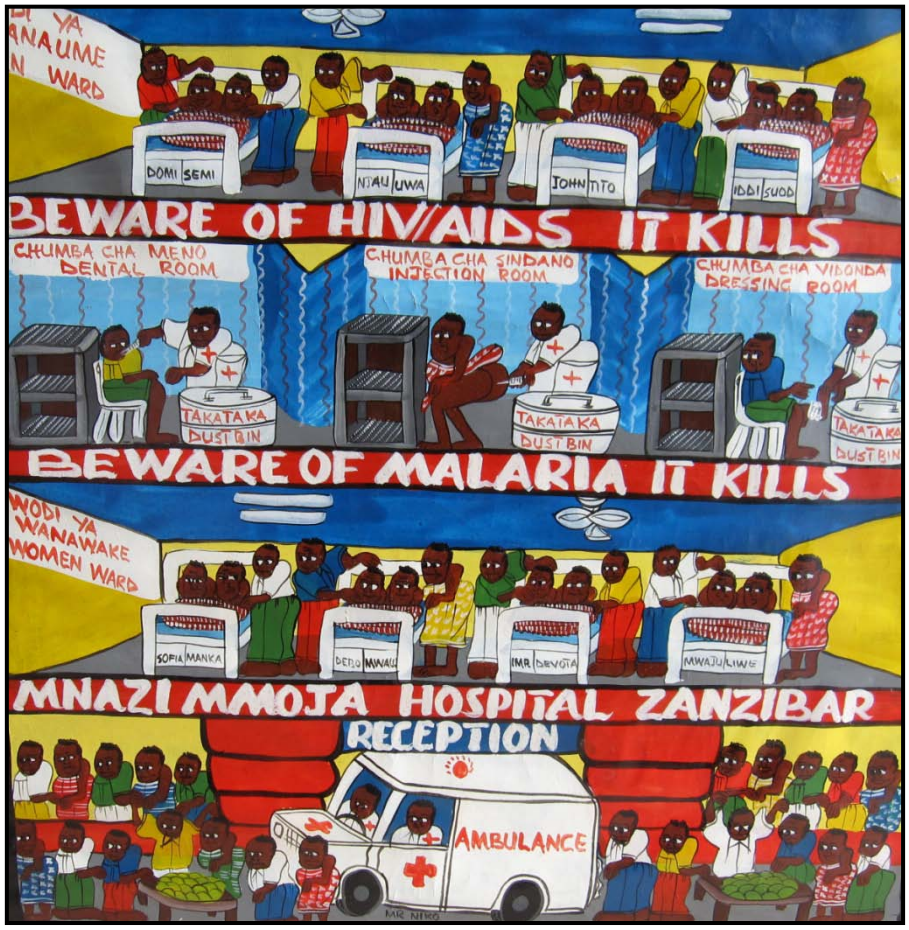
TESIS DOCTORAL

La malaria en pacientes pediátricos ingresados en un hospital rural de Mozambique, y el desarrollo clínico de nuevos fármacos antimaláricos.

Malaria in the paediatric wards of a rural Mozambican hospital and the clinical development of new antimalarial drugs



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Malaria in the paediatric wards of a rural Mozambican hospital and the clinical development of new antimalarial drugs

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A mi familia,
por una educación integral

A los United World Colleges,
por una educación comprometida
(y permitirme conocer a Pedro)

Y a María Maixenchs,
por una educación sentimental.

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1. Glossary

ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AIDS	Acquired immunodeficiency syndrome
AL	Artemether-Lumefantrine
AQ	Amodiaquine
AS	Artesunate
BCS	Blantyre Coma Scale
CFR	Case fatality rate
CISM	Centro de Investigação em Saúde da Manhiça
CQ	Chloroquine
DDT	Dichloro-diphenyl-trichloroethane
DHA-PQP	Dihydroartemisinin-Piperaquine
EIR	Entomological inoculation rate
EPI	Expanded programme of immunization
GMP	Good manufacturing practices
Hib	Haemophilus Influenzae type b
HIV	Human immunodeficiency virus
IMCI	Integrated management of childhood illness
IPTi	Intermittent Preventive Treatment in Infants
IPTp	Intermittent Preventive Treatment in Pregnancy
IRB	Institutional Review Board
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
LLIN	Long-lasting insecticidal nets
MDH	Manhiça District Hospital
NTS	Non-typhoid Salmonella
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
SP	Sulfadoxine-Pyrimethamine
WHO	World Health Organization

2. Resumen (Castellano)

A pesar del renovado afán por eliminar la malaria de la faz de la tierra, esta histórica enfermedad se mantiene como uno de los principales peligros para la salud infantil en amplias zonas del mundo. Las medidas de control existentes son claramente insuficientes a la hora de reducir globalmente el impacto de esta enfermedad, y a día de hoy la malaria es endémica en más de 100 países en dónde más de la mitad de la población mundial está expuesta al riesgo de infectarse. De hecho, la carga de esta enfermedad en términos de morbilidad y mortalidad persiste inaceptablemente alta con, anualmente, más de 250 millones de episodios clínicos y cerca de un millón de muertes. Mientras que la enfermedad clínica puede afectar a cualquier grupo de edad, la mortalidad por malaria se ve esencialmente circunscrita a los niños menores de cinco años infectados por *P. falciparum*.

