over quinine in non-endemic areas would be unethical. Other benefits of treatment with intravenous artesunate such as shorter ICU and hospital treatment were clearly demonstrated in European patients [8].

Quinine is still widely used in Europe, but the rate of patients receiving intravenous artesunate almost doubled in the 8-year study period. In the final year 2014, every second patient received intravenous artesunate. Current surveillance data from national reference centres indicate that the proportion of patients treated with artesunate is further increasing, particularly in countries where participating centres still reported frequent treatment with quinine during this study [24]. Although prospectively collected safety data from Europe is not available, this study adds to the evidence that artesunate is effective and can safely be used to treat patients in Europe. Artesunate is used in Europe despite considerable legal problems: The manufacturer of intravenous artesunate has been prequalified for good manufacturing practice-standard (GMP) by WHO [6], but the drug is not available in a European GMP-standard quality. It has an orphan designation for Europe by the European Medicines Agency since 2007, but no marketing authorization in Europe or in USA. Only in France, USA, Belgium, Denmark, and The Netherlands, named-patient programmes or similar

protocols are in place, providing a legal basis for treating patients with this lifesaving drug [25, 26]. Until intravenous artesunate receives approval from the European Medicines Agency and the US Food and Drug Administration, the legal context for physicians procuring and applying this drug in Europe will remain unsatisfactory. This study also highlights the differences in treatment practices and guidelines across Europe: from occasional use in some countries to exclusive use of artesunate to treat severe malaria in The Netherlands or Norway. This finding likely reflects the respective national legal framework for using non-licensed drugs as well as national treatment recommendations [27]. The treatment of patients with intravenous artesunate and quinine in parallel, reported in seven patients, might also be based on judicial reasons: physicians might want to combine the most effective but unapproved drug (artesunate) with the approved and recommended standard first-line treatment (quinine) in order to avoid a legal risk [28]. No benefit of this combination has been shown compared to treatment with artesunate alone, whereas the frequency of adverse events increased [29]. A small number of patients were treated exclusively with oral anti-malarials in this study. These patients were less severely ill, including three patients with jaundice, as the only criterion for severe disease, who would not have been classified as severe according to the current WHO classification. No treatment complications were reported for these patients, yet time to parasite clearance was comparatively long. Overall, there was a remarkable variety of altogether 56 different combinations of intravenous and oral anti-malarial drugs reported. Harmonized, evidence-based European treatment guidelines would be useful to support clinicians in their choice of anti-malarial treatments.

Adverse drug reactions

Following the initial description of an episode of severe prolonged haemolysis after treatment of a patient with severe malaria with intravenous artesunate in Japan in 2002 [30], late haemolytic reactions 2–6 weeks after treatment with intravenous artesunate were described in a case series in European patients in 2011 [12], and then confirmed in studies in European [15–17] and African [31] patients. Removal of parasites from RBCs in the spleen, leaving behind a once-infected 'pitted' erythrocyte with a shorter life span has been shown to be a potential mechanism causing late haemolysis [11], but the pathophysiology is not fully understood. The rate of haemolytic reactions and transfusions reported in the literature is variable depending on size, context, type, and setting of a study as well as on definitions of post-treatment haemolysis; the results of the present study are generally in line with previous observations [12, 16, 32] and late haemolysis can be expected to occur in approximately 20–30% of non-immune patients treated. As shown by the present data, a considerable proportion of them also receive blood transfusions. The results show once more that patients receiving intravenous artesunate for treatment of severe malaria should be routinely observed for signs of haemolysis at least on days 7 and 14 after treatment. An acute cerebellar syndrome 3 days after the end of anti-malarial treatment with intravenous artesunate and oral artemether lumefantrine was reported in one patient. Although the reported time until complete resolution (7 months) is longer than in most cases reported in the literature; symptoms and time of onset are suggestive of post-malaria neurological syndrome [33].

Mortality

Mortality in this patient population was very low, reflecting the high standard of intensive care in Europe. Previous single-centre and national studies reported mortality rates between 4 and 15% [3, 18, 19, 22, 34]. The proportion of patients who had criteria of severe malaria associated with adverse outcome and death was comparable to other studies (Table 2) [2, 21, 34]. Mortality might have been biased by the fact that most reporting centres are tertiary care institutions with long experience in treating severe malaria. The study did not capture cases of severe malaria in smaller remote hospitals, where mortality might be higher. The increasing use of intravenous artesunate as main first-line treatment may also have contributed to reduce mortality, e.g., through rapid parasite clearance and shorter length of ICU and inpatient treatment [7–9]. Age as risk factor for adverse outcome of imported severe malaria has been shown by numerous studies [2]. In the present study, population patients ≥60 years were more likely to suffer from acute renal failure or from cerebral malaria, yet there was no increased case fatality among older patients.

Anti-malarial chemoprophylaxis

Only 10% of patients with severe malaria had taken anti-malarial chemoprophylaxis and very few of them had been fully compliant. These data suggest that correct anti-malarial prophylaxis can effectively prevent severe malaria in European travellers. Counselling of travellers on malaria prevention should be improved and coverage increased, particularly for travellers going to West Africa, where 60% of infections in this study were acquired. Little is known about the proportion of European travellers who take prophylaxis. In a recent study only 60% of travellers from the UK to endemic areas used anti-malarial

chemoprophylaxis [35]. VFR travellers are a large traveller population to Africa with different perceptions of malaria and its prevention [4, 5]. This may also influence the longer delay between onset of symptoms of malaria and presentation in hospital. The fact that 37% of patients in the present study had a history of migration clearly demonstrates the risk of these patients to suffer from severe malaria. Moreover, one of the patients who died was a 34 years old, otherwise healthy patient with history of migration. She presented with hyperparasitaemia, acute renal failure, jaundice, and respiratory failure. There was altogether no difference in symptoms and clinical presentation between patients with or without history of migration, suggesting a waning of semi-immunity in migrants who left endemic areas [36].

Supportive treatments

Supportive treatments such as exchange transfusions and erythrocyte apheresis are a matter of controversy and their use is guided by national or local practices. European single-centre studies recently failed to demonstrate improved parasite clearance through whole blood or erythrocyte exchange, compared to patients treated with quinine or artesunate alone [37, 38]. Only 4% of patients received exchange transfusions in this study. It was performed in only 7 out of 28 participating centres, mainly in patients treated with intravenous quinine. Likewise, only five patients in two centres received automated erythrocyte apheresis. With the increased use of intravenous artesunate and its potential to rapidly reduce high parasite loads it needs to be determined whether particular patient populations might still benefit from these adjunctive treatments [39].

Limitations

This observational study has inherent limitations. As patient information was collected retrospectively in TropNet centres and not all patients treated may have been reported equally, selective under-reporting, e.g., for patients who died, may have occurred. Bias in reporting data on PADH must be assumed as the condition was not known at the beginning of the study and no universally accepted clinical definition exists to date. All TropNet centres are referral centres for tropical medicine and patient composition as well as treatment data may not fully reflect treatment practices and outcomes in non-referral hospitals. Moreover, data reported by the participating treatment centres may not always fully reflect treatment practices in the respective countries. For patients with elevated creatinine levels at the end of follow-up, it cannot be ruled out that unknown or undocumented elevation of creatinine had existed before the episode of severe malaria.

Conclusion

The data show that the majority of patients with severe malaria in Europe are tourists or migrants acquiring infection in West Africa. Intravenous artesunate is increasingly used for treatment of severe malaria; it is the most effective drug and can be safely given to European patients with severe malaria. There is need for harmonization of guidelines for the treatment of severe malaria in Europe. Patients treated with intravenous artesunate should be followed up to detect late haemolytic events.

Authors' contributions

TZ designed the study; TZ and FK performed acquisition, entry, cleaning and analysis of data, wrote the manuscript; MD, MM, DM, JC, SA, IEG, JG, KM, EN, MR, AB, LV, TR, PZ, GC, JSC, HN, GJN, AN, AH, MLS, PA, TL, PK, AK, JSdC, PP, ASA, MS, NS, and CH treated patients, gathered data in study centres, contributed to and corrected the manuscript. All authors read and approved the final manuscript.

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None.

Competing interests

The authors declare that they have no competing interests.

Availability of data
The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
The study was approved by the Ethics Committee of Charité University Hospital, Berlin (AE1/334/14). Ethical clearance for transfer of retrospective, pseudonymized patient data was sought at participating Tropnet centres according to local regulations.

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2.2. Erste klinische Daten zur Behandlung der komplizierten Malaria tropica mit intravenös verabreichtem Artesunat

Zoller T, Junghanss T, Kapaun A, Gjørup I, Richter J, Hugo-Persson M, Mørch K, Foroutan B, Suttorp N, Yürek S, Flick H. Intravenous Artesunate for Severe Malaria in Travelers, Europe. *Emerg Infect Dis. 2011;17(5):771-777. https://doi.org/10.3201/eid1705.101229*

Ausgangspunkt der Arbeit waren zu diesem Zeitpunkt – mit Ausnahme einer kleinen Fallserie aus Norwegen(25) - fehlende Daten zur Anwendung von intravenösem Artesunat in nicht-endemischen Ländern. Die Anwendung dieses Medikaments erfolgte seinerzeit nur in einzelnen erfahrenen klinischen Zentren in Europa im Rahmen von individuellen Heilversuchen, nachdem die SEQUAMAT-Studie(18) einen Überlebensvorteil im Vergleich zur Behandlung mit intravenösem Chinin bei Patienten in Südostasien gezeigt hatte.

Es wurden Behandlungsdaten von insgesamt 25 Patienten aus Zentren in Deutschland, Dänemark, Schweden und Norwegen retrospektiv analysiert.

Zu diesem Zeitpunkt erhielten die analysierten Patienten noch sehr unterschiedliche Behandlungsregime mit intravenösem Artesunat hinsichtlich Dosierung und Dauer der Gabe. Auffällig war die im Vergleich zu Chinin sehr schnelle Reduktion der Parasitämie um eine Logstufe innerhalb von 24-36 Stunden. In sechs Patienten aus fünf verschiedenen Behandlungszentren wurde eine unerwartete Hämolyse beginnend ab dem Tag 14-31 nach Behandlungsbeginn beobachtet. Fünf der sechs Patienten mit Hämolyse erhielten Bluttransfusionen zum Ausgleich der Anämie. Die hämolytischen Reaktionen ließen 3-6 Wochen nach der ersten Gabe von Artesunat nach. Es wurden ein direkter Coombs Test sowie Tests auf freie medikamenteninduzierte Antikörper als mögliche Ursache einer verzögert auftretenden Hämolyse durchgeführt. Diese Tests blieben negativ.

Diese Arbeit fasste frühe klinische Behandlungsdaten mit intravenösem Artesunat bei Patienten mit komplizierter Malaria aus Europa zusammen. Erstmalig wurde das Phänomen einer verzögert eintretenden Hämolyse nach Artemisinin-Therapie in einer Serie von Patienten beschrieben, welches künftig als „post-artemisinin delayed

haemolysis - PADH“ in der Literatur benannt wurde, und was in allen zuvor mit Artemisininen durchgeführten Studien sowohl bei komplizierter wie auch bei unkomplizierter Malaria noch nicht beobachtet werden konnte. Der zugrundeliegende pathophysiologische Mechanismus war zu diesem Zeitpunkt noch ungeklärt.

Intravenous Artesunate for Severe Malaria in Travelers, Europe

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Multicenter trials in Southeast Asia have shown better survival rates among patients with severe malaria, particularly those with high parasitemia levels, treated with intravenous (IV) artesunate than among those treated with quinine. In Europe, quinine is still the primary treatment for severe malaria. We conducted a retrospective analysis for 25 travelers with severe malaria who returned from malaria-endemic regions and were treated at 7 centers in Europe. All patients survived. Treatment with IV artesunate rapidly reduced parasitemia levels. In 6 patients at 5 treatment centers, a self-limiting episode of unexplained hemolysis occurred after reduction of parasitemia levels. Five patients required a blood transfusion. Patients with posttreatment hemolysis had received higher doses of IV artesunate than patients without hemolysis. IV artesunate was an effective alternative to quinine for treatment of malaria patients in Europe. Patients should be monitored for signs of hemolysis, especially after parasitologic cure.

Infection with *Plasmodium falciparum* malaria remains a major risk for European travelers returning from malaria-endemic areas. World Health Organization (WHO) guidelines recommend intravenous (IV) artesunate as first-line therapy for severe malaria (*1*). However, quinine is still the primary treatment for severe non–multidrug-resistant *P. falciparum* malaria in Europe (*2*) because IV artesunate is not registered for this indication, and the only commercially available product is not manufactured according to good manufacturing practice. Quinine has several adverse effects (e.g., cardiotoxicity, hypotension, hypoglycemia, and cinchonism), has a narrow therapeutic range, and must be administered 3×/d by rate-controlled infusion (*3*,*4*). In experienced hands, adverse effects can be minimized, but a major proportion of patients still experience moderate-to-severe side effects.

The efficacy and safety of artemisinins and their derivatives in oral, rectal, and intramuscular dosage forms have been widely studied (*5*–*11*). When administered intravenously, these drugs are useful for treatment of severe malaria because of their rapid parasite clearance, apparent absence of clinically relevant side effects, and simplicity of administration (e.g., by bolus injection). Since 1992, several studies in Asia (*5*,*6*,*8*–*10*) and a recent study of children in Africa (*11*) have shown better, or at least equivalent, survival rates for patients with severe malaria treated with artesunate than for those treated with quinine. This finding applies particularly to patients with severe malaria and hyperparasitemia (*10*).

Systematic data are not available for safety and efficacy of IV artesunate for treatment of severe *P. falciparum* malaria outside disease-endemic areas. In the United States, use of IV artesunate is monitored by the Centers for Disease Control and Prevention (Atlanta, GA, USA) under an investigational new drug protocol (*12*). In Europe, artesunate manufactured by the Guilin Pharmaceutical Factory No. 2 (Shanghai, People's Republic of China), which was used in all major trials of artesunate in Southeast Aia and Africa (*9*–*11*), is used. TropNetEurop (www.tropnet.net/about/contents/about_tropnet.html), a European surveillance network for tropical diseases, has been collecting data on artesunate use since 2005 (*13*).

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Severe malaria is rare outside disease-endemic regions. Thus, the limited numbers of patients in industrialized countries makes it difficult to conduct trials with sufficient statistical power to reproduce the survival benefit for IV artesunate observed in Southeast Asia (*10*). Nonetheless, these patients may benefit from the lower cardiotoxicity of artesunate than that of quinine and, because of more rapid parasite clearance, from reduction of time spent in intensive care units, in-hospital treatment, decreased use of exchange transfusion, and secondary complications. This finding is relevant for increased numbers of older persons who travel abroad to malaria-endemic areas, despite relevant cardiac or other medical conditions associated with a several-fold increased risk for complications and death caused by severe malaria (*14*). We report data for 25 patients with severe malaria who were treated with IV artesunate in 7 treatment centers in areas to which malaria was not endemic.

Study Characteristics

During January 2006–June 2010, we conducted a retrospective analysis of 25 patients from 7 treatment centers in Europe who were admitted to a hospital for *P. falciparum* malaria, which was classified as severe according to WHO criteria (*15*,*16*), and who received IV artesunate as the main antiparasitic therapy. The hyperparasitemia level for patients in a region to which malaria was not endemic was ≥5% (*15*). Patients treated at 7 centers, 4 in Germany (2 in Berlin, 1 in Heidelberg, and 1 in Düsseldorf), and 1 each in Denmark (Copenhagen), Sweden (Helsingborg), and Norway (Bergen), participated in the study. The Berlin (Charité University Medical Center), Heidelberg, Düsseldorf, Bergen, and Copenhagen centers are tertiary care academic teaching hospitals; the center in Helsingborg and the Armed Forces Hospital in Berlin are secondary care regional referral hospitals. The second Berlin center and the Bergen center provided data only for patients with posttreatment hemolysis; other centers provided data for all patients treated with IV artesunate. Anonymous treatment data were reported on case-reporting forms for severe malaria (TropNetEurop). The study was reviewed and approved by the ethics committee of the Charité Hospital in Berlin. Artesunate was obtained from the Guilin Pharmaceutical Factory No. 2 and stored at room temperature in all centers, according to the manufacturer's instructions.

Posttreatment Hemolysis

Serum and plasma of 3 patients with unusual posttreatment hemolysis in Berlin and Heidelberg (patients 6, 7, and 9) were tested for drug-induced autoantibodies, which react in the absence of the drug or its metabolites with erythrocytes, and for drug-dependent antibodies, which react only in the presence of the drug or its metabolites. Serum or plasma samples were available for testing from the time of artesunate treatment (patient 7), from the period of posttreatment hemolysis (patients 6, 7, and 9), or from the convalescent phase (7 and 16 months; patients 6 and 9).