La apasionante y casi palpable perspectiva de una vacuna efectiva contra la malaria, sumada a la promoción de innovadoras estrategias de control como el tratamiento intermitente en lactantes o mujeres embarazadas han centrado, en los últimos años, el debate científico relacionado con las estrategias de control. En contraste con estas innovadoras estrategias, el control de la malaria se ha visto sin embargo limitado en las últimas décadas a la identificación y tratamiento precoz de los episodios clínicos y a la reducción del contacto entre el reservorio humano y el vector. A menos que se produzca una importante renovación y optimización del arsenal terapéutico y de las estrategias frente a esta infección, acompañado por un despliegue masivo de las intervenciones en aquellas áreas dónde son más necesarias, la malaria continuará dictando el estado de salud global, y su erradicación no dejará de ser una utopía irrealizable a corto plazo.

La malaria es una enfermedad potencialmente mortal, y por lo tanto, un requerimiento fundamental para su control es la disponibilidad de fármacos antimaláricos efectivos para su tratamiento. El desarrollo de la farmacopea antimalárica, estrechamente relacionada a los diferentes conflictos bélicos del último siglo, ha representado un constante desafío y debido a los repetidos problemas de financiación ha resultado claramente inadecuado, especialmente en comparación con el desarrollo de fármacos para otras enfermedades. Como

ejemplo cabe destacar que, entre los años 1975-1999, apenas 4 de los cerca de 1400 fármacos registrados en el mundo, eran antimaláricos. Además, la aparición de resistencias por parte del parásito a los fármacos, un problema creciente y global, resulta un grave peligro para el control de esta infección, y representa una presión añadida para los pocos fármacos efectivos todavía disponibles. Con todo, en los últimos años, la malaria se está desprendiendo del status de enfermedad olvidada que arrastraba, e importantes esfuerzos han surgido para el desarrollo e investigación de nuevos fármacos con actividad antimalárica. Basado en el conocimiento adquirido con otras enfermedades infecciosas como la tuberculosis, la organización mundial de la salud promulga actualmente que el tratamiento frente a *P. falciparum*, la especie responsable de la mayoría de casos graves y la práctica totalidad de las muertes, debe basarse en la terapia combinada, incluyendo a ser posible un derivado de las artemisininas. La elección de los fármacos a combinar debe basarse en la potencial sinergia entre los perfiles farmacocinéticos y terapéuticos de los diferentes componentes, con el objetivo de conseguir una eliminación de la parasitemia rápida, protegiendo al mismo tiempo a los diferentes componentes frente al desarrollo de resistencias.

Los derivados de las artemisininas son actualmente considerados los antimaláricos con mayor eficacia y rapidez de acción. Por esta razón, la mayoría de países endémicos los han incluido como primera o segunda línea para el tratamiento de casos de malaria no complicada en sus guías terapéuticas, a pesar de su elevado coste en comparación con otros fármacos antimaláricos disponibles. Actualmente, la combinación de artemether y lumefantrina (Coartem®) es la más usada en el África subsahariana, considerándose la combinación estándar, y existen pocas alternativas disponibles con un perfil de eficacia similar. Frente al riesgo de aparición de resistencias frente a las artemisininas, indicios de las cuales ya han empezado a ser publicados, es necesario promover la investigación de nuevos fármacos antimaláricos o innovadoras combinaciones de los ya existentes. El primer artículo de esta tesis describe un importante ensayo clínico aleatorizado (más de 1500 pacientes), realizado en 5 países africanos, diseñado para demostrar la no-inferioridad de la combinación dihidroartemisina-piperaquina (DHA-PQP), manufacturada siguiendo prácticas estandarizadas de producción, frente a la

combinación estándar de AL para el tratamiento de niños africanos menores de cinco años con malaria no complicada. La estimación de la eficacia (corregida mediante determinación de PCR) de la combinación DHA-PQP fue alta (95.7%) y comparable a la observada para el fármaco control AL (95.7%), y por tanto la no-inferioridad pudo ser demostrada estadísticamente. El perfil de seguridad de ambas combinaciones fue similar, con la mayoría de efectos adversos detectados de leve o moderada intensidad, y en cualquier caso consistentes con síntomas atribuibles a la propia infección. Cabe destacar que la combinación DHA-PQP mostró un mejor efecto profiláctico post-tratamiento, sugerido por la reducida incidencia de nuevas infecciones durante los 42 días de seguimiento de estos pacientes. Aunque esta mejor prevención de futuros episodios parece jugar a favor de la nueva combinación, sobre todo en áreas de alta transmisión, debe tenerse en cuenta que el grupo tratado con DHA-PQP mostró sin embargo una mayor tasa de prevalencia de gametocitos durante el seguimiento, factor también asociado tradicionalmente con un aumento del riesgo de transmisión. Este estudio, cuyos resultados contribuirán de forma determinante para el registro internacional de esta nueva combinación, confirma que DHA-PQP es una nueva opción segura, tolerable, eficaz, y asequible para el tratamiento de la malaria en África.

La eficacia de un fármaco antimalárico viene determinada por una serie de características, entre las que se encuentran los patrones de sensibilidad farmacológica mostrados por los parásitos, las características farmacocinéticas del compuesto y su calidad adecuada, y la toma correcta por parte del paciente. Para actuar de forma eficaz contra el parásito infectante, el fármaco debe ser tomado siguiendo la prescripción correcta y ser absorbido satisfactoriamente para así lograr unas concentraciones plasmáticas óptimas para actuar. El adecuado cumplimiento de la prescripción, determinado por la posología, puede por tanto convertirse en un factor determinante para la eficacia del tratamiento. Así, este correcto cumplimiento puede verse comprometido tanto porque muchos pacientes dejan de tomar las siguientes dosis del tratamiento una vez empiezan a sentirse mejor, como porque no existen formulaciones adecuadas para el tratamiento en niños. Si en el primer artículo quedaba demostrado que DHA-PQP era una buena alternativa en

términos de eficacia antimalárica frente a AL, es importante destacar que DHA-PQP presenta una ventaja añadida, que es su posología simplificada, ya que requiere una dosis única diaria en vez de dos dosis al día, como AL. En este sentido, la adherencia y posterior cumplimiento del régimen de tratamiento posiblemente sean mejores. En el segundo artículo son descritos los resultados de otro importante ensayo clínico (900 pacientes) aleatorizado llevado a cabo en 5 países africanos, en el cual fue evaluada la no-inferioridad de una innovadora formulación pediátrica soluble de AL, con sabor a cereza, frente a su tradicional formulación en pastillas, para el tratamiento de episodios de malaria no complicados en niños menores de 12 años de edad. El ensayo mostró una adecuada tolerancia de la nueva formulación, sin apenas efectos secundarios importantes, y con una estimación de la eficacia similar y alta para ambas formulaciones (97.8% AL soluble, 98.5% AL en pastillas), confirmando de forma estadística la no-inferioridad del fármaco a estudio. Esta nueva formulación soluble, más adecuada para su uso en niños, puede contribuir a mejorar el cumplimiento terapéutico, y por tanto debería ser una alternativa útil a las pastillas convencionales de AL, mucho más difíciles de ingerir por los niños.

Los resultados de estos dos ensayos, reflejados en el artículo 1 y el artículo 2 de esta tesis, aportan a la comunidad de malariólogos datos relevantes desde el punto de vista de salud pública sobre nuevas alternativas al actual arsenal limitado de fármacos antimaláricos.

La presentación clínica de la malaria, y por lo tanto su pronóstico, son variables y, pueden depender de muchos factores entre los cuales destacan la intensidad de transmisión, la especie de plasmodio infectante, las características genéticas de la población, el comportamiento poblacional a la hora de procurar servicios sanitarios, el manejo a nivel local de la enfermedad y la coexistencia simultánea de otras enfermedades en el paciente. Existen pocas descripciones sistemáticas de la presentación clínica de la malaria en áreas endémicas africanas, y todavía no existe un concierto generalizado y consensuado acerca de cuáles deberían ser los criterios clínicos usados para categorizar un caso de malaria como grave. El conocimiento detallado de la presentación clínica de la malaria grave y las implicaciones pronosticas de sus diferentes síntomas y

signos clínicos puede contribuir a la comprensión de su fisiopatología, y por tanto dirigir de forma más adecuada y apoyándose en la evidencia, las intervenciones terapéuticas o de prevención. El tercer artículo de esta tesis es una descripción de la presentación clínica de la malaria grave en un hospital rural en Mozambique, con especial énfasis en detallar los factores de riesgo asociados con un pronóstico desfavorable. También son presentadas tasas de incidencia comunitarias de malaria grave, un indicador útil de la carga de malaria en la comunidad. En este hospital, alrededor de la mitad de los ingresos pediátricos (49.1%) fueron hospitalizados debido a su episodio de malaria, y más de una cuarta parte de todos los casos de malaria cumplían criterios de gravedad. Hasta un 18.7% de todas las muertes hospitalarias fueron atribuibles a malaria, y la tasa de letalidad calculada para malaria grave fue de 4.4%, con más de la mitad de las muertes por malaria aconteciendo en las primeras 48 horas de ingreso, una ventana demasiado corta para el correcto funcionamiento de los tratamientos antimaláricos. Por este motivo, la promoción de medidas enfocadas a la detección precoz de la malaria grave, a nivel de centros de salud o incluso comunitario, y el tratamiento anticipatorio con una primera dosis de antimalárico eficaz de camino al hospital podrían mejorar las probabilidades de supervivencia de estos pacientes graves. Este estudio destaca además que en Manhiça, la carga de enfermedad y mortalidad por malaria disminuye con la edad, afectando principalmente a los niños más pequeños, con una incidencia máxima en los menores de dos años de edad. El artículo concluye que las estrategias de intervención deben ser dirigidas a este grupo de edad, y que intervenciones como el tratamiento preventivo intermitente en lactantes (IPTi) pueden por tanto tener un impacto crítico a la hora de reducir la carga de enfermedad en esta área. A pesar de que la carga de enfermedad disminuye con la edad, este estudio destaca por fin que los niños más mayores no deben ser olvidados en la planificación de estrategias frente a la malaria, ya que también sufren una importante carga de la enfermedad, con hasta un 10% de las muertes por malaria evidenciables en este grupo de edad.