Serologic testing was conducted by using standard gel card techniques (DiaMed, Cressier sur Morat, Switzerland). Artesunate was diluted in 0.9% NaCl at a concentration of 1.0 mg/mL. Ex vivo antigens (urine) were obtained from 2 patients receiving IV artesunate to detect reactivity to artesunate metabolites. Serum samples were tested for reactivity with artesunate solution or urine metabolites by using the indirect antiglobulin test and a drug-dependent–antibody test with the gel card technique (*17*–*19*). Cumulative doses and treatment duration (days) were compared between adult patients with and without signs of posttreatment hemolysis by using the Mann-Whitney U test.

Patient Characteristics

One child and 24 adults (mean ± SD age 44.1 ± 16.1 years; 14 male and 11 female patients) treated with IV artesunate for severe malaria during January 2006–June 2010 were included in the study (online Appendix Table, www.cdc.gov/EID/content/17/5/771-appT.htm). Eighteen patients were travelers from Europe to malaria-endemic areas, and 7 patients were immigrants who returned from malaria-endemic countries after having visited friends and relatives. With the exception of patient 13, who was a short-term visitor to Germany from Chad, all other patients who visited friends and relatives had permanently left their home countries for >5 years before becoming infected.

Hyperparasitemia (range 5%–51% parasitized erythrocytes) in 20 (80%) patients and cerebral malaria in 8 (32%) patients were the most common severe malaria-defining criteria observed. Seven patients (28%) had renal failure, and 2 (8%) required hemodialysis. Respiratory failure caused by severe shock developed in 1 patient; this patient required therapy with vasopressors and mechanical ventilation for 6 days. Repeated chest radiographs did not show pulmonary edema or pneumonia. Shock developed in 4 patients (patients 3, 4, 13, and 25); these patients required vasopressor therapy.

Antimalarial Therapy

Details on dosage, treatment duration, and concomitant therapy are shown in the online Appendix Table. All but 3 patients received IV artesunate as first-line therapy. Therapy for patient 1 was changed to IV artesunate after complications (bradycardia) caused by the first dose of quinine. Therapy for patients 10 and 13 was 1 dose of artemether/lumefantrin or IV quinine, respectively, before transfer to a treatment center to avoid a delay in treatment initiation.

Patients 3–8, 15, 16, 18, and 19 received the dosing regimen for artesunate initially recommended by WHO (*16*): after an initial dose of 2.4 mg/kg, therapy was continued with 1.2 mg/kg every 12 hours and then 1.2 mg/kg every 24 hours. Patients 9–13 and 19–25 received artesunate, 2.4 mg/kg/dose. Therapy for all but 6 patients was changed to oral artemether/lumefantrine or atovaquone/proguanil after rapid clinical improvement and ability to swallow on days 3–4 of treatment. Different batches of artesunate were used in the Berlin and Heidelberg treatment centers. Batch information was not available from centers in Helsingborg, Copenhagen, and Bergen and the second center in Berlin. Six patients in whom posttreatment hemolysis occurred were treated for 4 years.

Efficacy

In all patients with hyperparasitemia, parasite load was reduced ≈1 $\log_{10}$ after 24–36 hours. All but 1 patient were free of parasites 36 hours–134 hours after the initial dose of artesunate. Parasite clearance was delayed (158 hours) in 1 patient (patient 7). In this patient, infection with HIV was diagnosed (CD4 count 382 cells/μL). Mean ± SD parasite clearance time was 81.2 ± 35.4 hours for all patients treated with IV artesunate as first-line drug, who had an initial parasitemia levels >1% and for whom data were available (patients 2–12, 14, and 20–24), and 78.9 ± 29.5 hours for patients not infected with HIV.

Tolerability

IV artesunate was generally well tolerated; there was no evidence of hemodynamic, cardiac, or allergic adverse reactions. Six patients from 5 treatment centers showed unusual hemolytic anemia, which recurred after clearance of parasites and was diagnosed 14–31 days after the first dose of IV artesunate (patients 6, 11, and 23) or persisted after the end of treatment until the end of the fourth week after the first dose of IV artesunate (patients 7, 9, and 25). Laboratory findings and typical patterns of hemolysis are shown in the Table and the Figure.

Patient 6 was treated with artesunate and doxycycline; she had malaria-related hemolysis and an initial hemoglobin level of 11.3 g/dL. This patient was discharged in good clinical condition on day 10 (hemoglobin level 7.7 g/dL, which had been stable for the past 4 days) and had a decreased lactate dehydrogenase (LDH) level (317 U/L). On day 15, this patient was readmitted because of severe anemia caused by recurring hemolysis (hemoglobin 5.7 g/dL, LDH 1,437 U/L). Glucose-6-phosphate dehydrogenase (G6PD) deficiency and antibody-mediated hemolysis were excluded as causes (negative result for Coombs test). The reticulocyte count was high (10.2%). After receiving 2 units of packed erythrocytes, the hemoglobin level of this patient remained stable.

Patient 11, who was treated in Helsingborg, received IV artesunate for 7 days. On day 15, laboratory parameters were indicative of secondary hemolysis. The reticulocyte count was within reference limits initially and was near the upper reference value during secondary hemolysis. Patient 23, who was treated in Bergen, had a similar episode of recurring and intense hemolysis after 4 days of treatment with IV artesunate, beginning on day 15, which required readmission to the center and blood transfusion. Results of the Coombs test were repeatedly negative, G6PD deficiency was ruled out, and reticulocytes values were 2.3× the upper reference value.

Other patients showed patterns of persisting hemolysis. Patient 7 was discharged from the hospital in Berlin 14 days after the first dose of IV artesunate (treatment duration 7 days) with a hemoglobin level of 8.2 g/dL, which was stable for 10 days. On day 32, this patient was readmitted to the University Hospital in Heidelberg with a hemoglobin level of 6.1 g/dL and signs of hemolysis (LDH 805 U/L). The patient received 2 units of packed erythrocytes and was discharged 3 days later in good clinical condition. Hemolytic activity decreased over the next 10 days.

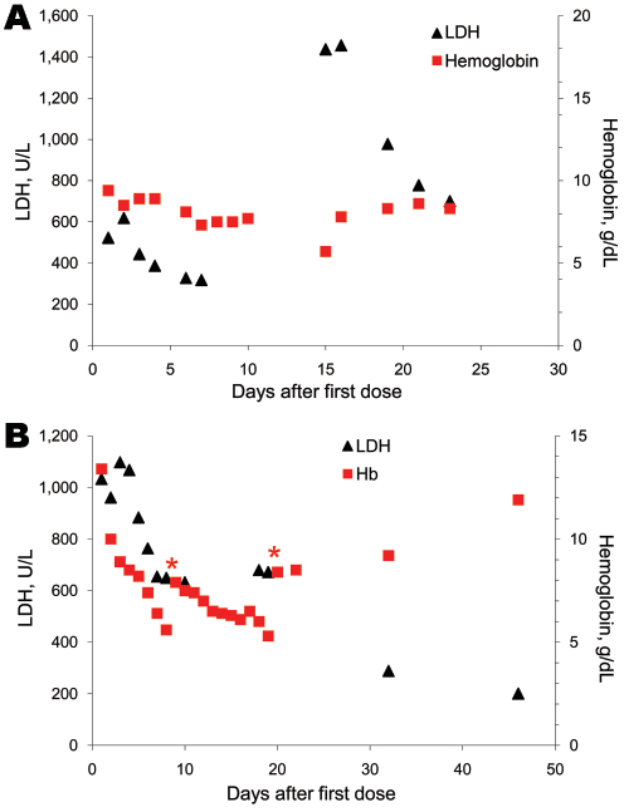

Figure. Typical patterns of hemolysis in 2 travelers with severe malaria treated with intravenous artesunate, Europe, January 2006–June 2010. A) Patient 6 with recurring hemolysis. B) Patient 9 with persisting hemolysis. LDH, lactate dehydrogenase. * indicates blood transfusion. Gaps between symbols indicate periods when samples were not obtained.

Table. Laboratory test results for 6 patients with posttreatment hemolysis who had been treated with intravenous artesunate for severe malaria, Europe, January 2006–June 2010*

Patient no.	Initial parasitemia level, %	Levels at first examination		Treatment duration, d	Parasite clearance, d	Levels at end of treatment		Day of diagnosis of hemolysis†	Levels at diagnosis of hemolysis		Other test results
		Hb, g/dL	LDH, U/L			Hb, g/dL	LDH, U/L		Hb, g/dL	LDH, U/L	
6	30	11.3	765	7	4	7.7	317	15	5.7	1,437	Coombs negative, reticulocytes 10.2%, G6PD deficiency ruled out
7	20	13.2	1,359	7	7	8.2	NA	32‡	6.1	805	None
9	30	13.4	1,033	4	5	7.6	650	19‡	5.3	672	None
11	4	13.4	904	7	2	9.8	311	15	7.8	660	Standard reticulocyte count
23	9	15.5	490	4	2	11.1	571	15	5.7	1,489	Reticulocytes >2× upper reference value, haptoglobin <0.1g/L, Coombs negative
25	10	14.2	570	3	NA	7.8	454	16‡	5.8	444	Reticulocytes 3× upper reference value, haptoglobin <0.08 g/L (day 14), G6PD deficiency ruled out

*Hb, hemoglobin; LDH, lactate dehydrogenase; G6PD, gluose-6-phosphate dehydrogenase; NA, not available.
†After first dose of artesunate.
‡Patients had persistent hemolytic activity after the end of malaria treatment.

Patient 9 was treated in Heidelberg and received IV artesunate for 3 days, followed by oral artemether/lumefantrine for another 3 days, and was parasite free after 96 hours. Intense and persisting hemolysis resulted in a hemoglobin level of 5.6 g/dL on day 8 (LDH 633 U/L). This patient received 2 units of packed erythrocytes, and hemoglobin level increased to 7.9 g/dL. On day 19, hemoglobin level decreased to 5.3 g/dL because of persistent hemolysis (LDH 672 U/L); he was again given transfusions of packed erythrocytes. Hemolytic activity decreased after day 22, and LDH levels returned to reference values on day 46. Patient 25 showed a similar pattern of persistent hemolysis, which gradually decreased after day 21, after malaria therapy.

In multiple repeat thin blood films used to determine parasitemia levels, no abnormalities in erythrocyte morphology were observed. To identify causes of posttreatment hemolysis, cumulative doses of IV artesunate and treatment durations were compared among all adult patients. Patients with posttreatment hemolysis had received higher doses of IV artesunate than patients without observed hemolysis (mean ± SD cumulative dose 12.8 ± 3.3 mg/kg vs. 7.6 ± 2.9 mg/kg; p = 0.006 in all adult patients) and were treated for longer periods (mean ± SD 5.8 ± 1.6 days vs. 3.6 ± 1.7 days; p = 0.038).

Immunohematologic Tests

Free indirect antibodies against globulin were not detected in serum or plasma from 3 patients (patients 6, 7, and 9) at the Berlin and Heidelberg treatment centers who had prolonged posttreatment hemolysis. Presence of drug-dependent antibodies in serum or plasma of patients was investigated by using as test substrates an artesunate solution and urine (artesunate metabolites) of patients receiving artesunate therapy; antibodies were not detected.

Clinical Outcome

All patients survived and all complications related to severe malaria resolved at time of hospital discharge for all but 1 patient. Patient 2 had a more severe clinical course (respiratory and renal failure), and required further rehabilitation and physiotherapy because of critical illness (neuropathy) that developed while he received prolonged intensive care and immobilization. Unusual hemolysis in 6 patients resolved spontaneously during weeks 3–6 after the first dose of IV artesunate.

Conclusions

Data from large multicenter trials on use of parenteral artesunate are limited to malaria-endemic regions, particularly Southeast Asia. We report data on use of parenteral artesunate for patients with severe malaria outside malaria-endemic areas, who were treated according to intensive care standards in Europe. In these patients, treatment with IV artesunate was effective and induced rapid parasite clearance. The only other report of a series of patients treated with IV artesunate for severe malaria outside malaria-endemic areas was from Norway; outcomes for 9 patients were good, and adverse reactions related to IV artesunate were not observed (*21*).

Parasitemia levels took longer to clear for participants in our study than those in a study in Thailand (*9*) (mean ± SD 81.2 ± 35.4 hours vs. 62.5 hours, 95% confidence interval 53.4–71.8 hours). In contrast with uncomplicated malaria, parasite clearance times for severe malaria are difficult to compare when different drug regimens (concomitant and sequence therapy) have been used. In addition, all patients in our study were considered nonimmune.

High parasitemia levels in severe malaria are more likely to develop in nonimmune patients; such patients are more likely to receive exchange transfusions. Physicians treating patients in our study decided not to use exchange transfusions because they have unproven benefits. Artesunate has been shown to be particularly effective in reducing mortality rates among patients with parasitemia levels >10% (*10*). Therefore, patients from Europe may benefit more from treatment with artesunate than with quinine.

Unusual episodes of hemolysis developed in 6 patients in our study. These patients had clinical signs caused by anemia during the third week of treatment with IV artesunate or had persistent signs of hemolytic activity until 6 weeks after the first dose of IV artesunate. In all cases, physicians in different treatment centers were unaware of other cases at that time, and hemolysis was not immediately considered to be induced by IV artesunate. Thus, a follow-up of patient serum samples and pharmacologic analysis of drugs used was not conducted. An additional case of Coombs-negative, posttreatment hemolysis in a European traveler during the third week after receiving IV artesunate/quinine therapy in Tanzania was reported from the Netherlands (R.M. Peerenboom, unpub. data). Despite these observations, it is not appropriate to infer incidence rates from our study regarding the incidence of posttreatment hemolysis in patients treated with IV artesunate because in 2 centers not all patients were available for inclusion. Known and possible causes of hemolytic anemia in association with malaria or antiparasitic therapy include blackwater fever, artemisinin-induced reticulocytopenia, direct hemolytic effects of the drug, and drug-induced immune hemolytic anemia.

G6PD deficiency is the basis for primaquine-, quinine- (*22*), and tafenoquine- (*23*) induced hemolytic anemia, but it was not identified in patients tested in our study. Blackwater fever, which causes acute hemolysis and hemoglobinuria in the early course of malaria treatment, was observed in the South East Asian Quinine Artesunate Trial (*10*) for quinine and artesunate (5% vs. 7%). Late onset, prolonged duration, and recurrence are not typical for blackwater fever as the cause of hemolysis in our patients. A temporary depression of reticulocytopoesis 3–7 days after the first dose of artemisinin derivatives has been reported in other studies (*5*,*24*,*25*). This phenomenon was not found for patients in our study for whom reticulocyte levels were determined.

IV artesunate is rapidly hydrolyzed to the active metabolite dihydroartemisinin. Because dihydroartemisinin has a short half-life (*26*), prolonged hemolysis after stopping treatment with IV artesunate and the recurring hemolysis in 2 patients suggest that a direct hemolytic effect of the drug is unlikely, although contaminants may have a longer half-life. Drug-induced immune hemolytic anemia, which involves production of drug-induced, irregular autoantibodies against erythrocytes, is typically associated with administration of different drugs (e.g., cephalosporins, quinine, penicillin, diclofenac, or rifampin) (*19*,*27*,*28*).

Formation of drug-dependent immunoglobulin (Ig) G or IgM leads to hemolysis and complement activation only in the presence of the causative drug; other drug-independent IgG types can cause hemolytic anemia in the absence of the drug (*29*). This second type of hemolysis occurs without complement activation and, in most cases, has a less severe clinical course. This unusual pattern of hemolysis was not observed in large clinical trials conducted with IV artesunate obtained from the same manufacturer in China (*9*,*10*) or in studies of oral artesunate. One case of a similar phenomenon was reported in Japan (*30*). However, in previous trials, patients were not routinely followed up, and cases of prolonged or recurring hemolysis might have been missed. Results of immunohematologic tests in our study did not indicate drug-induced or drug-dependent hemolysis. Because these tests had to be performed with frozen blood samples, results may have been influenced by the quality of the samples. However, the consistently negative Coombs test result for fresh blood from 2 of our patients with hemolysis suggests that hemolysis induced by autoimmune mechanisms is unlikely.

Patients with posttreatment hemolysis had received a higher cumulative dose of IV artesunate and were treated for longer periods. This observation supports the hypothesis that hemolysis might occur as a consequence of artesunate treatment in a dose-dependent manner. However, because this study did not have a prospective design and patients were not routinely followed up for signs of hemolysis in all centers, we cannot exclude undetected cases of hemolysis in our study.

The underlying cause of posttreatment hemolysis in our study of travelers is still unknown. Because IV artesunate currently produced in China is not manufactured according to standards of good manufacturing practice used in Europe, contaminants might have caused direct or antibody-mediated hemolysis in our patients. However, the manufacturer of IV artesunate recently passed the WHO drug prequalification program (*31*). Hemolysis in 5 centers in Europe over a period of 4 years suggests that contamination in a batch of IV artesunate is unlikely. Other reported artesunate-related adverse reactions, such

as hypersensitivity reactions (*32*) and vestibulocochlear disturbances (*33*,*34*), were not observed in our study.