En la mayoría de países en vías de desarrollo, en dónde la transición demográfica todavía no ha ocurrido, las principales causas de mortalidad

infantil son enfermedades infecciosas. La organización mundial de la salud ha producido históricamente revisiones periódicas que desglosan de forma adecuada las principales causas de mortalidad infantil en las diferentes regiones del mundo. La malaria, junto con las infecciones bacterianas (siendo su paradigma las neumonías bacterianas) se mantienen como las dos principales causas de morbilidad y mortalidad infantil en África. A pesar de las dificultades inherentes para su correcto diagnóstico, las infecciones bacterianas invasivas son consideradas como una de las principales causas de ingreso y mortalidad hospitalarias, siendo *S. pneumoniae* y *Salmonella* las bacterias consistentemente reportadas como principales microorganismos aislados en niños africanos. A pesar de que el impacto de la infección por ciertos microorganismos en la salud infantil ha sido bien descrito, el diagnostico etiológico preciso en la gran mayoría de zonas rurales de los países africanos sigue presentándose como un enorme desafío. Por ejemplo, los signos y síntomas clínicos de la malaria son poco específicos, y comunes a los de la mayoría de otras enfermedades infecciosas pediátricas. Al mismo tiempo, en las zonas más rurales, las facilidades diagnósticas son inexistentes, y el manejo de los pacientes se decide en base de la presencia o ausencia de signos y síntomas clínicos. Precisamente, en esta premisa se basan las guías de manejo integrado de las enfermedades pediátricas (IMCI), que sugieren que en zonas endémicas para malaria, todo niño con fiebre deberá ser tratado con antimaláricos. Además, como consecuencia del desarrollo de la inmunidad natural adquirida frente a la malaria, es relativamente frecuente detectar parasitemias maláricas periféricas sin ninguna traducción clínica asociada. Todos estos factores contribuyen a la incertidumbre diagnóstica alrededor de una enfermedad como la malaria en las áreas endémicas, y dificultan en gran medida la clasificación etiológica precisa de las enfermedades pediátricas causantes de la sintomatología clínica. Como consecuencia directa de esta incertidumbre diagnóstica, niños con sintomatología poco especifica compartida por varias enfermedades acaban recibiendo de forma simultánea antibióticos y antimaláricos, incluso si esto significa un mal uso de las escasas opciones terapéuticas disponibles en estos países. Para acabarlo de complicar, se ha sugerido que la presencia de parásitos de malaria puede predisponer a la aparición de otras infecciones sobreañadidas, como por ejemplo una

bacteremia. Por otro lado, la presencia de una bacteriemia concomitante podría hipotéticamente aumentar la severidad de un episodio no complicado de malaria convirtiéndolo en potencialmente letal. En este sentido, parece por tanto necesario producir datos relevantes describiendo la interacción y el grado de solapamiento entre las infecciones bacterianas y la malaria, con especial énfasis en las implicaciones pronosticas de su coexistencia. El cuarto y último artículo de esta tesis versa sobre la relación entre malaria grave y las infecciones bacterianas invasivas en niños menores de cinco años de edad ingresados en el hospital rural de Manhiça, Mozambique. Este estudio concluye que esta coexistencia es frecuente (hasta en un 5.4% de los casos de malaria grave) y conlleva implicaciones pronosticas desfavorables. La presencia de una bacteremia concomitante multiplica las tasas de letalidad y aumenta de forma independiente el riesgo de muerte (OR 6.2). Estos datos también destacan la importancia de *S. pneumoniae*, más que cualquier otra bacteria, como el principal aislado bacteriano en casos de malaria grave en Manhiça, posiblemente como consecuencia directa de las altas tasas de prevalencia de HIV en esta comunidad. El artículo concluye destacando que las estrategias diseñadas para disminuir la carga de enfermedad bacteriana invasiva, como por ejemplo la vacuna conjugada anti pneumocócica, pueden tener un impacto importante añadido reduciendo la carga de mortalidad por malaria.

La malaria es una enfermedad histórica con terribles consecuencias para la humanidad. Todos los esfuerzos destinados a reducir sus devastadores efectos en aquellas áreas dónde es endémica deben ser integrados de forma coordinada para luchar contra ella desde todos los frentes posibles. Entender los determinantes clínicos de la malaria y su relación con otras infecciones coexistentes puede permitirnos comprender mejor su fisiopatología, y a partir de allí guiar de forma más dirigida las estrategias de manejo. Paralelamente, es necesario desarrollar nuevos fármacos antimaláricos altamente eficaces, junto con formulaciones más adecuadas para la edad pediátrica, y garantizar que éstos lleguen allí dónde son más necesarios.

3. Summary (English)

Despite a renewed impetus on bringing together efforts to wipe malaria from the globe, the truth is that this ancient disease remains one of the principal threats to child survival in vast areas of the world. The existing control measures are largely insufficient to reduce globally the malaria burden, and as of today, malaria remains endemic in more than 100 countries, where at least half of the world's population live exposed to the risk of being infected. Indeed, the burden of malaria morbidity and mortality continues to be unacceptably high, with more than 250 million clinical episodes and at least one million deaths per year. While morbidity due to malaria affects all age groups and is scattered throughout the world, Sub-Saharan Africa concentrates the brunt of the malaria deaths, which overwhelmingly occur in children under five years of age infected with *P. falciparum*.