The role of IV artesunate for treatment of severe malaria in patients treated in Europe remains to be defined. However, it should be considered for patients with hyperparasitemia, patients with medical conditions limiting or prohibiting use of quinine, or patients in whom quinine-related adverse reactions are observed. Efficacy and safety profiles of IV artesunate should be prospectively evaluated, and patients should be monitored for signs of hemolysis after parasitologic cure. Reducing the cumulative dose of IV artesunate by early initiation of oral treatment might help reduce risk for posttreatment hemolysis. Improving availability of IV artesunate produced according to standards of good manufacturing practice used in Europe or the United States would constitute a major step in improving therapy for severe malaria.

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2.3. Intravenöses Artesunat zur Behandlung der komplizierten Malaria tropica reduziert die Zeit bis zur Parasitenelimination sowie die Behandlungsdauer auf Intensivstation und im Krankenhaus

Kurth F, Develoux M, Mechain M, Clerinx J, Antinori S, Gjørup IE, Gascon J, Mørch K, Nicastri E, Ramharter M, Bartoloni A, Visser L, Rolling T, Zanger P, Calleri G, Salas-Coronas J, Nielsen H, Just-Nübling G, Neumayr A, Hachfeld A, Schmid ML, Antonini P, Pongratz P, Kern P, Saraiva da Cunha J, Soriano-Arandes A, Schunk M, Suttorp N, Hatz C, **Zoller T** for the TropNet Severe Malaria Investigator Group. Intravenous Artesunate Reduces Parasite Clearance Time, Duration of Intensive Care, and Hospital Treatment in Patients With Severe Malaria in Europe: The TropNet Severe Malaria Study.
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Da in Europa aufgrund der niedrigeren verfügbaren Patientenzahlen und vieler anderer Begleitfaktoren ein Unterschied in der Mortalität wahrscheinlich nicht beobachtet werden kann, konzentrierte sich diese Analyse auf sekundäre Faktoren, die hinsichtlich Morbidität und Mortalität im Rahmen der Behandlung einer komplizierten Malaria tropica von Bedeutung sein könnten. Die Beobachtungsstudie des Netzwerkes TropNet zur komplizierten Malaria schloss als einzige multizentrische Studie in nicht-endemischen Gebieten sowohl Patienten ein, die intravenöses Artesunat als auch Patienten, die intravenöses Chinin zur Therapie erhalten hatten, ein.
Es konnte gezeigt werden, daß die Zeit bis zur Reduktion der Parasitendichte um 99% sowohl allgemein, aber insbesondere bei hyperparasitämischen Patienten unter Behandlung mit intravenösem Artesunate signifikant geringer ist. Auch die Dauer der intensivstationären Behandlung sowie die Dauer der gesamten stationären Behandlung waren unter Gabe von intravenösem Artesunat signifikant geringer als unter intravenösem Chinin. Diese Vorteile galten nur für Patienten mit einer Hyperparasitämie (Parasitendichte ≥5%). Ferner war die Behandlungsdauer auf der Intensivstation – relevant für das Risiko nosokomialer bzw. intensivmedizinisch assoziierter Komplikationen – niedriger bei Patienten ohne cerebrale Malaria, ohne respiratorisches Versagen und ohne Nierenversagen.

Da absehbar keine Daten aus prospektiven kontrollierten Studien gewonnen werden können, zeigte diese aus Daten einer Beobachtungsstudie stammende Analyse klinisch relevante Vorteile in der Behandlung der komplizierten Malaria tropica in nicht-endemischen Gebieten mit intravenösem Artesunat. Die Daten tragen dazu bei, die Grundlage für die Indikationsstellung und Durchführung der Therapie mit intravenösem Artesunat zu verbessern.

Intravenous Artesunate Reduces Parasite Clearance Time, Duration of Intensive Care, and Hospital Treatment in Patients With Severe Malaria in Europe: The TropNet Severe Malaria Study

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Intravenous artesunate improves survival in severe malaria, but clinical trial data from nonendemic countries are scarce. The TropNet severe malaria database was analyzed to compare outcomes of artesunate vs quinine treatment. Artesunate reduced parasite clearance time and duration of intensive care unit and hospital treatment in European patients with imported severe malaria.

Keywords. severe malaria; artesunate; quinine; *Plasmodium falciparum*; clinical study.

Intravenous artesunate has been shown to be a life-saving drug for patients with severe malaria in multicenter trials in Southeast Asia and Africa, improving survival compared with quinine by 34.7% and 22.5%, respectively. The survival benefit was most pronounced in hyperparasitemic patients [1, 2].

In nonendemic countries where nonimmune travelers with imported malaria may exhibit high parasite loads, introduction of intravenous artesunate into treatment practice has been slow because a formulation meeting standards of Good Manufacturing Practice (GMP) as well as prospective clinical safety data required for regulatory approval are not available. Intravenous artesunate is generally safe and well tolerated. A delayed self-limiting hemolytic reaction can occur several weeks after treatment, making follow-up examinations necessary [3–5].

Given the documented life-saving effect of artesunate in endemic countries, controlled clinical trials to confirm better survival for patients in nonendemic countries are no longer justified. Moreover, limited patient numbers and low case fatality of patients treated under intensive care standards in industrialized countries curtail their feasibility. Observational studies are therefore the only source of information on artesunate in this patient population.

The European Network for Tropical Medicine and Travel Health (TropNet) has conducted an observational multicenter study over 9 years (2006–2014) to monitor treatment practices and outcomes of severe malaria treatment across 12 European countries. For this brief report, data from study patients treated with intravenous artesunate or intravenous quinine were analyzed to compare clinical outcomes such as parasite clearance,

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treatment duration in hospital (particularly in the intensive care unit [ICU]), and survival.

METHODS

All patients with confirmed severe *Plasmodium falciparum* malaria according to the 2006 World Health Organization criteria [6] treated at one of the 28 participating TropNet centers between 2006 and 2014 were eligible for inclusion. The treatment remained the responsibility of the treating physician. Demographic, travel, clinical, laboratory, and treatment data were collected retrospectively from clinical records at the treatment center, pseudonymized, and reported using an electronic case report form.

The primary objective of this analysis was to analyze differences among patients treated with intravenous artesunate vs intravenous quinine with regard to survival, duration of ICU and inpatient treatment, and 99% and complete parasite clearance time. The Mann–Whitney *U* test at a 2-sided significance level of $\alpha = .05$ was used for comparative analysis. Data are displayed as median (interquartile range). To further assess the association between treatment with artesunate and ICU treatment time, multiple regression analysis with backward elimination ($P < .05$) was performed, with duration of ICU treatment as a dependent variable including (1) known risk factors for longer treatment (age, renal failure, respiratory failure, coma, and the number of comorbidities); (2) variables with statistically significant association with ICU treatment time in univariate analysis; and (3) the year of treatment. The selected variables were used as independent variables in multifactor analysis of variance (ANOVA) of ICU treatment time using the *F* test.

Statistical analysis was performed using JMP software version 7.0 (SAS Institute Inc, Cary, North Carolina). The study was approved by the Ethics Committee of Charité University Hospital Berlin. Ethical clearance for transfer of pseudonymized patient data was sought at participating TropNet centers according to local regulations.

RESULTS

The TropNet severe malaria study comprises 185 cases with severe falciparum malaria. The majority of patients were European tourists (106/185 [57%]), followed by patients with a history of migration (68/185 [37%]) and tourists from endemic areas (11/185 [6%]). The overall 28-day survival rate was 98.4% (182/185). Of 3 patients who died, 2 had been treated with intravenous quinine and 1 with quinine and artesunate simultaneously. All deaths occurred within the first 4 days after admission. Due to the low number of deaths, a survival analysis was not performed. Adverse events in patients treated with quinine consisted of transient cinchonism and hypoglycemia and were predominantly mild. In 70 patients who received intravenous artesunate, 19 episodes of delayed hemolysis were observed, a known adverse drug reaction [3–5].

For the following analysis of treatment duration and parasite clearance, patients who died (n = 3), who underwent erythrocyte apheresis (n = 5), who were treated with artesunate and quinine simultaneously (n = 7, 1 of whom also underwent apheresis and 1 of whom died), or who received oral antimalarial combination therapies as first-line treatment (n = 21) were excluded. Thereafter, 151 patients were available for comparative analysis, of whom 60 received intravenous artesunate and 91 received intravenous quinine as main first-line treatment.

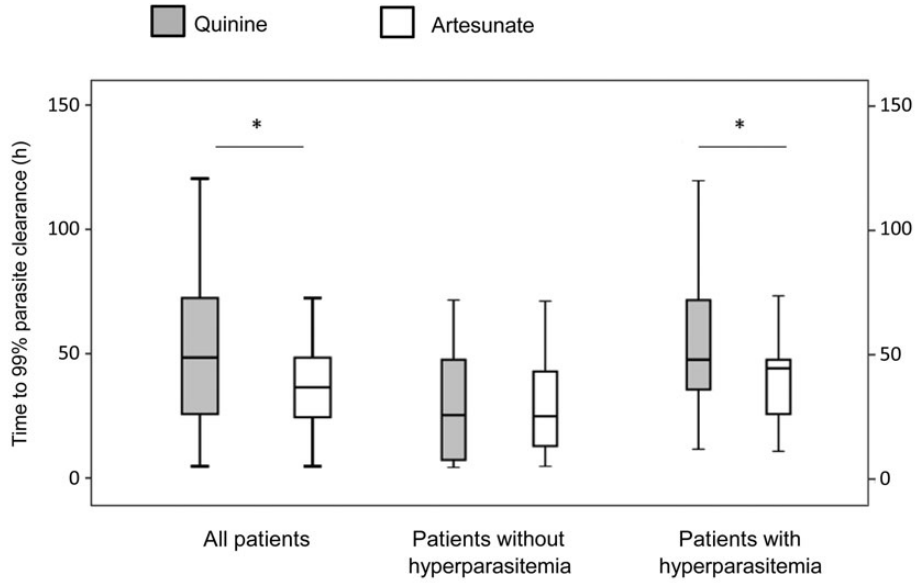
Patients treated with either artesunate or quinine showed similar baseline characteristics such as median age (44 vs 40 years; $P = .16$), median baseline parasitemia (5% vs 6%; $P = .4$), median number of criteria for severe disease (2 in both groups; $P = .3$), median proportion of patients with comorbidities (32% vs 39%; $P = .3$), median proportion of patients with European origin (60% vs 55%), history of migration (34% vs 39%), and visitors from endemic countries (6% in both groups; overall $\chi^2 = 0.62$, $P = .73$), respectively.

Patients treated with intravenous artesunate exhibited a faster 99% parasite clearance time (median, 36 vs 48 hours; $P = .02$, n = 100) and a faster complete parasite clearance time (median, 72 hours vs 96 hours; $P = .005$, n = 84) compared with patients treated with intravenous quinine.

Similarly, median length of ICU treatment (2 vs 3 days; $P < .05$, n = 117) and median length of hospital inpatient treatment (6 vs 7 days; $P < .01$, n = 151) were shorter for patients treated with artesunate compared to patients treated with quinine (Figure 1). Absence of signs of cerebral malaria (median 2 vs 4 days; $P < .0001$), absence of renal failure (median, 2 vs 4 days; $P < .0001$), and absence of respiratory failure (median, 2 vs 6 days; $P < .0001$) were equally associated with a shorter ICU treatment.

In multiple regression analysis, the same 4 variables were selected in the final model and showed independent statistically significant association with shorter length of stay at ICU in multifactor ANOVA ($F = 16.0$, $df = 4$, $P < .0001$): treatment with artesunate ($F = 6.1$, $P = .01$) compared with quinine, absence of cerebral malaria ($F = 4.33$, $P = .04$), absence of renal failure ($F = 20.0$, $P < .0001$), and absence of respiratory failure ($F = 12.2$, $P < .001$). Age, sex, citizenship (European/history of migration/visitor from endemic area), year of presentation, hyperparasitemia, presence of comorbidities, and multiplicity of criteria for severe malaria did not show significant association with duration of ICU treatment.

Subgroup analysis revealed that faster parasite clearance and shorter length of ICU and inpatient treatment for artesunate was only evident in patients presenting with hyperparasitemia ≥5% (108/151), whereas in patients without



	All patients			Patients **without** hyperparasitemia (parasitemia < 5%)			Patients **with** hyperparasitemia (parasitemia ≥ 5%)		
	Quinine	Artesunate	*P* value	Quinine	Artesunate	*P* value	Quinine	Artesunate	*P* value
Median time to 99% parasite clearance in hours (IQR)	48 (29–72)	36 (24–48)	**.02 n = 100**	24 (6–48)	24 (12–45)	.9 n = 23	48 (36–72)	45 (24–48)	**.02 n = 77**
Median time to total parasite clearance in hours (IQR)	96 (72–120)	72 (48–74)	**.005 n = 84**	56 (36–144)	54 (42–72)	.7 n = 21	96 (72–120)	72 (60–92)	**.004 n = 63**
Median length of stay at ICU in days (IQR)	3 (1–5)	2 (1–4)	**<.05 n = 117**	2 (1–5)	3 (1–5)	.5 n = 34	3 (2–5)	2 (1–3)	**.003 n = 83**
Median length of stay in hospital in days (IQR)	7 (6–10)	6 (4–8)	**<.01 n = 150**	7 (6–9)	6 (4–9)	.25 n = 43	7 (6–10)	6 (4–9)	**.01 n = 107**

Figure 1. Median time to 99% parasite clearance, median time to total parasite clearance, median length of stay in intensive care unit (ICU), and median length of stay in hospital in patients treated with either intravenous artesunate or intravenous quinine. Hyperparasitemia is defined as parasitemia ≥5%. *Statistically significant difference. Abbreviation: IQR, interquartile range.

hyperparasitemia (43/151), there was no statistically significant difference (Figure 1).

DISCUSSION

This TropNet study is the largest multicenter study outside endemic areas comparing intravenous quinine vs intravenous artesunate for the treatment of severe malaria. Only 1 single-center study from the United Kingdom reported similar data in a limited number of patients with artesunate treatment [7]. As an overwhelming benefit of artesunate treatment has already been shown in principle, controlled clinical trials are no longer justified. Regulatory approval in industrialized countries is therefore delayed, preventing patients from having regular access to the best treatment available. A GMP-compliant formulation is only available in the United States from the Centers for Disease Control and Prevention [8]. The present data may help to improve clinical decision making in nonendemic countries and provide useful information for regulatory authorities.

As observed in endemic countries, intravenous artesunate cleared high parasitemias in our patient population more rapidly than intravenous quinine. This is of particular relevance for nonimmune patients who carry a comparatively high risk of hyperparasitemia. Knowledge from malaria-endemic settings shows that survival benefit was most pronounced in hyperparasitemic patients [1, 2].

Our data demonstrate that intravenous artesunate reduces duration of ICU and hospital treatment. Both factors have relevant influence on outcome as many patients with severe malaria die from nosocomial complications rather than from malaria itself [9]. This applies in particular to patients in ICU treatment with respiratory failure (including comatose patients with cerebral malaria on mechanical ventilation), in whom the risk for ventilator-associated infections increases over time [10].

We did not find an association of sex, age, or comorbidities with duration of ICU treatment despite age being a known risk factor for increased mortality from severe malaria [11]. Shorter duration of ICU and hospital treatment was only found for patients with high parasitemia, underlining that rapid parasite clearance is likely to be a decisive factor for the clinical and overall benefit of artesunate.

This is a retrospective observational study on a heterogeneous patient population with inherent limitations. In particular, biases in physicians' judgment and patient selection may have occurred. The proportion of nonhyperparasitemic patients in our population was comparatively small. The absence of statistically significant different outcomes in these patients might therefore be caused by type II error. As postartemisinin delayed hemolysis was first described in the year 2011 [3], underreporting of this adverse reaction may have occurred in this retrospective study.

In conclusion, this analysis shows that the therapeutic benefit of artesunate to patients in nonendemic areas is beyond doubt. Faster parasite clearance is likely to be the relevant factor that reduces ICU treatment duration, hospital stay, and, therefore, the risk of nosocomial complications. Prospective collection of data—for example, in patient registries—should continue to improve the evidence base on the safety of artesunate in nonendemic countries.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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2.4. PADH nach Behandlung der unkomplizierten Malaria tropica mit oralen Artemisininen

Kurth F, Lingscheid T, Steiner F, Stegemann MS, Bélard S, Menner N, Pongratz P, Mayer B, Kim J, von Bernuth H, Damm G, Salama A, Suttorp N, **Zoller T**. Hemolysis after Oral Artemisinin Combination Therapy of Uncomplicated Plasmodium falciparum Malaria. *Emerging Infect Dis. 2016; 22(8):1381-1386. http://dx.doi.org/10.3201/eid2208.151905*

Für die Therapie der unkomplizierten Malaria tropica sind Artemisinin-Kombinationstherapien (ACT) seit über 10 Jahren das Medikament der 1. Wahl und spielen für die WHO-Strategie „roll back malaria“ eine zentrale Rolle. Effektivität und Sicherheit der oralen ACT wurden in zahlreichen Zulassungsstudien in endemischen und nicht-endemischen Gebieten (siehe auch 2.5.) prospektiv ausführlich untersucht. Durch die kürzliche Erstbeschreibung der PADH (siehe 2.2) sollte mit dieser Arbeit die Frage geklärt werden, ob auch bei der unkomplizierten Malaria tropica unter oraler Artemisinin-Therapie eine PADH beobachtet werden kann.