The exciting and almost palpable perspective of an effective malaria vaccine, added to the promotion of innovative control strategies such as intermittent preventive treatment for infants or pregnant women, have in the recent years centred the scientific discussions regarding the approach to malaria control. However, in contrast to these innovative perspectives, malaria control has remained in the last two decades essentially dependent on the early identification and prompt treatment of clinical malaria cases and the reduction of man-vector contact. Unless there is a major renewal, optimisation and scale-up of the antimalarial arsenal and strategies, malaria will continue to dictate the health status of the world, and eradication will not be achievable in a realistic timeframe.

As malaria is a life-threatening infection, a fundamental requirement for its adequate control is the availability of effective antimalarial drugs. The development of the antimalarial pharmacopeia, closely linked to the different military conflicts of the past century, has been challenging and time-consuming, and clearly remains inadequate and underfunded when compared to other diseases. As a blatant example, only four of the approximately 1400 drugs registered worldwide during 1975-1999 were antimalarials. Moreover, the advent of antimalarial drug resistance, now widespread and affecting almost all antimalarial drugs, poses a serious threat to the control of this infection and

further pressures the few available effective treatments. In recent years, however, malaria seems to be abandoning the neglected disease status, and renewed efforts are being mobilised into research and development of new compounds with antimalarial efficacy. Based on the experience with other infectious diseases such as tuberculosis, the World Health Organization now advocates that treatment against *P. falciparum*, the parasite species which causes the vast majority of the severe cases and mortality, should be based on combination therapy, including if possible an artemisinin-derivative. The choice of the different partner drugs should be based on the synergy of the therapeutic and pharmacokinetic profiles with the objective of achieving a fast parasite clearance and protecting the partner drugs from the emergence of resistance.

Artemisinin derivatives are currently considered the fastest acting and more efficacious antimalarial drugs, and for this reason most endemic countries in the world have included them in their policies, as first or second-line treatment against uncomplicated malaria, despite their elevated costs when compared to hitherto available therapies. The combination of artemether and lumefantrine (AL, commercial name Coartem®), currently used in most of Sub-Saharan Africa, has become the front-runner and gold standard for combination therapies, and few alternatives are available with similar efficacy profiles. In the light of potential emergence of artemisinin resistance, reports of which are already being published, there is a need to research, develop and register new antimalarial drugs, or innovative combinations of the already available. The first paper in this thesis describes a large randomised clinical trial (around 1500 children) performed in five different African countries to assess the non-inferiority of a novel combination, dihydroartemisinin-piperaquine, produced under strict good manufacturing practices, when compared to the standard combination therapy AL, for the treatment of uncomplicated malaria in African children under the age of five years. The efficacy estimates (PCR corrected) observed for DHA-PQP in the primary efficacy population (per protocol) was high (95.7%), and comparable to that observed for the comparator drug AL (95.7%), and thus non-inferiority was statistically confirmed. The safety profile of both drugs was also similar, with the majority of adverse events of mild or moderate severity and consistent with symptoms attributable to malaria. Importantly, DHA-PQP showed the added advantage of a better post-treatment

prophylactic effect, suggested by the lower rate of new infections occurring up to day 42 of follow-up. While this could clearly be an advantage in areas of high transmission, it needs to be balanced out with the higher gametocytaemia prevalence found in the DHA-PQP group, as gametocytes are known to enhance transmission also. This study, which shall contribute significantly to the registration of this new drug combination, confirms that DHA-PQP is a safe, efficacious, tolerable and affordable new antimalarial treatment option in Africa.

The efficacy of an antimalarial drug is determined by a series of characteristics, including the sensitivity patterns to that drug present among the parasite populations, its pharmacokinetic characteristics, and the drug's adequate quality and correct intake by the patient. To act effectively against the infecting parasite, the drug needs to be correctly up-taken and absorbed by the patient, according to a pre-specified regimen, so that it can reach its optimal plasmatic levels to act. Compliance becomes therefore a critical step that often negatively affects its effectiveness, not only because many patients stop taking their medication as soon as they start feeling better, and prior to the completion of the pre-specified regimen, but also because for the younger children, no adequate paediatric formulations are available. While in the first article, DHA-PQP was shown to be a good alternative in terms of efficacy to AL, it also has the added advantage that it requires a single daily dose, compared to the two standard daily doses in the AL regimen. This simplification of the regimen may surely improve adherence and compliance to the treatment. In the second paper, we describe the results of another large (around 900 patients) randomised clinical trial in which we assessed in 5 different African countries the non-inferiority of a new, paediatric-suited cherry-flavoured, dispersible formulation of AL, when compared to standard crushed AL tablets, for the treatment of uncomplicated malaria in children younger than 12 years of age. The trial showed adequate tolerability, no important safety issues, and similar high efficacy estimates in both treatment arms (97.8% dispersible AL, 98.5% crushed AL), confirming statistically the non-inferiority of the new dispersible tablets. Thus, this new dispersible formulation, better suited for children's use, may contribute to a better therapeutic compliance and consequently become a

useful alternative to the conventional crushed tablets for the treatment of uncomplicated malaria in children.
Thus, the results of these two trials, reflected in paper 1 and paper 2 of this thesis, bring to the malaria community public-health relevant data on alternatives to the currently limited antimalarial drug arsenal.