Es wurde eine prospektive Beobachtungsstudie an Patienten mit unkomplizierter Malaria an der Charité – Universitätsmedizin Berlin begonnen. Nach einem Jahr wurde bereits eine erste Zwischenanalyse durchgeführt. Zu diesem Zeitpunkt waren 20 Patienten mit vollständigen Datensätzen zur Auswertung verfügbar. Für diese Patientengruppe wurde die PADH definiert als ein Haptoglobin von <0,3g/l und eine Laktat-Dehydrogenase über dem oberen Referenzbereich am Tag 14 nach Behandlungsbeginn. 40% der Patienten erfüllten diese Definition der PADH und Patienten mit PADH hatten einen deutlich geringeren Hämoglobinwert als Patienten ohne PADH. Ferner fielen zwei Untergruppen von Patienten mit einerseits kompensierter Hämolyse (stabiler Hämoglobinwert, hoher Retikulozyten-Produktionsindex) und unkompensierter Hämolyse (Abfall des Hämoglobins, niedriger Retikulozyten-Produktionsindex) auf. Die erstgenannte Gruppe bestand ausschließlich aus Patienten afrikanischer Abstammung, wohingegen die letztgenannte Gruppe aus Patienten europäischer Abstammung bestand. Die beobachteten hämolytischen Reaktionen hielten über eine Dauer von bis zu 8 Wochen an.

Durch diese Arbeit konnte nach dem Nachweis der PADH bei der komplizierten Malaria nach Artemisinin-Therapie erstmalig auch die PADH bei der unkomplizierten Malaria nach oraler ACT-Therapie beschrieben werden. Diese zeigt einen klinisch milderen Verlauf, führt jedoch ebenfalls zu lang anhaltenden hämolytischen Reaktionen. Kurz vor Erscheinen dieser Arbeit wurde durch Jauréguiberry(26) die mögliche Rolle von O-IE bei der Pathophysiologie der PADH beschrieben. Die Daten dieser Arbeit unterstützen die Rolle der O-IE bei der PADH: Patienten mit dem stärksten Abfall des Hämoglobinwerts zwischen Tag 3 und 14 (unkompensierte Hämolyse) hatten einen geringen Anstieg des Hämoglobinwertes am Anfang der Therapie (Tag 0-3). Dies unterstützt die Hypothese, dass bei Patienten mit PADH zu Beginn eine Hämolyse durch Entfernung von Parasiten aus dem Erythrozyten in der Milz verhindert wird; diese Erythrozyten hämolysieren jedoch später alle synchronisiert in einem relativ engen Zeitraum und führen so zum klinischen Bild der PADH. Die Unterschiedlichen Reaktionen auf durch PADH verursachte Anämie in Patienten unterschiedlicher Abstammung ist eine Beobachtung, deren Bedeutung in weiterführenden Studien untersucht werden muss. Durch die Fortführung der Studie werden nach deren Abschluss noch wesentlich detailliertere klinische Daten zur PADH unter oraler ACT-Therapie erwartet.

Hemolysis after Oral Artemisinin Combination Therapy for Uncomplicated *Plasmodium falciparum* Malaria

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Episodes of delayed hemolysis 2–6 weeks after treatment of severe malaria with intravenous artesunate have been described. We performed a prospective observational study of patients with uncomplicated malaria to investigate whether posttreatment hemolysis also occurs after oral artemisinin-based combination therapy. Eight of 20 patients with uncomplicated malaria who were given oral artemisinin-based combination therapy met the definition of posttreatment hemolysis (low haptoglobin level and increased lactate dehydrogenase level on day 14). Five patients had hemolysis persisting for 1 month. Patients with posttreatment hemolysis had a median decrease in hemoglobin level of 1.3 g/dL (interquartile range 0.3–2.0 g/dL) in the posttreatment period, and patients without posttreatment hemolysis had a median increase of 0.3 g/dL (IQR –0.1 to 0.7 g/dL; p = 0.002). These findings indicate a need for increased vigilance for hemolytic events in malaria patients, particularly those with predisposing factors for anemia.

Artemisinin-based drugs are the mainstay of current antimalarial treatment and play a key role in the World Health Organization (WHO) global strategy to reduce malaria illness and death caused by malaria. These drugs act rapidly against *Plasmodium* spp. and are usually well tolerated. Artemisinin-based combination therapies (ACTs) are the recommended first-line treatment for uncomplicated malaria in most countries (*1*).

Episodes of delayed hemolysis 2–6 weeks after treatment for severe malaria with intravenous artesunate have been observed in non–malaria-immune patients in Europe (*2*). This phenomenon, recently referred to as postartemisinin-delayed hemolysis (PADH) (*3*,*4*), has been confirmed in other nonimmune patients (*4*,*5*) and in children in Africa (*6*). Approximately 20%–30% of nonimmune patients given intravenous artesunate show signs of PADH that vary in intensity and duration (*5*,*7*). Hemolysis is usually self-limiting, but patients need to be actively followed up because transfusion of erythrocytes and rehospitalization might be necessary (*2*,*5*).

The pathophysiology of hemolysis after artemisinin therapy is not fully understood. Once-infected erythrocytes that have been cleared of parasites in the spleen have a shorter life span and play a role. Patients with higher concentrations of once-infected erythrocytes after artemisinin treatment are at higher risk for PADH (*4*). However, other features of posttreatment hemolysis, such as prolonged hemolytic reactions over several weeks (*2*), are not explained by this mechanism. Several reports suggest involvement of a drug-dependent autoimmune hemolysis mechanism (*8*), but systematic investigations have not been performed in most published cases (*2*,*9*). Given the key role of artemisinins in malaria treatment, WHO calls for prospective clinical studies and further research to improve the understanding of delayed hemolysis after artemisinin therapy (*10*).

Only 2 single cases and 2 patients in a recent analysis of surveillance data in the United States have been reported with signs of delayed hemolysis after oral ACT treatment (*11–13*). We hypothesize that delayed hemolysis occurs not only after intravenous treatment for severe malaria but also in a substantial number of patients given oral ACTs for uncomplicated malaria. Because delayed hemolysis has not been captured by safety studies on ACTs, we assume that delayed hemolysis after oral ACTs is less pronounced and occurs to a subclinical degree.

We conducted a study of patients with uncomplicated *Plasmodium falciparum* malaria to investigate the clinical, laboratory, and immunohematologic features of hemolysis and anemia during and after antimalarial treatment. This article presents data for patients investigated during the first 12 months of this ongoing study.

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Methods

This prospective observational study was conducted at the University Hospital of Charité–Universitätsmedizin Berlin (Berlin, Germany). The study protocol was approved by the ethical committee of Charité–Universitätsmedizin Berlin and is registered at the WHO International Clinical Trials Registry Platform (DRKS00007104). All laboratory analyses and parasitologic examinations were performed in accredited laboratories at Charité-Universitätsmedizin, Berlin.

All patients who sought treatment at the hospital and were found to have microscopically confirmed uncomplicated *P. falciparum* malaria were included in the study after written informed consent was obtained. Patients were excluded if they had received antimalarial treatment (excluding prophylaxis) within 12 weeks before inclusion; had medical conditions that potentially cause hemolysis (e.g., glucose-6-phosphate dehydrogenase deficiency, hemoglobinopathy, mechanical heart valve, lymphoproliferative disease); or were taking medication that potentially causes hemolysis.

Patients were seen for study visits at admission before treatment (day 0). After the first treatment, they were seen again after the last treatment dose on day 3, on day 7 (range day 6–day 10), and on day 14 (range day 14–day 20). If symptoms or signs of hemolysis were detected, patients were seen on day 30 (range day 27–day 31) and thereafter if clinically indicated.

Study visits included obtaining a medical history and conducting a physical examination. Laboratory investigations were parasitologic (thick and thin blood smears), hematologic (differential blood count), and biochemical (haptoglobin, lactate dehydrogenase [LDH], C-reactive protein, potassium, and sodium levels and renal and liver function tests) examinations; screening for glucose-6-phosphate dehydrogenase deficiency; and immunohematologic examinations (direct and indirect antiglobulin test, including testing with enzyme-treated erythrocytes).

This analysis evaluated data for all patients given oral ACTs during the first 12 months of the study. The primary objective was to assess the proportion of patients with posttreatment hemolysis, which was defined as a low haptoglobin level (<0.3 g/L) and an LDH level above the age-dependent upper normal level 14 days after treatment. Secondary objectives were to compare in patients with posttreatment hemolysis and those without it possible risk factors (age, ethnicity, sex, initial parasitemia) and the course of anemia (hemoglobin [Hb] level, reticulocyte production index) during treatment, after treatment, and overall. Hemolysis with a loss of Hb ≥1.5 g/dL during days 3–14 was classified as uncompensated hemolysis; hemolysis without a decrease in Hb level or a decrease <1.5 g/dL during days 3–14 was classified as compensated hemolysis.

Differences between patients with and without signs of posttreatment hemolysis and between patients with compensated and uncompensated hemolysis were analyzed by using the Mann-Whitney U test for continuous data and the Fisher exact test for binary data at a 2-sided significance level of $\alpha = 0.05$. Data are presented as median and interquartile range (IQR). Statistical analysis was performed by using JMP version 7.0 (SAS Institute Inc., Cary, NC, USA).

The sample size of the ongoing study was calculated to detect an incidence of posttreatment hemolysis of 20% with a 95% CI, ± 7.5% precision, and 15% lost to follow-up. This calculation resulted in a sample size of 130 patients. This study evaluated 27 patients, which represented 21% of the intended total sample size. Because we could find no published prospective data for hemolysis after oral ACT treatment, we decided to communicate the findings of this interim analysis before completion of the study.

Results

During May 2014–April 2015, a total of 27 patients with uncomplicated *P. falciparum* malaria and a standard 3-day treatment course of oral ACT were included in the study. All malaria infections had been acquired in Africa, and none of the patients had taken antimalarial prophylaxis. Six patients did not complete all necessary follow-up visits, and 1 patient was excluded because of sickle cell disease. Twenty patients with ≥4 study visits until day 20 were available for this interim analysis; of these patients, 3 were children (Table). All patients showed rapid clinical improvement with clearance of peripheral asexual parasitemia no later than 72 hours after initiation of treatment. There were no treatment failures.

The criteria for posttreatment hemolysis (haptoglobin and LDH levels) on day 14 were met by 8 (40%) of 20 patients. An additional 2 patients showed signs of in vitro hemolysis on day 14 (increased LDH and potassium levels but haptoglobins level within reference ranges). The LDH values of these patients were excluded from further analysis and these patients were classified as patients without hemolysis. Patient characteristics showed no differences between those with and without hemolysis, with the exception of slightly higher Hb levels on day 0 and day 3 in patients with posttreatment hemolysis (Table).

After treatment (during days 3–14), patients with posttreatment hemolysis showed a decrease in Hb level (median change –1.3 g/dL, IQR –2.0 to –0.3), and patients without posttreatment hemolysis showed an increase in Hb level (median change 0.3 g/dL, IQR –0.1 to 0.7; $p = 0.002$) (Figure 1, panel C). During treatment (during days 0–3), patients with posttreatment hemolysis showed a tendency toward a smaller decrease in Hb level (median change –0.15 g/dL, IQR –0.6 to 0.6) than did patients without

Table. Baseline characteristics and follow-up laboratory data for patients with uncomplicated *Plasmodium falciparum* malaria who were given ACT*

Characteristic	All, n = 20	Without posttreatment hemolysis, n = 12	With posttreatment hemolysis, n = 8	p value	With compensated posttreatment hemolysis, n = 4	With uncompensated posttreatment hemolysis, n = 4	p value
Baseline							
Age, y	35 (26–40)	31 (17–40)	38 (30–43)	0.18	32 (22–42)	40 (27–46)	0.15
Children	3/20 (15.0)	3/12 (25.0)	0/8 (0)	0.24	0/4 (0)	0 (0)	
African ethnicity	13/20 (65.0)	9/12 (75.0)	4/8 (50.0)	0.35	4/4 (100.0)	0/4 (0)	0.001
Female sex	9/20 (45.0)	7/12 (58.3)	2/8 (25.0)	0.19	2/4 (50.0)	0/4 (0)	0.42
Treatment with ARM/LUM	5/20 (25.0)	4/12 (33.3)	1/8 (12.5)	0.60	0/4 (0)	1/4 (25.0)	1.0
Treatment with DHA/PPQ	15/20 (75.0)	8/12 (75.0)	7/8 (87.5)	0.60	4/4 (100.0)	3/4 (75.0)	1.0
Parasitemia†	0.4 (0.2–1.1)	0.3 (0.1–0.9)	0.9 (0.4–1.4)	0.12	1.15 (0.4–1.9)	0.8 (0.2–1.1)	0.40
Hb d0‡	12.5 (11.1–14.0)	11.3 (10.5–13.5)	13.1 (12.5–14.1)	0.11	12.7 (12.4–13.8)	13.7 (12.6–14.6)	0.30
Laboratory follow-up‡							
Hb d3	12.2 (10.6–13.6)	11.1 (9.7–12.7)	13.2 (12.2–14.3)	0.02	12.8 (11.9–13.4)	14.2 (12.5–14.6)	0.15
Hb d7	12.1 (11.1–13.0)	11.7 (10.5–12.5)	12.5 (11.9–12.6)	0.33	12.6 (11.8–13.1)	12.7 (11.5–12.9)	0.66
Hb d14	12.0 (10.9–12.6)	11.7 (10.5–12.6)	12.2 (11.9–12.6)	0.33	12.5 (12.2–12.8)	11.9 (10.5–12.5)	0.11
ΔHb d0–d3	−0.4 (−0.8 to 0.0)	−0.5 (−1.0 to −0.3)	−0.1 (−0.6 to 0.6)	0.07	−0.4 (−1.1 to 0.7)	0.1 (−0.2 to 0.6)	0.40
ΔHb d3–d7	0.0 (−0.8 to 0.5)	0.3 (0.3–0.8)	−0.8 (−1.5 to −0.1)	0.007	−0.3 (−0.6 to 0.2)	−1.5 (−1.7 to −1.0)	NA
ΔHb d7–d14	0.1 (−0.5 to 0.5)	0.3 (0.1–0.5)	−0.4 (−0.9 to 0.1)	0.04	0.0 (−0.4 to 0.4)	−0.8 (−1.2 to −0.3)	NA
ΔHb d3–d14	0.0 (−0.7 to 0.5)	0.3 (−0.1 to 0.7)	−1.3 (−2.0 to −0.3)	0.002	−0.3 (−0.6 to 0.3)	−1.9 (−2.6 to −1.9)	NA
ΔHb d0–d14	−0.7 (−1.1 to 0.1)	−0.4 (−0.8 to 0.4)	−1.3 (−2.1 to −0.3)	0.03	−0.4 (−1.3 to 0.2)	−1.9 (−2.8 to −1.3)	NA
LDH d7, U/L	250 (225–331)	244 (222–274)	327 (229–407)	0.16	329 (211–495)	327 (248–381)	0.77
LDH d14, U/L	256 (210–283)	210 (197–247)	280 (256–365)	0.006	273 (255–394)	298 (234–365)	1.0
RPI d3	0.5 (0.3–0.7)	0.4 (0.2–0.7)	0.7 (0.4–0.7)	0.22	0.7 (0.4–0.7)	0.6 (0.5–0.7)	1.0
RPI d7	1.4 (0.9–1.6)	1.4 (0.9–1.5)	1.3 (0.8–2.0)	0.66	1.7 (1.2–2.7)	1.0 (0.4–1.8)	0.24
RPI d14	1.4 (1.0–1.8)	1.1 (1.0–1.6)	1.9 (1.4–2.6)	0.015	2.5 (1.9–2.9)	1.5 (1.1–1.9)	0.04

*Values are median (interquartile range) or n/N (%). ACT, artemisinin-based combination therapy; ARM, artemether; d, day; Δ, period between indicated days; DHA, dihydroartemisinin; Hb, hemoglobin; LDH, lactate dehydrogenase; LUM, lumefantrine; NA, not applicable; PPQ, piperaquine; RPI, reticulocyte production index.
†Percentage of erythrocytes infected.
‡Hb levels are in grams/deciliter.

posttreatment hemolysis (median change –0.5 g/dL, IQR –1.0 to –0.3; p = 0.07) (Figure 1, panel B). Overall (during days 0–14), patients with posttreatment hemolysis showed a larger decrease in Hb level (median change –1.35 g/dL, IQR –2.1 to –0.3) than did patients without posttreatment hemolysis (median change –0.45 g/dL, IQR –0.8 to 0.4; p = 0.03) (Figure 1, panel A).

Analysis of the course of anemia in the 8 patients with posttreatment hemolysis showed that a decrease in Hb level during days 3–14 occurred in only 4 patients (uncompensated hemolysis). The other 4 patients with hemolysis maintained stable Hb levels during days 3–14 (compensated hemolysis) (Figure 2, Panel C). Consistent with this observation, we found that patients with compensated posttreatment hemolysis showed a higher reticulocyte production index on day 14 than did patients with uncompensated posttreatment hemolysis or without hemolysis on day 14. No differences were observed in median LDH levels and initial parasitemia between patients with compensated or uncompensated posttreatment hemolysis (Table). All patients with compensated posttreatment hemolysis were of African ethnicity, and all patients with uncompensated posttreatment hemolysis were Caucasian.

Five patients with posttreatment hemolysis (4 patients with uncompensated hemolysis and 1 patient with compensated hemolysis) were followed-up until day 30. All of these patients had persistent low haptoglobin levels (<0.3 g/L) on day 30, and 2 patients still had LDH levels above the age-dependent upper reference level on day 30. Exemplary cases of a patient without hemolysis (Figure 3, panel A), a patient with compensated hemolysis (Figure 3, panel B), and a patient with uncompensated hemolysis (Figure 3, panel C) show the course of laboratory values over time. The patient with compensated hemolysis had a low haptoglobin level

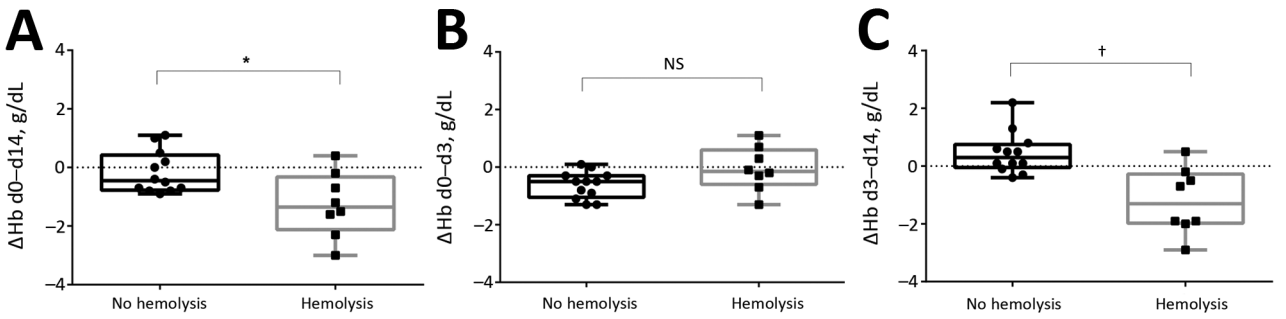

Figure 1. Changes in hemoglobin levels (DHb) for patients with and without posttreatment hemolysis after treatment with oral artemisinin-based combination therapy for uncomplicated *Plasmodium falciparum* malaria. A) Day (d) 0 to d 14 (overall); B) d 0 to d 3 (treatment period); C) d 3 to d 14 (posttreatment period). Horizontal lines indicate median values, boxes indicate interquartile ranges, whiskers indicate ranges, and solid squares and circles indicate individual patient data points. The Mann-Whitney U test was used for comparative analysis. *p<0.05; †p<0.01; NS, not significant.

(<0.3 g/L) and an increased reticulocyte count 8 weeks after treatment on day 56 (Figure 2, panel B).

Immunohematologic testing showed that serum samples from 5 (25%) of 20 patients were reactive only with enzyme-treated erythrocytes after therapy (3 patients with and 2 patients without posttreatment hemolysis). The direct antiglobulin test result was weakly positive for 3 patients; none of them had posttreatment hemolysis. None of the patients with hemolysis showed coating of erythrocytes with IgG, IgM, or C3d.

Discussion

Hemolytic anemia after treatment of severe malaria with intravenous artesunate has been described in malaria-endemic and non–malaria-endemic countries. However, evidence of hemolytic anemia after treatment of malaria with oral ACTs is limited to 2 case reports. Data from the current prospective study confirm our hypothesis that delayed posttreatment hemolysis also occurs after oral artemisinin treatment and provide insight into its frequency and clinical course. In 40% of the patients in our study with uncomplicated malaria and oral ACT treatment, laboratory signs of hemolysis were detected 2 weeks after therapy. In 5 patients, hemolysis persisted 1 month after treatment. Patients with posttreatment hemolysis showed a larger decrease in Hb levels after treatment than did patients without hemolysis. The intensity of hemolysis was mild compared with that after intravenous artesunate. In many reported cases of PADH after intravenous artesunate, patients received blood transfusions (*2,9*). In other studies, patients with hemolysis after oral ACT treatment had decreases in Hb levels of 2.1 g/dL–3.6 g/dL in the posttreatment period (*11–13*).

The decrease in Hb levels during treatment in the current study was smaller in patients with posttreatment hemolysis than in patients without posttreatment hemolysis. Consistent with this finding, we found that the patient group with the largest decrease in Hb levels after treatment (i.e., patients with uncompensated hemolysis) showed a small increase in Hb levels during treatment (Figure 2). This observation could be explained by involvement of once-infected erythrocytes: during treatment, erythrocytes are spared by removal of parasites without destruction of the cell. The Hb level therefore remains stable. After treatment,

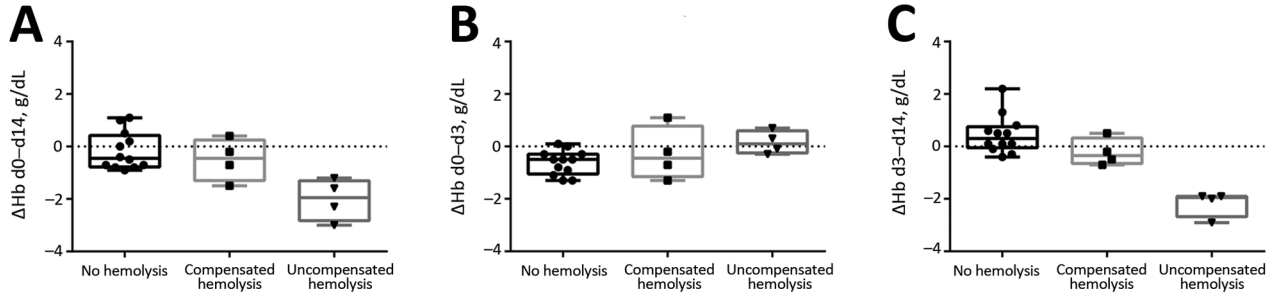

Figure 2. Changes in hemoglobin levels (DHb) for patients without posttreatment hemolysis, with compensated posttreatment hemolysis, and with uncompensated posttreatment hemolysis after treatment with oral artemisinin-based combination therapy for uncomplicated *Plasmodium falciparum* malaria. A) day (d) 0 to d 14 (overall); B) d 0 to d 3 (treatment period); C) d 3 to d 14 (posttreatment period). Horizontal lines indicate median values, boxes indicate interquartile ranges, whiskers indicate ranges, and solid squares, circles, and triangles indicate individual patient data points.

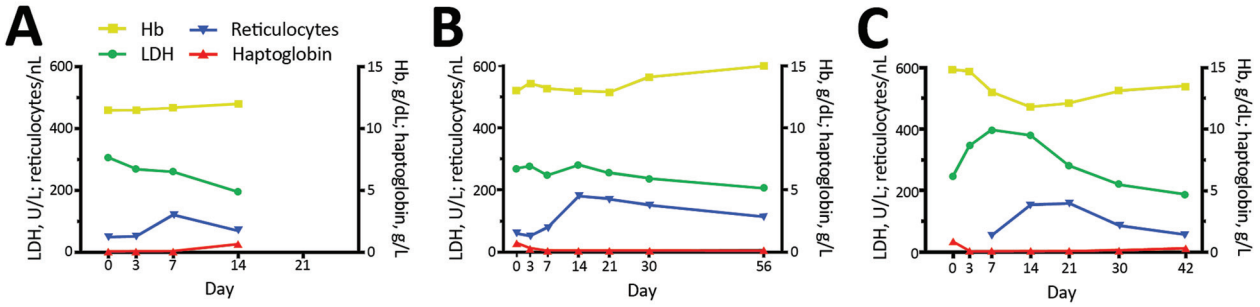

Figure 3. Laboratory values over time for exemplary patients with and without posttreatment hemolysis after treatment with oral artemisinin-based combination therapy for uncomplicated *Plasmodium falciparum* malaria. A) Patient without posttreatment hemolysis, B) patient with compensated posttreatment hemolysis, and C) patient with uncompensated posttreatment hemolysis. Hb, hemoglobin; LDH, lactate dehydrogenase.

the once-infected, pitted erythrocytes hemolyze because of their shorter life spans, which results in a postponed loss of Hb during the posttreatment period (*4*). Our data therefore give additional support to the relevance of this mechanism as a cause of late hemolysis.

Half of the patients with posttreatment hemolysis showed erythropoietic activity at day 14 that was sufficient for compensating the postponed loss of Hb. These patients were all of African origin, unlike those with uncompensated hemolysis, who were all Caucasian. The reason for this observation is unknown. Malaria-related dyserythropoiesis (*14*) might be less pronounced in African patients than in European patients. Impairment of erythropoiesis by artemisinins has been described in vitro (*15*), but no differences regarding ethnicity have been reported.

Different reported clinical courses of delayed hemolysis after artemisinin therapy suggest involvement of mechanisms other than pitting (*4,16*). In some patients, the decrease in Hb level far exceeds the loss of erythrocytes expected from destruction of once-infected erythrocytes (*16*). In a recent case report, drug-dependent autoimmune hemolysis was reported as a probable cause of PADH (*8*). Several other reports failed to demonstrate immune-mediated hemolysis or drug-induced antibodies in patients with severe malaria (*2,9,17*). In our patients, results from immunohematologic testing were inconclusive. No antibody or complement coating of erythrocytes was found that could trigger bystander hemolysis of uninfected erythrocytes.

Baseline Hb levels were comparatively high in our patients. The mild loss of Hb therefore did not result in clinical symptoms. However, in settings in which chronic anemia is common because of concomitant infections and nutritional deficiencies, posttreatment hemolysis after antimalarial treatment might be a clinically relevant factor. Recently, a large study in Nigeria reported a >5% decrease in hematocrit levels in 23% of African children with uncomplicated malaria 14–28 days after ACT treatment (*18*). Although no further assessments were performed in this study, the authors assumed an association with postartemisinin hemolysis. Further prospective investigations of this phenomenon in malaria-endemic areas are needed and should include markers for detection of hemolysis.

The dataset used for this analysis has several limitations. The most relevant limitation is that this study has, so far, not included patients who are receiving oral antimalarial drugs other than ACTs. At this time, we cannot rule out that similar hemolytic reactions occur after non-ACT antimalarial treatment because no prospective studies are available with a comparable method to detect hemolysis. More data on posttreatment anemia, hemolysis, and erythropoiesis after non-ACT treatment are therefore needed for comparison. However, this analysis also included patients with no evidence of posttreatment hemolysis after malaria and ACT therapy; it is therefore unlikely that hemolysis generally occurs after antimalarial therapy. The main objective of this study was to prospectively collect evidence for posttreatment hemolysis after oral ACT treatment. Some uncertainty might arise from the limited number of patients regarding other conclusions, such as different reticulocyte responses in patients from Africa and Europe. These conclusions have to be confirmed with larger sample sizes.

In conclusion, our study provides evidence that a mild form of posttreatment hemolysis commonly occurs after oral ACT treatment for uncomplicated malaria. The role of this observation for clinical practice in malaria-endemic and non–malaria-endemic settings remains to be defined but should prompt increased vigilance for hemolytic events, particularly for patients with preexisting anemia or those for whom mild anemia constitutes a clinical problem. Larger studies are needed to investigate observations and hypotheses concerning underlying pathophysiology and to eventually identify potential risk factors.

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2.5. Effektivität, Sicherheit und Pharmakokinetik der Behandlung der importierten unkomplizierten Malaria tropica mit Artemether/Lumefantrin, einer neuen Artemisinin-Kombinationstherapie

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Chinin und Mefloquin waren über viele Jahrzehnte die Standardtherapien für die Malaria tropica sowohl in endemischen wie auch in nicht-endemischen Ländern. Diese Therapien waren zum einen durch schlechte Verträglichkeit hinsichtlich Herzrhythmusstörungen, Chinonismus und neuropsychiatrische unerwünschte Wirkungen gekennzeichnet. Andererseits wurden – nachdem aufgrund von Resistenzen die Therapie mit Chloroquin bei der Malaria tropica bereits weitgehend wirkungslos geworden war – in Südostasien Resistenzen gegen Mefloquin beschrieben(9), sodass von einer Ausbreitung dieser Resistenz auch nach Afrika auszugehen war. Nachdem aus Südostasien(14,27–29) und Afrika (15–17) zahlreiche Studien zur Effektivität und Sicherheit von Artemisinin-Kombinationstherapien veröffentlicht worden waren, sollte erstmals Artemether-Lumefantrin als eine moderne ACT bei Patienten in Europa evaluiert werden. Artemether ist ein nicht-wasserlösliches Artemisininderivat und Lumefantrin eine antiparasitär wirkende Substanz mit langer Halbwertszeit (bis zu 24 Wochen im Blut nachweisbar); diese soll Rekrudeszenzen nach Gabe von Artemisininen mit schneller Metabolisierung verhindern.
Es wurde eine prospektive, nicht-randomisierte Studie an 15 Zentren in Europa durchgeführt; wegen geringerer Rekrutierung als erwartet wurde nach Änderung des Studienprotokolls noch ein Zentrum in Kolumbien in einem Gebiet mit nicht-endemischer Malaria hinzugefügt.
Es wurden 165 Patienten eingeschlossen. 118 stammten aus Europa und 47 aus Kolumbien. Artemether-Lumefantrin führte zur Parasitenfreiheit in 98,4% der Fälle am Tag

7 sowie 96% an Tag 28. Die mediane Zeit bis zur Parasitenfreiheit betrug 41,5 Stunden, und die Zeit bis zur Fieberfreiheit 36,8 Stunden. Ab Tag 7 waren bei allen Patienten keine Gametozyten mehr nachweisbar. Es wurden keine lebensbedrohlichen oder tödlichen unerwünschten Ereignisse registriert; typische unerwünschte Wirkungen waren Kopfschmerzen, Schlaflosigkeit und Durchfall. Es konnten keine klinisch relevanten QT-Verlängerungen beobachtet werden. Die pharmakokinetische Untersuchung ergab eine Nachweisbarkeit von Lumefantrin bis zu 168 Stunden nach erster Einnahme. In einem Fall konnte eine PCR-bestätigte Rekrudeszenz gesichert werden; diese war mit einem sehr niedrigen Lumefantrin-Spiegel verknüpft.

Diese Studie war die bislang umfassendste prospektive Studie zur medikamentösen Behandlung der importierten Malaria tropica in nicht-endemischen Gebieten. Sie zeigte, dass Artemether-Lumefantrin als neues ACT sehr effektiv und sicher für die Behandlung dieser Patientengruppe angewendet werden kann. Vorteilhaft sind insbesondere die schnelle Parasitenfreiheit, was eine frühere Entlassung aus stationärer Behandlung ermöglicht. Im Vergleich zu den bisher verfügbaren Therapieoptionen Chinin und Mefloquin stellt die gute Verträglichkeit von Artemether-Lumefantrin ebenfalls einen deutlichen Fortschritt dar.

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Treatment of Acute Uncomplicated Falciparum Malaria with Artemether-Lumefantrine in Non-immune Populations: A Safety, Efficacy, and Pharmacokinetic Study

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Abstract. The efficacy and safety of artemether-lumefantrine for the treatment of malaria in non-immune populations are not well defined. In this study, 165 non-immune patients from Europe and non-malarious areas of Colombia with acute, uncomplicated falciparum malaria or mixed infection including *P. falciparum* were treated with the six-dose regimen of artemether-lumefantrine. The parasitologic cure rate at 28 days was 96.0% for the per protocol population (119/124 patients). Median times to parasite clearance and fever clearance were 41.5 and 36.8 hours, respectively. No patient had gametocytes after Day 7. Treatment was well tolerated; most adverse events were mild to moderate and seemed to be related to malaria. There were few serious adverse events, none of which were considered to be drug-related. No significant effects on ECG or laboratory parameters were observed. In conclusion, the six-dose regimen of artemether-lumefantrine was effective and well tolerated in the treatment of acute uncomplicated falciparum malaria in non-immune patients.