The clinical presentation of malaria and therefore its prognosis, are variable and may depend, among others, on several factors, including the intensity of transmission, the parasite species involved, the genetic characteristics of the population, the health seeking behaviour and local case management, and the coexisting non-malaria co-morbidities. Few detailed and systematic descriptions regarding the clinical presentation of malaria in African settings are available, and the clinical criteria used to categorize a malaria episode as severe remain still a matter of debate. Understanding the clinical presentation of severe malaria and its prognostic implications may give us insights into the pathophysiology of the disease, and thus produce evidence-based guidance for better-targeted curative or preventive interventions. The third paper in this thesis describes the clinical presentation of severe malaria in a rural Mozambican Hospital, and the associated risk factors for a negative outcome. It also presents minimum community based incidence rates, a useful indicator of the malaria burden in the area. In this hospital, about half of the admitted patients (49.1%) under the age of 15 were hospitalised because of their malaria episode, and more than a quarter of all the malaria cases fulfilled the definition of severe disease. Malaria accounted for 18.7% of all in-hospital paediatric deaths, and the case fatality rate for severe malaria was 4.4%, with more than 50% of all malaria-attributable deaths occurring within the first 48 hours of admission, a limited time window for antimalarial therapies to start working. For this reason, measures designed to enhance early recognition of severe malaria cases at home or at the community level, and prompt pre-referral treatment with effective and easy to administer antimalarials may improve the survival likelihood of the sickest children. This study also highlighted that the burden of malaria morbidity and mortality decreases with increasing age, and is borne mainly by infants and young children, thus suggesting that malaria control interventions specifically targeted at early infancy (such as IPTi, for instance)

may therefore have a significant impact in reducing the burden of malaria in this area. Importantly, it also brings to the attention that children older than 5 years of age should not be neglected in the design of anti-malaria policies, as they still suffer a considerable burden of the disease, with significant morbidity and even mortality (up to 10% of all malaria deaths).

In the majority of developing countries, where the demographic transition has not yet occurred, the principal killers of children are infectious diseases. WHO has produced accurate reviews of the burden of the different infections causing child mortality in the different regions of the world. Bacterial infections (the paradigm of which are bacterial pneumonia cases) and malaria remain the two leading causes of paediatric mortality and morbidity in Africa. Invasive bacterial disease is nowadays, despite the difficulties in confirming its diagnosis, one of the principal killers of children *per se*, and *S. pneumoniae* and *Salmonella* species have been consistently reported as the bacterial isolates causing major morbi-mortality in African children. Even if the impact of certain microorganisms as a cause of paediatric morbidity and mortality has been well studied, reaching a precise aetiological diagnosis in the vast majority of rural African settings remains considerably challenging. Clinical signs and symptoms of malaria are often poorly specific, and common to many other paediatric infectious diseases. On top of that, in rural settings, diagnostic facilities are often unavailable, and management of patients is decided on the basis of the presence or absence of clinical signs and symptoms. This is the basis of the IMCI guidelines, which suggest considering all fever cases in malaria-endemic areas with antimalarials. Moreover, as a consequence of the development of naturally acquired immunity, it is quite frequent to detect peripheral malaria parasitaemias without accompanying clinical symptomatology. All of these factors contribute to malaria misdiagnosis in endemic areas, and do not permit a clear-cut picture of the precise aetiological nature of the infection causing clinical symptoms. As a direct consequence of these diagnostic difficulties, children with overlapping symptoms may end-up receiving simultaneously antibiotic and antimalarial treatments, even if this implies a misuse of the scarce available therapeutic strategies. Furthermore, it has been suggested that the presence of malaria parasites or clinical malaria, may predispose to the appearance of other

superimposed infections, such as for instance bacteraemia. Conversely, the presence of concomitant bacteraemia may hypothetically enhance the severity of an otherwise benign malaria episode and turn it life-threatening. It becomes therefore necessary to produce relevant data describing the interaction and the extent to which infectious bacterial diseases overlap with malaria, and specifically of the prognostic implications of their coexistence. The fourth paper addresses the relationship between severe malaria and bacterial invasive disease in children younger than 5 years admitted to a Manhiça's rural hospital, Mozambique. This study concludes that the coexistence of these two infections is frequent (5.4%) and has detrimental prognostic implications, as concomitant bacteraemia substantially increases the risk of death among severe malaria patients (OR 6.2). These data also highlight the importance of *S. pneumoniae*, more than any other bacteria, as the principal isolate related with severe malaria in Manhiça, possibly in relation to the high underlying HIV prevalence in the area. The paper concludes that measures envisaged to decrease the burden of invasive bacterial disease, such as the *pneumococcus* conjugate vaccine, may also have an impact in reducing malaria mortality.

Malaria is an ancient disease of terrible consequences for mankind. All efforts devised to diminish the burden that malaria imposes in endemic areas need to be embraced and brought together on a coordinated manner to fight this infection from all possible fronts. Understanding the clinical determinants of the infection and its relation with other coexistent infectious diseases will give an insight to the basic pathophysiology of malaria, and thus guide appropriate management strategies. New highly effective drugs, and their paediatric-orientated formulations, need to be developed and made available to those most at need.

4. General Introduction

4.1 The global burden of malaria

When in 1955, the notorious malariologist Paul Russell predicted without hesitation the imminent end of malaria[1], little could he imagine that half a century later malaria would still constitute one of the most important public health challenges in the world. Despite recent reports of an important yet not unprecedented decline in malaria-related morbidity and mortality in certain areas of the world[2-4], malaria remains at the beginning of the 21st century a significant scourge for humankind. Almost half the world's population, that is more than 3300 million people, live at the moment in areas where malaria is endemic, in up to 109 countries in the world[5]. WHO estimates that, every year, around 250 million cases of malaria occur worldwide[5], leading to an estimated 1 million fatalities, mostly due to *P. falciparum*[5, 6]. Globally, 9% of all deaths in children younger than 5 years of age are attributed to malaria[7], and this age group carry the brunt of this disease, with up to 85% of all deaths affecting them.

Sub-Saharan Africa (SSA) represents the paradigm of the devastating effects of infectious disease affecting child survival[8, 9]. This is even truer for an infection like malaria, endemic in 45 of the 46 countries defining the WHO African region[5], an area which accounts for around 85% of all the world's malaria cases[5], and 91% of the world's malaria deaths. Indeed, malaria was responsible in 2006 for at least a fifth of the estimated 4.4 million annual child deaths in this continent[5, 10].

Malaria is essentially a malady of the poor, but also heavily contributes to increasing poverty in the countries where it is endemic. The global distribution of per-capita gross domestic product shows a striking correlation between malaria and poverty, and malaria-endemic countries also have lower rates of economic growth[11]. At the same time, poverty enhances the risk of transmission, and specially hinders the possibilities of malaria control, closing down a poverty-disease vicious circle, from which it becomes very difficult to exit.

Malaria needs therefore to be addressed not only as a major health problem but also as a human development crisis[6].

4.2 Historic perspective

The history of malaria can be traced back to the prehistory of humankind, possibly originating in Africa to later expand to the warmer regions of the planet. The name of the disease comes from the Italian *mal aria* (bad air), as during the 17th century it was believed that it originated from the putrid fumes arising from the Tiber river in Rome. The disease is also known with the French name *paludisme* deriving from the Latin word *palus* meaning "swampy". It was only at the end of the 19th century when the aetiology of this disease started to be understood. In 1880, Laveran described the blood stages of the parasite as observed in a peripheral blood smear taken from a patient with malaria, and a few years later, and almost simultaneously, the Italian zoologist Giovanni Battista Grassi and the British doctor Ronald Ross described the detailed life cycle of the parasite in mosquitoes and humans, finally untangling the mechanisms of transmission of the disease.

The World Health Organization became determined to eradicate malaria, launching in 1955 the "Global malaria eradication campaign". The geographic distribution of malaria enclosed vast areas of the world until the 50s and 60s, including, apart from the tropical and subtropical regions where it still remains endemic, the south of Europe, the south of the United States, the North of Africa, the Middle East, the north of Australia and some regions of East Asia, areas where malaria was successfully eliminated. The control of malaria in such areas was made possible through the combination of efficient environmental actions, the use of powerful and cheap insecticides such as DDT and the development of antimalarial drugs during the 20th century. These historic achievements led to the optimistic belief that malaria would soon be erased from the rest of the planet. The rapid emergence of resistances in mosquitoes to insecticides, and in parasites to the available antimalarial drugs (especially to chloroquine), and the evidence that eradication was not anymore realistic, made the WHO reconsider their campaign and opt for a less ambitious goal of decreasing the associated morbi-mortality in endemic areas. Efforts were then switched into treatment, rather than prevention.

The 21st century has seen great advances in the understanding of the disease, with the description of the full genome of both *Plasmodium falciparum*[12] and its main vector *Anopheles Gambiae*[13], and together with the publication of the full

human genome, the three vertices of the malaria triangle are finally connected. The new fields of genomics and proteomics, together with the new available technologies applied to research in molecular biology, should contribute substantially to the development of new control tools aimed at solving a millenary problem.

4.3 Three actors in the play: The mosquito, the parasite and the human host

Malaria is the principal parasitic infection in the world, and results from the infection of a vulnerable host by a Plasmodium parasite. The three actors involved in the transmission of this infection are the mosquito vector, the parasite and the human host.

The anopheline vector

The vector responsible for the parasite's transmission is a mosquito from the Anopheles genus. Although more than 400 species of Anopheles exist, only 30-40 are considered potential transmissors of the disease. The parasite undergoes sexual reproduction inside the mosquito, and this process does not occur adequately when temperatures rise above 35ºC or fall below 16ºC. The effect of climatic conditions on both the parasite's and the vector's life cycle have been critical at shaping the geographical distribution of the parasite, and therefore of malaria itself. Similarly, altitude (due to its relation to temperature) had been traditionally considered a protective factor, and malaria transmission was believed to be difficult above 2,000 metres. The climatic changes that the planet is undergoing have contributed to recent deviations from this paradigm.

The parasite

There are currently more than 100 known plasmodium species, most of them causing animal malaria, but only 5 of which are considered to cause disease in humans: *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi* and *P. falciparum*. *Plasmodium falciparum* is responsible for the vast majority of severe disease and the practical totality of deaths attributed to this infection. *P. vivax* is increasingly recognised as a less harmless parasite than traditionally considered, but still accounts for a very small proportion of the severe cases, and the other three are associated with relatively benign infections, with P. knowlesi, a parasite usually affecting monkeys, having been recently described

as capable of infecting humans also. Contrarily to the other species which specifically invade different stages of maturation of the erythrocyte, *P. falciparum* can invade red blood cells of any age, thus accounting for a greater virulence and faster potential to multiply and reach higher peripheral parasitaemias. Likewise, *P. falciparum* is the only species capable of invading the same red blood cell simultaneously by different parasites.