INTRODUCTION

The vast majority of cases of malaria occur in endemic countries in Africa, Asia, and Latin America. Imported malaria, however, remains a significant problem in industrialized countries and in non-malarious areas of endemic countries. Travelers from such regions lack the partial immunity to malaria that is considered to develop in residents of endemic areas after repeated infections. The non-immune individuals fall into two broad categories: those who, by virtue of being native to a non-endemic area, have never been exposed to malaria, and those who may originally have lived in endemic areas but subsequently have settled in non-malarious areas and have lost the partial immunity they previously had. Immigrants from endemic areas who have become resident in non-malarious areas are an important group because they may visit endemic areas for professional or personal reasons.[1]

The authors are aware of the fact that there is no standard definition of non-immune status. Travel to malaria-endemic areas places non-immune individuals at risk of infection with *Plasmodium falciparum* and of complications of malaria. This is particularly the case where chemoprophylaxis is not used or is ineffective. As a result, a significant number of patients return from travel with imported malaria. It has been estimated that ~16,000 cases of imported malaria occur in Europe each year.[2] Deaths from falciparum malaria also occur; for example, in the United Kingdom, > 2,000 cases of imported malaria are reported annually, with an average of 9 cases being fatal.[3]

A number of treatments are currently used in cases of imported malaria in industrialized countries. A survey in France in 2001[2] found that, even for uncomplicated falciparum malaria, intravenously quinine was the most commonly used treatment in 41% of cases. Mefloquine (in 18% of cases) and atovaquone-proguanil (Malarone), used in 14% of cases, were the next most commonly used therapies. Quinine and mefloquine have both been associated with potentially severe side effects. Quinine causes highly unpleasant adverse effects, and mefloquine is associated with severe neuropsychological problems,[4] especially when used at a curative dose. Discontinuation because of adverse reactions has been reported in 11% of patients receiving mefloquine for the treatment of imported uncomplicated falciparum malaria in France.[2] Atovaquone-proguanil, whereas effective and well tolerated as prophylaxis and in the treatment of falciparum malaria in endemic countries, has been relatively little studied in the treatment (rather than prophylaxis) of malaria in non-immune travelers, and there are few data on its effectiveness against other *Plasmodium* species.[5]

Against this background, there remains a need for additional effective, well-tolerated treatments for imported uncomplicated falciparum malaria. Artemether-lumefantrine (co-artemether) is the first artemisinin-based combination therapy registered in industrialized countries. This fixed combination treatment has been extensively studied in endemic countries, mainly in Southeast Asia[6–9] and sub-Saharan Africa,[10–13] and the six-dose regimen has been shown to be associated with high parasitologic cure rates and rapid clearance of parasites and resolution of fever in these settings. However, experience with the six-dose regimen of artemether-lumefantrine in non-immune patients is limited. Here we report the results of a multicenter, open-label, non-comparative study in which adult non-immune travelers with imported uncomplicated falciparum malaria (or mixed infections including *P. falciparum*) were treated with the six-dose regimen of artemether-lumefantrine.

MATERIALS AND METHODS

The study protocol and amendments were approved by the ethics committees of all participating institutions, and all pa-

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tients gave written informed consent before participating in the study. Patients were eligible to enter the study if they were at least 18 years of age and had microscopically confirmed acute uncomplicated *Plasmodium falciparum* malaria or mixed infections including *P. falciparum*. Patients who had received prior antimalarial prophylaxis (but not artemisinin derivatives within the previous 7 days) could enter the study. In the initial inclusion criteria for the study, it was simply stated that patients had to be non-immune. After a protocol amendment (implemented after 80 of the 165 patients had been recruited), a precise definition of non-immune status was introduced (i.e., not having spent either the first 5 years of their life or long periods during the 5 years before the study in a malaria-endemic area and not having had acute *P. falciparum* malaria diagnosed during the past 5 years).

Key exclusion criteria were signs/symptoms indicative of severe/complicated malaria (as defined by the World Health Organization[14]); known hypersensitivity to the study medication; having received artemisinin derivatives within the previous 7 days; and concurrent use of other treatment/prophylaxis for malaria.

Methods. This was an open-label, non-comparative study performed at 16 centers in Europe and Colombia. The single center in Colombia was in Villavicencio, in an area without endemic malaria. This center had a history of treating non-immune patients, typically patients from non-endemic areas who had to move to endemic areas either in search of employment or as a result of conflict.[15] It was included in the study after a protocol amendment (the original protocol included only European centers) to accelerate recruitment of patients, which before the amendment had been slower than anticipated.

Treatment. Doses of 80 mg artemether and 480 mg lumefantrine (four tablets, each containing 20 mg artemether and 120 mg lumefantrine) were given on diagnosis and at 8, 24, 36, 48, and 60 hours. Patients were followed up for 28 days after diagnosis. Most patients took their medication with fat-containing food.

Efficacy evaluations. Efficacy was assessed in terms of parasitologic cure rates at 7 and 28 days, time to fever clearance, time to parasite clearance, and the proportion of patients with *P. falciparum* gametocytes at each evaluation on days 0, 1, 2, 3, 7, and 28. Confidence intervals on proportions were calculated using the exact Pearson-Clopper method. Time to fever and parasite clearance were analyzed using Kaplan-Meier estimation (with appropriate censoring for patients lost to follow-up). The original sample size was chosen on the basis that, assuming at most a 10% recrudescence rate and requiring a precision of ± 5% (95% CI), ~140 patients would be needed.

Pharmacokinetics. It was planned for one 2-mL blood sample to be collected from all patients on Day 3, 4–10 hours after the last dose of study medication, for determination of lumefantrine and desbutyl-lumefantrine. In addition, it was planned to take a blood sample from any patient with treatment failure to assess drug levels. The blood sample was drawn by venipuncture into a heparinized tube. Blood was centrifuged without delay at 1,000 rpm for 15 minutes, and the plasma was transferred in polypropylene tubes and stored at −70°C until shipment for analysis. Samples were shipped to the analytical center packed with dry ice. In practice, samples were available for 27 patients, and only 1 patient with treatment failure had a sample for determination of lumefantrine and desbutyl-lumefantrine.

A subset of 15 patients (recruited at the Colombian center after closure of the main study) had more extensive blood sampling for pharmacokinetic analysis. In these patients, samples were taken pre-dose and 2–4 hours after study medication doses 2, 3, 4, and 5 on Day 3 (72 hours after first dose), Day 4 (96 hours after first dose), and on Day 7 (168 hours after first dose). Lumefantrine and desbutyl-lumefantrine plasma concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods at Novartis Pharma, BAPK-F, Rueil-Malmaison, France. These methods were developed by the sponsor and were fully validated. The lower limit of quantitation (LLOQ) of the methods was 50 ng/mL for lumefantrine and 5 ng/mL for desbutyl-lumefantrine. Pharmacokinetic parameters (derived from extensive samplings in the subset of 15 patients) were determined using model-independent methods (WinNonlin Pro., Version 4.0.1; Pharsight Corp., Mountain View, CA).

Safety assessments. Safety was assessed in terms of adverse events and laboratory parameters. Adverse events were summarized in terms of all adverse events occurring after baseline and in terms of treatment-emergent signs and symptoms (TESS; i.e. adverse events occurring after baseline but before re-appearance of asexual parasites in the blood).

Blood samples to assess clinical laboratory parameters (hematology: hematocrit; hemoglobin; red blood cell count; white blood cell count; platelet count; and biochemistry: glucose; bilirubin; creatinine [serum]; ALT [SGPT]; and alkaline phosphatase) were taken at baseline and Day 28. Laboratory values were analyzed in terms of summary statistics for changes from baseline, shift tables based on the normal ranges, and shift tables based on National Cancer Institute Common Toxicity Criteria (NCI CTC) where these were available.

At baseline and Days 1, 2, and 3 (e.g., 6–10 hours after last dose), a standard 12-lead ECG (25 mm/s) was recorded followed by a tracing for rhythm evaluation using standard ECG recording equipment. The ECGs were analyzed qualitatively and quantitatively including measurements of the PQ- and QT-interval and the duration of the QRS-complex. QTc-interval was calculated by using the formulae of Bazett[16] and Fridericia.[17] ECGs were reviewed in a blinded manner by cardiologists at a Clinical Research Organization (eResearch Technology, Philadelphia, PA) to guarantee the quality and homogeneity of the interpretation of these data.

RESULTS

Patient population. A total of 165 patients entered the study, 118 from Europe and 47 from Colombia, of whom 154 completed the 3-day treatment period (and received the full course of treatment, although 3 of these patients took a replacement dose after vomiting the first dose) and 135 completed the study. The most common reasons for discontinuation were loss to follow-up and protocol violations, and the most common major protocol violation being incomplete documentation of parasite counts up to Day 7 (19 patients) and on Day 28 after parasite clearance on Day 7 (14 patients).

Demographic and disease characteristics at baseline are shown in Table 1. Patients were predominantly men (69%) and young to middle-aged (median, 37 years). Median body

TABLE 1
Baseline characteristics (all treated patients)

Variable	Statistic	Treated patients (N = 165)
Age (years)	Mean (± SD)	37.7 (± 12.44)
	Median (range)	37.0 (17–66)
Sex—*n* (%) patients	Male	113 (68.5)
	Female	52 (31.5)
Race—*n* (%) patients	White	80 (48.5)
	Black	40 (24.2)
	Other	45 (27.3)
Body weight (kg)[a]	Mean (± SD)	72.9 (± 13.72)
	Median (range)	73.0 (41–119)
Body weight categories—*n* (%) patients	≤ 65 kg	55 (33.3)
	> 65 kg	107 (64.8)
Parasitological diagnosis, *n* (%) patients	*P. falciparum*	162 (98.2)
	Other species	8 (4.8)
	P. vivax	2 (1.2)
	P. malariae	6 (3.6)
Parasite density per 1,000 red cells†	Mean ± SD	6.2 ± 9.45
	Median (range)	2.4 (0–70)
Body temperature (°C)	Mean (± SD)	38.1 ± 1.29
	Median (range)	38.0 (35.1–40.7)
Time since diagnosis (days) at study entry	Distribution, *n* (%) patients	
	1 day	150 (90.9)
	2 days	14 (8.5)
	≥ 3 days	1 (0.6)
Time since return from most recent travel (days)	Mean ± SD	15.2 (± 20.77)
	Median (range)	12.0 (1–240)

* Body weight was not reported for three patients.
† For 51 (30.9%) patients, parasite density was reported in other units, and is not included in the statistics given here.

TABLE 2
Efficacy results (per protocol population)

Parameter statistic	PP population (N = 126)
28-day parasitologic cure rate n/M (%) patients (95% CI*)	119/124 (96.0 [90.8, 98.7])
7-day parasitological cure rate n/M (%) patients (95% CI*)	123/125 (98.4 [98.4 94.3, 99.8])
Time to parasite clearance (hours) Median [95% CI]	41.5 [40.0 42.6]
Time to fever clearance (hours) Median [95% CI]	36.8 [24.5, 40.0]
n (%) patients with parasite clearance by time after initiation of therapy	
PCT ≥ 24 h	30 (23.8)
PCT > 24–≤ 48 h	62 (49.2)
PCT > 48 h	32 (25.4)
Parasite clearance not achieved	2 (1.6)
n/M (%) patients with gametocytes by time window	
Days 0–3†	26/126 (20.6)
Day 4 to day 7‡	7/113 (6.2)
Day 8 to day 42‡	0/122 (0.0)

* Using Pearson Clopper limits.
† Percentage of patients with at least one positive slide between baseline and 72 hours after start of treatment.
‡ Percentage of patients with positive slide at the weekly follow-up visits evaluated by time window.
For time to parasite clearance and time to fever clearance, patients who had no or parasites or fever (as appropriate) at baseline were censored at the time-point hour = 0.
M = number of patients with observations.

weight was 73.0 kg (range, 41–119 kg), with 65% of patients weighing > 65 kg (including 20 patients, 12.1%, who weighed > 90 kg). Most patients had pure falciparum malaria, with only 5% having mixed infections. Other *Plasmodium* species detected on admission were *P. vivax* (in two patients) and *P. malariae* (in six patients), but were not found subsequently. In another three patients, *P. vivax* was first detected at their final evaluation (Day 28, Day 13, and Day 15).

Parasite density was generally relatively low, with a mean of 6.2 asexual forms per 1,000 erythrocytes. Median body temperature was 38.0°C. More than 30% of patients had a baseline temperate of at least 39°C, and 37% were afebrile (body temperature < 37.5°C). More than 90% of patients entered the study within 1 day of diagnosis. Median time since return from most recent travel (as reported by the patients) was 12.0 days, but the range was very wide (1–240 days). Most of the patients from the European centers had recently returned from traveling in sub-Saharan Africa (most commonly Ghana, Cameroon, or Kenya, but including a wide range of countries). All patients at the Colombian center had traveled in other parts of Colombia. Of the 80 patients enrolled before the protocol amendment, 20% had used antimalarial prophylaxis within 7 days of study entry. Of the 85 patients recruited once the protocol amendment was in force, 17.6% had used antimalarial prophylaxis within 4 weeks of study entry. The sub-analyses of various subgroupings of the study cohort, especially with regard to the Colombian patients, did not reveal any difference.

Efficacy. Efficacy results are shown in Table 2. Results are presented for the per protocol (PP) population (defined as all patients who completed the study and did not have major protocol violations). It was originally planned to analyze efficacy primarily on the intention-to-treat (ITT) population (all patients with confirmed malaria who received at least one dose of study drug). The pre-specified ITT analysis, however, counted patients who had incomplete documentation of parasite counts after parasite clearance as treatment failures. An unexpectedly high proportion of patients in the study had incomplete documentation of parasite counts after parasite clearance, most commonly because of discontinuation before the Day 28 visit. This would have led to an underestimation of the parasitologic cure rate in the ITT population. The PP population was therefore considered to provide the most relevant parasitologic cure rates.

At 7 days, the parasitologic cure rate was 98.4%, with Pearson-Clopper 95% CIs of 94.3%, 99.8%. Of the two patients who were not cured at Day 7, one did not clear parasites until Day 10, and the other was withdrawn from the study because of unsatisfactory therapeutic effect after the second dose of study medication. This latter patient was successfully treated with quinine and doxycycline.

The parasitologic cure rate at 28 days was 96.0% (95% CI, 90.8%, 98.7%). In addition to the two patients who did not clear parasites by Day 7, three patients had late reappearance of parasites (at Days 22, 24, and 28). All three of these patients were treated successfully with atovaquone-proguanil (Malarone), and one patient also received chloroquine. All three patients had been infected in sub-Saharan Africa, but in different countries: Kenya, the Gambia, and either Rwanda or Angola.

Subgroup analysis according to body weight category in the PP population showed 28-day parasitologic cure rates of 100% (95% CI, 92.5%, 100.0%) in patients of body weight ≤ 65 kg and 93.4% (95% CI, 85.3%, 97.8%) in patients weighing > 65 kg. The median weight of the five patients with treatment failure was 85 (range, 73–97 kg) versus 70.5 kg (range, 47–115 kg) in the 119 patients who were successfully cured.

The effects of age on treatment outcome were not examined by subgroup analysis (there were very few patients > 60 years of age in the study); the patients in the PP population who had treatment failure ranged between 21 and 62 years of age.

Of the three patients who vomited and replaced doses of artemether-lumefantrine, two were cured at Day 28, and the other was discontinued from the study because of a protocol violation (incomplete documentation of parasite counts after clearance). This latter patient also violated the protocol by only taking three doses of study medication.

Median times to parasite clearance and fever clearance were 41.5 (95% CI, 40.0, 42.6) and 36.8 hours (95% CI, 24.5, 40.0), respectively. Between baseline and Day 3, > 20% of the patients had *P. falciparum* gametocytes. No patient had gametocytes after Day 7.

Safety and tolerability. There were no deaths or life-threatening adverse events (AEs) during the study. Six patients had AEs that needed prolonged hospitalization. In three of the patients, the AEs were primarily related to malaria (in two cases to the severity of the signs/symptoms of the initial infection and in the other to recrudescence). The pattern of AEs in two of the remaining three patients (anemia, thrombocytopenia, liver function test abnormalities, hematuria, malaise, and abdominal pain in one patient and hepatocellular damage in the other) also suggests an association with malaria, particularly with one patient who discontinued artemether-lumefantrine treatment after the second dose and received intravenously quinine as antimalarial rescue medication from Day 0 to Day 3 because of complications of malaria. The endocarditis reported in one patient seems to have been a coincidental infection. None of the three patients, including one with reported progression of malaria, who had either hepatocellular damage or liver function test (LFT) abnormalities had serum transaminase levels greater than NCI CTC Grade 2. One patient had NCI CTC Grade 3 serum bilirubin levels at baseline, but at Day 28, this had decreased to NCI CTC Grade 1. Resolution of all of the AEs that needed prolonged hospitalization was reported, with the exception of the malaria (reported on Day 22) in one patient, for which no further information was available.

The most common AEs (those reported in at least 5% of patients) are shown in Table 3. In total, 75% of patients reported at least one adverse event. The most frequent AEs reported were headache, insomnia, diarrhea, vertigo, malaise, and cough. Most of the common AEs, such as headache, malaise, and gastrointestinal disturbances such as diarrhea, nausea and vomiting, together with anorexia and vertigo and chills, were probably related to signs and symptoms of malaria. The proportions of patients experiencing common AEs that were suspected by the investigators to be related to study treatment are also shown in Table 3. It can be seen that insomnia was the most frequently reported adverse event considered to be treatment-related. For the other common adverse events, only a small proportion were considered to be treatment-related. No allergic reactions were reported in any of the patients.

The overall profile of changes from baseline in laboratory parameters (hematology and biochemistry) observed was consistent with the resolution of acute uncomplicated malaria. Both hematology and biochemistry values tended to normalize over the course of the study. None of the patients had shifts from baseline to NCI CTC Grade 3 or 4 for any parameter. Four patients had anemia reported as an AE, one of whom also had reported microcytic anemia and thrombocytopenia. In all of these patients, the hemoglobin level, hematocrit, and platelet count were all within NCI CTC grade 0 by Day 28. Also, nine patients had liver function test abnormalities (elevated transaminases and/or bilirubin) reported as AEs. In most cases, the AEs were reported between the two scheduled sampling times for laboratory analysis. In all but three cases, all LFT values were within the normal range by Day 28. In the remaining cases, SGPT was elevated outside the normal range, to NCI CTC Grade 1 (i.e., up to 2.5 times upper limit of normal [ULN]); in one case, this was a slight worsening from the baseline value.

ECG evaluations revealed only very small and clinically irrelevant changes in mean and median QTc, as calculated using either Bazett's or Fridericia's formula. The proportions of patients with predefined QTc signal values are shown in Table 4. The majority of patients had QTc increases from baseline of < 30 ms. There were no patients with absolute QTc values (using either formula) of > 500 ms, and none of the patients had QTc increases from baseline of > 60 ms according to both formulae. One patient had an increase from baseline of > 60 ms in QTc interval calculated using Bazett's formula from 393 ms at baseline to 456 ms at Day 2. Two

TABLE 3

Most frequently reported adverse events (those occurring in at least 5% of patients)

	n (%) with AEs (*N* = 165)	
	All AEs	Drug-related AEs
Total no. of patients with AEs	124 (75.2)	48 (29.1)
Adverse events[a]		
Headache	48 (29.1)	6 (3.6)
Insomnia	22 (13.3)	11 (6.7)
Diarrhoea	22 (13.3)	5 (3.0)
Vertigo	21 (12.7)	6 (3.6)
Malaise	19 (11.5)	2 (1.2)
Cough	18 (10.9)	2 (1.2)
Anorexia	17 (10.3)	4 (2.4)
Vomiting	14 (8.5)	6 (3.6)
Asthenia	13 (7.9)	1 (0.6)
Nausea	11 (6.7)	5 (3.0)
Chills	11 (6.7)	4 (2.4)
Hyperhidrosis	10 (6.1)	3 (1.8)
Abdominal pain	10 (6.1)	2 (1.2)

* MedDRA preferred term, presented by descending order of overall frequency.

TABLE 4

Number (%) of patients with signal QTc values or signal QTc increases from baseline

	QTc increases from baseline to highest post-baseline value [*n* (%) patients] (*N* = 147)	
QTc increase (ms)	Bazett's formula	Fridericia's formula
≤ 0	38 (25.9)	25 (17.0)
> 0–< 30	62 (42.2)	56 (38.1)
30–60	16 (10.9)	34 (23.1)
> 60	1 (0.7)	2 (1.4)
Baseline ECG not done	30 (20.4)	30 (20.4)

Percentages are calculated using the total number of patients with post-baseline visit day 3 as denominator.

Bazett's formula: QTc = QT/(RR × ⅓). Fridericia's formula: QTc = QT/(RR × 1/3).

TABLE 5
Pharmacokinetic variables for lumefantrine and desbutyl-lumefantrine

Variable	Lumefantrine	Desbutyl-lumefantrine
C_{max} (μg/mL) – mean ± SD	5.72 ± 2.91	0.0193 ± 0.0079
(CV%)	(50.8%)	(40.7%)
t_{max} (h) – median [range]	52.42 [12.08–93.50]	62.67 [50.00–93.50]
$AUC_{(0-t)}$ (μg · h/mL) – mean ± SD	272 ± 159	0.905 ± 0.738
(CV%)	(58.4%)	(81.5%)
AUC_{∞} (μg · h/mL) – mean ± SD	335 ± 196*	NA
(CV%)	(58.5%)	

* $N = 8$.
NA, not available.

patients had an increase from baseline of > 60 ms in QTc interval calculated using Fridericia's formula. One patient had an increase of 71 ms from baseline to Day 2 (from 331 to 402 ms). The only AE reported by this patient was mild vertigo from Day 2 to Day 3. The other had a QTc increase of 64 ms from 401 ms to 465 ms at Day 1. Days 2 and 3 QTc values were both within the normal range, at 420 and 427 ms, respectively. This patient reported mild vertigo on Day 1 but otherwise experienced no AEs.

Pharmacokinetics. Lumefantrine concentrations could be determined from single samples in 27 patients (body weight range, 49–115 kg) drawn between 5:80 AM and 11:00 AM after the last dose of treatment. Concentrations ranged from 0.457 to 17.6 μg/mL. Median value for patients sampled at similar time-points (i.e., between 5.50 and 6.92 hours, likely to reflect time at which C_{max} occurs) was 5.58 μg/mL (mean ± SD: 6.55 ± 4.81 μg/mL). Descriptive and linear regression analyses both suggested that exposure was not directly related to body weight (e.g., the patient with the highest body weight [115 kg] showed a concentration of 5.59 μg/mL 6 hours after dose). The linear regression showed only a very weak negative correlation ($R^2 = 0.09$, $P = 0.20$) between log-transformed lumefantrine concentrations and body weights. Lumefantrine concentration was available for one patient who had treatment failure. This was the patient described above with polymerase chain reaction–confirmed recrudescence at Day 28. This patient had the lowest lumefantrine plasma concentration observed (0.457 μg/mL, at 5.5 hours, expected to be close to t_{max}), which was indicative of a very low absorption of the drug in this patient. His body weight was 85 kg. Because lumefantrine concentration was only available for one patient with treatment failure, no meaningful evaluation of any correlation between parasitologic cure and lumefantrine levels was possible.

For the 15 patients who provided more detailed pharmacokinetic data, plasma concentration-time profiles for lumefantrine and its metabolite desbutyl-lumefantrine are shown in Figure 1. Pharmacokinetic parameters for both compounds are shown in Table 5. The metabolite-to-parent ratio was 0.36% ± 0.15% (C_{max}) and 0.33% ± 0.19% ($AUC_{(0-t)}$). Linear regression analyses of log-transformed pharmacokinetic parameters (C_{max} and $AUC_{(0-t)}$) against body weight did not show any significant relationship between body weight and the pharmacokinetic parameters ($R^2 = 0.22$, $P = 0.078$ for C_{max} and $R^2 = 0.13$, $P = 0.182$ for $AUC_{(0-t)}$ for lumefantrine and $R^2 = 0.08$, $P = 0.303$ and $R^2 = 0.002$, $P = 0.866$, respectively, for desbutyl-lumefantrine). All 15 patients achieved parasite clearance without recrudescence and with no gametocytes present at end of study. This was despite a large range of individual exposure levels to lumefantrine (C_{max} from 2.12 to 11.2 μg/mL and $AUC_{(0-t)}$ from 98.8 to 761 μg · h/mL) and desbutyl-lumefantrine (C_{max} from 0.006 to 0.034 μg/mL and $AUC_{(0-t)}$ from 0.276 to 2.76 μg · h/mL). Therefore, no relationship between drug exposure levels and parasite clearance could be established. Neither descriptive analysis nor linear regression analyses of QTc changes from baseline (calculated using either the Bazett or Fridericia formula) showed any significant relationship between the pharmacokinetic parameters and QTc changes from baseline.

DISCUSSION

This study, which included a total of 165 patients, is (to our knowledge) probably the largest prospective clinical trial performed to date on imported falciparum malaria in non-immune patients. Originally intended to be conducted in centers in Germany and Switzerland, slow recruitment (because of decreasing numbers of patients returning to these countries with imported malaria) led to the inclusion of centers in other countries through amendments to the protocol. The patient population described here were non-immune, defined as not having spent either the first 5 years of their life or for long periods during the 5 years before the study in a malaria-endemic area and not having had acute *P. falciparum* malaria diagnosed during the past 5 years. Whereas there are no malarious regions in Western Europe, the disease is endemic in

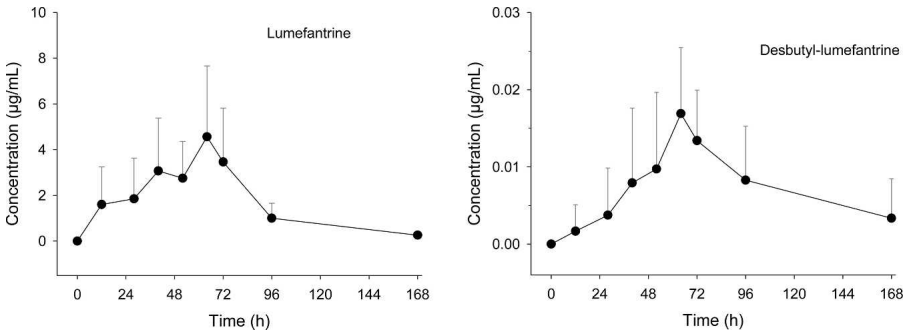

FIGURE 1. Mean ± SD plasma concentration--time profiles of lumefantrine and desbutyl-lumefantrine.

some areas of Colombia. The patients recruited at the Colombian center were from non-malarious areas of the country and had, at most, infrequent travel to malarious regions, in addition to satisfying the definition of non-immunity given above.

At baseline, patients tended to have relatively low parasite counts. There was a wide range of time between returning from travel and diagnosis of malaria; the median was 12 days, but one patient had apparently been infected for at least 240 days before diagnosis. The majority of infections were pure *P. falciparum*. Mixed infections with other species (*P. vivax* and *P. malariae*) were found in < 5% of patients. Data from Thailand suggest mixed infections (*P vivax* and *P. falciparum*) cause more anemia, but this fact does not seem relevant in the present context to influence the interpretation of our data.[18]

Most patients completed the study as planned, but there was a relatively high rate of premature discontinuation and of protocol violations. This was unexpected on the basis of studies with artemether-lumefantrine in malaria-endemic countries. Most patients who discontinued prematurely were lost to follow-up or did so as a result of protocol violations, specifically as incomplete documentation of parasite counts after initial clearance. It seems that a significant proportion of patients failed to return to the study centers once their malaria symptoms had resolved. This high rate of discontinuation and protocol violations led us to present the efficacy analysis based on the PP population, rather than the planned primary analysis based on an ITT population, providing the most representative parasitologic cure rate.

The original intention to conduct a study comparing the most commonly used drugs such as mefloquine, atovaquone/proguanil, and artemether/lumefantrine proved not to be feasible because of declining numbers of non-immune malaria patients in industrialized countries. In addition, it also became evident that atovaquone-proguanil was used extensively as a chemoprophylactic agent and was therefore less likely to be used as drug of choice for treatment.

Treatment was effective, with a high 28-day parasitologic cure rate. Two patients did not clear parasites by Day 7, and three patients had late re-appearance of parasites (at Days 22, 24, and 28). The observed 28-day parasitologic cure rate was comparable with those seen in trials in endemic countries (ranging from 94% to 97%, although analysis populations were not always defined as in this study),[6,9,10,12,13] suggesting that treatment is as effective in the non-immune population as in semi-immune patients. Gametocytes also appeared to be cleared rapidly.

The 28-day parasitologic cure rate was chosen as the primary efficacy endpoint on the basis of WHO recommendations in place at the time the study was designed. It is therefore possible that not all cases of recrudescence were detected, because those occurring later than Day 28 would have been missed. A recent publication reporting a comparative study of artemether-lumefantrine and artesunate plus amodiaquine performed in Zanzibar[12] found that the Day 28 and Day 42 parasitologic cure rates for artemether-lumefantrine (uncorrected for re-infection) were 93% and 77%, respectively. This difference was largely caused by re-infection: the adjusted cure rates after polymerase chain reaction analysis to identify new infections were 97% at Day 28 and 92% at Day 42. Re-infection should not be an issue in non-malarious areas, but a 42-day follow-up period would still have been preferable in this study to detect late recrudescence. In practice, however, a follow-up period of > 28 days would seem to be impracticable in the European setting, as shown by the proportion of patients who were lost to follow-up in this study.

Artemether-lumefantrine was well-tolerated, with most reported AEs appearing to be related to malaria. Laboratory evaluations were consistent with malaria and its resolution. ECG evaluations were performed because of the chemical similarity between lumefantrine and halofantrine, an antimalarial known to be associated with prolongation of the QTc interval.[8] The possibility of a cardiotoxic effect of lumefantrine has been extensively studied in *in vitro* and *in vivo* studies. These studies unequivocally showed that lumefantrine lacks the cardiotoxicity of halofantrine.[7,8,19] In this study, no significant effects on cardiac safety in terms of QTc interval were observed; changes in QTc interval and rates of QTc prolongation were low and consistent with those previously observed.

The pharmacokinetic data obtained in this study suggest that lumefantrine and desbutyl-lumefantrine concentrations are not strongly correlated with body weight. No clear relationship between lumefantrine or desbutyl-lumefantrine levels and either parasite clearance or changes in QTc interval were apparent. However, the higher median body weight in patients with treatment failures (although based on very few patients) highlights the need for additional data to ensure that the dose does not need to be adapted in overweight patients. No allergic reactions were seen, although these have been reported elsewhere.[20]

In conclusion, the six-dose regimen of artemether-lumefantrine is a good choice for treating acute uncomplicated falciparum malaria in non-immune patients, with a high efficacy, a rapid resolution of clinical symptoms, and a good tolerability. It may prove to be the most appropriate option when considering the range of treatments available in industrialized countries, particularly when some alternative therapies such as quinine and mefloquine are associated with tolerability problems. Another alternative, atovaquone-proguanil, although apparently well tolerated, has not been well studied in the treatment of malaria in the non-immune population,[21,22] but may be as effective as artemether-lumefantrine. The results of this study also underline the fact that all non-immune patients treated for malaria need to be informed about the possibility of re-appearance of parasite and clinical recrudescence for several weeks after treatment.

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3. Diskussion

Durch die vorliegende Arbeit konnten Beiträge zu relevanten Aspekten der importierten Malaria tropica geleistet werden. Schwerpunkte hierbei waren die Epidemiologie und die Behandlung mit neuen artemisinbasierten Medikamenten, die Erstbeschreibung der PADH sowohl bei der komplizierten wie auch bei der unkomplizierten Malaria, sowie auch erstmals die Erhebung und Darstellung der Informationen und Ergebnisse zentren- und länderübergreifend auf europäischer Ebene.

Hinsichtlich der Epidemiologie der komplizierten Malaria tropica waren bislang nur wenige, überwiegend aus einzelnen Zentren kommende Daten verfügbar. Die Mortalität der importierten Malaria tropica wird durch die komplizierte Malaria tropica bedingt und steigt mit dem Alter an. Es ist bekannt, daß sich bei Patienten über 60 bzw. 65 Jahren die Mortalität um den Faktor 6 bis 10 erhöhen kann(6,30). Hier konnte in europäischen Daten ergänzend gezeigt werden, dass die beiden häufigsten Komplikationen (cerebrale Malaria und akutes Nierenversagen) ebenfalls deutlich häufiger bei Patienten >60 Jahren sind(31). Es kann somit ein als sicher geltender Zusammenhang zwischen höherem Lebensalter einhergehend mit höherem Risiko der Entwicklung von Komplikationen der komplizierten Malaria und damit auch der Mortalität festgestellt werden. Pathophysiologisch ist hierbei möglicherweise die Alterung der Gefäße bzw. eine Gefäßsklerose in Zusammenhang mit der Sequestration von Parasiten und dadurch verursachte kapilläre Obstruktion und Inflammation in den jeweiligen Organen der für das Risiko bestimmende Faktor.

Die unterschiedlichen Gruppen von Reisenden konnten im Bezug auf das Risiko zur Entwicklung der komplizierten Malaria tropica in einem europäischen Datensatz aufgetrennt dargestellt werden; die mit Abstand meisten VFR-Reisenden kamen aus Westafrika, wohingegen Reisende europäischer Abstammung aus den touristisch häufig besuchten Regionen sowohl in West- wie auch in Ostafrika kamen. Diese Daten erlauben die gezielte Ansprache und Interventionen wie z.B. Aufklärung und Malaria-Prophylaxe in den Gruppen mit dem höchsten Risiko.

Wie eingangs beschrieben wurde bei Patienten in endemischen Gebieten in Südostasien(18) und Afrika(19) die Überlegenheit hinsichtlich der Mortalität bei Anwendung von intravenösem Artesunat zur Behandlung der komplizierten Malaria tropica bestätigt. Es ist bis heute jedoch ungeklärt, ob das dort beobachtete höhere Überleben der Patienten auch für Patienten, die in Europa oder den USA behandelt werden, ebenfalls zutrifft. Auch für die orale Behandlung der unkomplizierten Malaria tropica lagen keine ausreichenden Daten vor. Die weiteren Bemühungen zur klinischen Entwicklung von Artesunat und zur Schaffung einer legalen Grundlage zu dessen Anwendung müssen sich daher auf Klärung der Sicherheit in der Anwendung konzentrieren. Das Ziel bleibt eine Zulassung des Medikaments durch die europäische bzw. US-amerikanische Arzneimittelbehörde.

Erstmals wurde die Behandlung von Patienten mit intravenösem Artesunat in einer Fallserie an 9 Patienten aus Norwegen(25) mit guter Verträglichkeit dokumentiert. Im Anschluss daran wurde im Rahmen dieser Arbeit eine weitere Fallserie von Patienten aus verschiedenen europäischen Ländern veröffentlicht, bei welcher erstmals eine verzögert auftretende Hämolyse (PADH) als wesentliches Ereignis im Hinblick auf die Arzneimittelsicherheit beschrieben wurde (hierzu siehe auch folgender Abschnitt). Diese Beobachtung wurde anschließend in weiteren Fallserien(32,33) bestätigt. Abgesehen von der PADH war intravenöses Artesunat auch in einer größeren eigenen Studie im Rahmen des Netzwerkes TropNet im Vergleich zu Chinin sehr gut verträglich(31). Eine weitere prospektive Studie an 123 Patienten in Frankreich im Rahmen eines „Temporary Use Authorization“ Programmes ergab ebenfalls mit Ausnahme der Anämie ein sehr gutes Sicherheitsprofil(26). Zusammenfassend können neben der PADH bzw. der damit zusammenhängenden Anämie allergische Reaktionen, Hautreaktionen, Erhöhung der Leberwerte und ZNS-Nebenwirkungen im Sinne von Ataxie oder Tremor benannt werden(31,34). Alle unerwünschten Wirkungen lösen sich jedoch im Verlauf vollständig auf. Aufgrund der insgesamt noch geringen Fallzahlen und des Fehlens kontrollierter Studien bleibt jedoch weiterhin ungeklärt, inwiefern diese beobachteten unerwünschten Wirkungen nicht auch mit der Grunderkrankung zusammenhängen, was insbesondere für den Anstieg der Transaminasen und der ZNS-Nebenwirkungen anzunehmen ist.

Längere Liegezeiten im Krankenhaus und insbesondere auf der Intensivstation erhöhen die Rate an krankenhausassoziierten Komplikationen; dies wurde insbesondere für das Risiko der beatmungsassoziierten Pneumonie gezeigt, welche mit der Beatmungsdauer zunimmt(35). Die im Rahmen dieser Arbeit gewonnenen klinischen Daten zum Einsatz von intravenösem Artesunat konnten eine signifikante Verkürzung der Behandlungsdauer im Krankenhaus wie auch auf der Intensivstation im Vergleich zu Chinin zeigen(36). Dies unterstützt die Annahme, dass auch außerhalb endemischer Gebiete die Verwendung von intravenösem Artesunat klinisch vorteilhaft ist und zu einer Reduktion von Komplikationen und Mortalität beitragen kann.
In Südostasien konnte der Überlebensvorteil durch Gabe von intravenösem Artesunat im Vergleich zu intravenösem Chinin nur bei Patienten mit einer Parasitämie von >10% beobachtet werden(18). Dasselbe gilt für die zuvor erwähnte Verkürzung der Liegezeiten auf der Intensivstation und im Krankenhaus(36). Obwohl die Endpunkte und Umstände der zugrundeliegenden Studien sehr unterschiedlich sind, unterstützen diese Beobachtungen insgesamt die Annahme, dass die Vorteile der Behandlung mit intravenösem Artesunat zu einem wesentlichen Anteil auf die im Vergleich zu Chinin deutlich schnellere Reduktion hoher Parasitämien zurückzuführen ist.
Auch für die orale Behandlung der unkomplizierten Malaria tropica in nicht-endemischen Gebieten konnten wichtige Daten gewonnen werden, die letztlich auch für die Zulassung der Medikamentenkombination aus Artemether/Lumefantrin in Europa relevant waren. Hierbei wurde ebenfalls ein sehr gutes Sicherheitsprofil festgestellt, vor allem wenn der Vergleich mit bisher verwendeten Substanzen zugrunde gelegt wird(37).

Die PADH als relevante unerwünschte Wirkung der Therapie mit Artemisinen konnte im Rahmen dieser Arbeit zuerst für die komplizierte Malaria tropica und anschließend für die unkomplizierte Malaria tropica jeweils erstmalig beschrieben werden(31,36,38). Die Daten führten dazu, dass Patienten künftig nach Gabe von intravenösem Artesunat nachbeobachtet wurden, die Studiendaten der SEQUAMAT-Studie(18) aus Südostasien diesbezüglich re-evaluiert wurden und angesichts der Bedeutung der Artemisinine für den weltweite Malaria-Strategie der WHO die Medicines for Malaria Venture und die WHO eine Expertenkonferenz zur weiteren Klärung dieses Phänomens einberiefen(39,40).

Dass diese unerwünschte Wirkung in Studien in Südostasien, Afrika und bei der hier im Jahr 2003 durchgeführten Studie mit Artemether/Lumefantrin bislang nicht aufgefallen war, ist am ehesten der nicht ausreichenden Nachbeobachtungszeit der Patienten bei früheren Studien zuzuschreiben.
Die PADH tritt in 20-30% der mit intravenösem Artesunat(31,34) und in bis zu 40% der mit oralem Artemether behandelten Patienten auf(38), wobei für die unkomplizierte Malaria bislang nur Daten im Rahmen dieser Arbeit mit von bislang 20 Patienten verfügbar sind. In der Folge wurden auch in endemischen Gebieten Patienten mit komplizierter Malaria tropica auf das Eintreten einer PADH untersucht; in einer Studie aus Westafrika konnte eine PADH in 5 von 72 (7%) Kindern zwischen 6 Monaten und 5 Jahren nachgewiesen werden(41). In 19% bis 60% der Fälle von PADH in einzelnen Studien war eine Transfusion erforderlich(32,34,42); der Hämoglobin-Gehalt des Blutes kann bis auf Werte unter 6g/dl bei Erwachsenen(32,34,42) und bis auf Werte unter 3g/dl bei Kindern absinken(41).

Die PADH weist unterschiedliche klinische Ausprägungen auf. In der ersten Beschreibung des Phänomens ließ sich eine „rekurrierende“ von einer „persistierenden“ Hämolyse abgrenzen(42), eine andere Studie unterteilte „ansteigende“, „persistierende“, „komplexe“ und „PADH“-Formen der Hämolyse aufgrund des unterschiedlichen zeitlichen Ablaufes(34). Erste Zeichen der Anämie – welche nicht direkt der Malaria-induzierten Hämolyse zuzuschreiben sind - sind ab Tag 8 nach Behandlungsbeginn zu beobachten; der Nadir der Hämoglobin-Konzentration im Blut liegt bei den meisten Patienten zwischen Tag 10 und Tag 21(32,33,38,42), kann beim Typ der „kontinuierlichen Hämolyse“ aber auch später liegen. Dies hat Bedeutung für die Klärung der pathophysiologischen Mechanismen der PADH. Zuerst wurde eine autoimmunhämolytische Anämie als Mechanismus in Betracht gezogen. Bei den bislang publizierten Patienten wurden in eigenen wie auch anderen Arbeiten jedoch sowohl positive wie auch negative Anti-Humanglobulin-Tests beobachtet(32,33,42); ein klares Muster war jedoch nicht erkennbar. Ein Mechanismus der Parasitenelimination ist die Extraktion ringförmiger Parasiten aus dem Erythrozyten während der Passage durch die Milz; aufgrund der schnellen und effektiven antiparasitären Wirkung von Artemisininen auf alle Entwicklungsstadien kommt

dieser Mechanismus unter Artemisintherapie offenbar deutlich stärker zum Tragen als unter anderen Therapien(43). Nach Extraktion des Parasiten aus dem Erythrozyten in der Milz bleibt das „ring-stage erythrocyte antigen“ (RESA) auf der Erythrozytenoberfläche nachweisbar und markiert so die zuvor infizierten Erythrozyten („once-infected erythrocytes“, o-iE)(43). Diese haben eine verkürzte Lebensdauer von ca. 7-8 Tagen im Vergleich zu 43 Tagen bei normalen Erythrozyten(44). In einer folgenden prospektiven Studie an Patienten mit importierter komplizierter Malaria und intravenöser Artesunatgabe bestätigte sich, dass Patienten mit einer PADH (definiert als 10% Abfall des Hämoglobinwerts oder 10% Anstieg des LDH-Wertes) signifikant höhere Anteile an o-iE aufwiesen als Patienten ohne PADH(26). Unter Anwendung eines Grenzwerts von 180 Millionen o-iE pro Liter konnte eine PADH mit einer Sensitivität von 89% und einer Spezifität von 83% vorhergesagt werden(26). Hiermit konnte eine zentrale Rolle der o-iE im Rahmen der PADH nachgewiesen werden.

Die unkomplizierte Malaria unterscheidet sich unter anderem von der komplizierten Malaria durch eine häufig geringere Parasitämie. Dementsprechend sind auch die zu erwartenden Zahlen an o-iE geringer. Wie im Rahmen dieser Arbeit gezeigt werden konnte, sind ebenfalls hämolytische Reaktionen nach oraler Artemisintherapie bei unkomplizierter Malaria zu beobachten, bei allerdings deutlich geringerem Ausprägungsgrad der Anämie (medianer Abfall des Hämoglobinwerts um 1,3g/dl (IQR -2,0 bis -0,3)(38). Interessanterweise konnten Patienten afrikanischer Abstammung die Hämolyse wesentlich effektiver kompensieren als Patienten europäischer Abstammung. Die Ursache dieser Beobachtung ist zum gegenwärtigen Zeitpunkt noch ungeklärt und Gegenstand weiterer Untersuchungen.

Ebenso wie bei der Therapie der komplizierten Malaria mit Artesunat sind auch bei der unkomplizierten Malaria nach Artemisintherapie unterschiedliche Verläufe der Hämolyse zu beobachten. Zusammenfassend betrachtet lässt sich am ehesten eine kürzere Verlaufsform mit einer intensiveren Hämolyseaktivität zwischen den Tagen 8-21 nach Behandlungsbeginn einerseits und eine längere Verlaufsform mit einer bis zu 56 Tagen kontinuierlichen, aber weniger intensiven Hämolyseaktivität andererseits abgrenzen(38,42).

Einhergehend mit dem aktuellen pathophysiologischen Verständnis der PADH dürfte jedoch mit dem Ablauf der verkürzten Lebensdauer der o-iE von maximal 10-12 Tagen die Hämolyseaktivität an Tag 21 nach Behandlungsbeginn weitgehend erloschen sein. Die bei einigen Patienten beobachtete und über diesen Zeitpunkt weit hinaus anhaltende Hämolyseaktivität lässt sich zum gegenwärtigen Zeitpunkt mit dieser Hypothese nicht eindeutig erklären und sollte in weiteren Studien geklärt werden. Insbesondere bleibt zu klären, ob auch zu späteren Zeitpunkten noch o-iE nachweisbar sind und warum nur ein Teil der Patienten bei vergleichbarer Parasitämie eine PADH entwickelt.

Obwohl bei *P. falciparum* mittlerweile auch für Artemisine in Südostasien eine zunehmende Resistenz beobachtet werden kann(45), spielen artemisinbasierte Therapien heutzutage weltweit die zentrale Rolle bei der Medikamentösen Therapie der Malaria. Zusammenfassend betrachtet weisen sie im Vergleich zu bisher verwendeten Chinolinderivaten eine wesentlich schnellere Wirkung und eine deutlich bessere Verträglichkeit auf. Sofern eine ausreichende Überwachung der Patienten im Anschluss an die Therapie gewährleistet ist, rechtfertigt das verbesserte Überleben die Gabe von intravenösem Artesunat als Mittel der ersten Wahl zur Behandlung der komplizierten Malaria tropica. Die Zulassung von intravenösem Artesunat durch die zuständigen Arzneimittelbehörden auch in Europa und USA wäre ein wichtiger Schritt, um die Verfügbarkeit des Medikaments und die rechtlichen Rahmenbedingungen der Anwendung für Patienten in Europa entscheidend zu verbessern.
Bei der unkomplizierten Malaria tropica führt eine geringer ausgeprägte Hämolyse in der Regel nicht zu schweren Komplikationen; für anämiebedingte Komplikationen gefährdete Patienten wie z.B. Patienten mit fortgeschrittener ischämischer Kardiomyopathie sollten ebenfalls auf Zeichen einer Hämolyse auch nach Behandlung einer unkomplizierten Malaria tropica überwacht werden. Das Auftreten der PADH bei Patienten in endemischen Gebieten ist insgesamt seltener und tritt mit geringerer Ausprägung auf(46); zudem bestehen offenbar bessere Kompensationsmechanismen als bei Patienten in nicht-endemischen Gebieten(38). Die klinischen Konsequenzen der PADH in endemischen Gebieten, wo zudem zahlreiche andere Ursachen für eine Anämie vorliegen, bleibt der künftigen Bewertung vorbehalten.

Zusammenfassend zeigen die gewonnenen Erkenntnisse ebenfalls, dass auch im Rahmen netzwerkbasierter Forschung an Reiserückkehrern in Europa wertvolle Erkenntnisse für die Behandlung von in den Tropen endemischen Erkrankungen gewonnen werden können.

4. Zusammenfassung

An der Malaria erkranken weltweit ca. 212 Millionen Menschen pro Jahr und ca. 430.000 Todesfälle an Malaria werden pro Jahr registriert. Die Malaria tropica wird durch Reisende und Migranten jedoch auch häufig nach Europa importiert. Auch in Europa kommt es durch die importierte Malaria tropica zu Todesfällen.

Artemisine, gewonnen aus der Pflanze *Artemisia annua* sind die mit Abstand wichtigste Substanzklasse in der Therapie der Malaria tropica weltweit. Intravenöses Artesunat war zur Behandlung der komplizierten Malaria tropica in einer Studie in Südostasien der bisherigen Standardtherapie mit intravenösem Chinin hinsichtlich der Mortalität überlegen. Die Effektivität und Sicherheit der Therapie mit Artemisinen wurde primär in endemischen Ländern untersucht, wohingegen Untersuchungen an Patienten aus nicht-endemischen Gebieten nicht vorlagen.

Es konnte gezeigt werden, dass eine orale Therapie mit Artemether-Lumefantrin auch unter den Bedingungen der Behandlung in nicht-endemischen Ländern eine sichere und effektive Behandlungsoption für die nach Europa importierte Malaria tropica ist.

Die Untersuchung der Epidemiologie der importierten komplizierte Malaria tropica – gekennzeichnet durch einen Verlauf mit hoher Parasitämie und Organversagen – ergab eine deutliche Altersabhängigkeit hinsichtlich des Risikos des Auftretens von Komplikationen. Die meisten Patienten mit importierter komplizierter Malaria tropica kamen aus den Ländern Westafrikas.

In einer Fallserie von Patienten mit importierter komplizierter Malaria tropica konnte zum einen die sehr gute Effektivität von intravenös verabreichtem Artesunat auf die Parasitenelimination demonstriert werden; andererseits wurde erstmals eine starke hämolytische Reaktion als unerwünschtes Ereignis im Zeitraum nach Abschluss der Malaria-Therapie erstmals beschrieben. Hierbei fielen ein „rekurrierendes“ Muster der Hämolyse mit einem Beginn ab ca. Tag 10 nach Erstgabe von Artesunat auf, sowie ein „persistierendes“ Muster mit einer anhaltenden hämolytischen Aktivität bis zum Tag 50 nach Behandlungsbeginn. Eine autoimmunhämatologische Ursache der beobachteten Hämolyse konnte nicht eindeutig nachgewiesen werden. Ferner konnte im Anschluß ebenfalls nach oraler Therapie der unkomplizierten Malaria eine vergleichbare, aber in

der Ausprägung deutlich mildere Form der Hämolyse nach Therapie mit oralen Artemisinderivaten erstmalig nachgewiesen werden. Die beschriebenen hämolytischen Reaktionen stellen den zentralen Stellenwert der Artemisine für die weltweite Therapie der Malaria nicht infrage; eine entsprechende Nachbeobachtung ist jedoch insbesondere bei der Behandlung der komplizierten Malaria angezeigt, da ca. 60% der Patienten mit Transfusionen behandelt werden mussten. Die Behandlung der komplizierten Malaria tropica bietet gegenüber der Behandlung mit Chinin klinische Vorteile auch im Bezug auf die Reduktion der Behandlungsdauer auf Intensiv- und Normalstation und somit ein erniedrigtes Risiko für behandlungsassoziierte Komplikationen.

Im Rahmen der vorliegenden Arbeit konnte zahlreiche Beiträge zur Epidemiologie und Verbesserung der Behandlung bzw. der Behandlungssicherheit bei der nach Europa importierten Malaria tropica erbracht werden.

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7. Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,

die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,

mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

.....	.....
Datum	Unterschrift