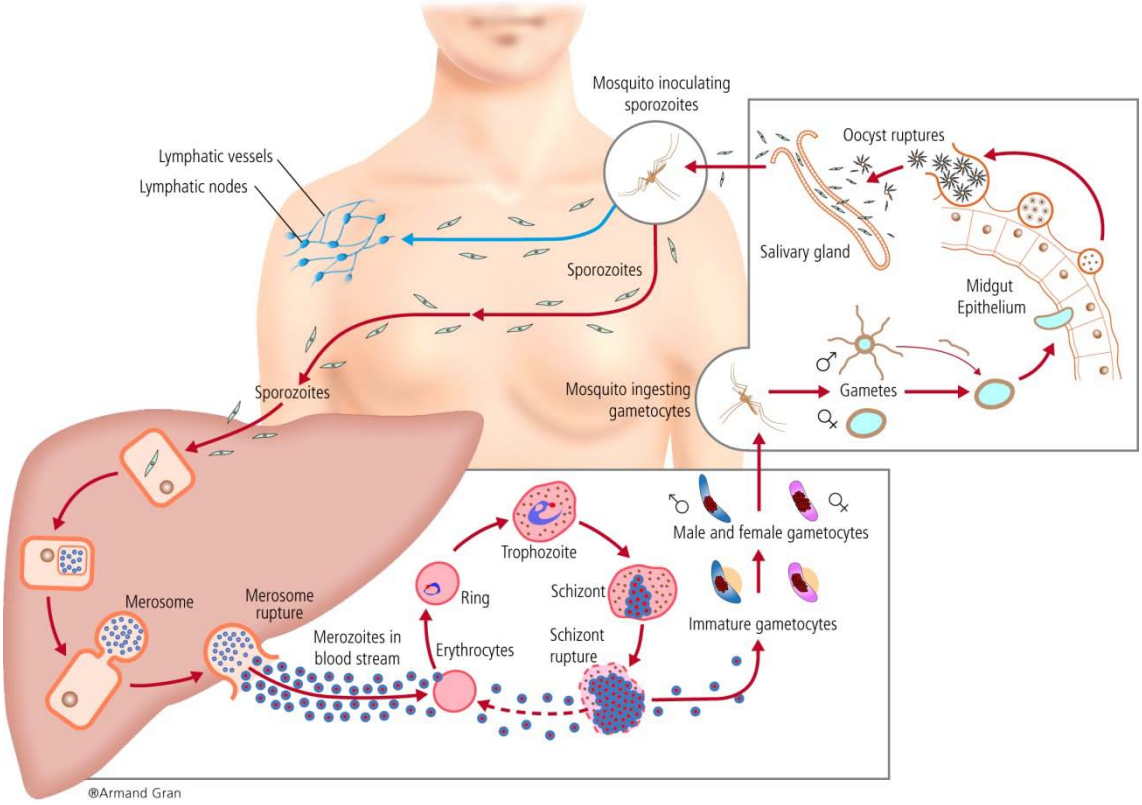
The human host

Once an infected mosquito bites a human host, transmitting the malaria parasites, the severity and progression of this infection will depend on multiple factors, including among others, the degree of immunity of the bitten individual, the person's age, health status and genetic characteristics, the species and strain of the parasite, and the antimalarial therapy received. Thus, the degree of immunity versus the malaria parasite will be a critical determinant for the prognosis and the evolution of the disease. When faced by a continuous exposure to infective bites, as occurs in malaria endemic areas, individuals acquire naturally certain level of immunity against the disease. Thus, an infection occurring in a person "naïve" to malaria, which therefore has not had the chance of developing any kind of immunity (i.e. a traveler), will certainly have greater risk than one occurring in a person previously exposed to the parasite. Similarly, certain genetic factors have been associated with protection against malaria, and this is believed to occur as an evolutionary mechanism in detriment of the parasite and in favour of the host's survival. As an example, the haemoglobin variant HbAS (sickle cell trait), present in certain African countries in up to 25% of the population, has been linked with a protection against severe malaria, although the frequency of infections among those individuals may not differ from that in the non-affected population. Likewise, the absence of the erythrocytic membrane Duffy antigens A and B among black Africans, especially in West Africa, is also known to protect against *Plasmodium vivax* infection, as this parasite needs such membrane markers to invade the red blood cell. Among many other genetic variations, certain HLA antigens have also been linked with protection against severe disease[14].

4.4 Life cycle of the malaria parasite

The life cycle of the Plasmodium falciparum parasite is summarized in figure 1. In humans, the infection arises from the bite of a vector (female mosquito of the *Anopheles* species) already infected with the sexual stages of the parasite. With the bite, the mosquito inoculates the infective stages of the parasite, known as sporozoites, which briefly (a few minutes) circulate in the bloodstream in their direction to the liver, where they invade the hepatocytes. Replication within these hepatic cells will give rise, after a few days, to the hepatic schizonts, containing thousands of merozoites that are released into the bloodstream, thus starting the asexual erythrocytic component of the cycle. For *P. falciparum*, all hepatic schizonts burst synchronically, but this does not occur for two of the other species capable of relapsing (*P. vivax* and *P. ovale*), as, regardless of producing normal schizonts, these species can develop "hibernant" ones, known as hypnozoites. Such sleeping forms will remain inactive for weeks or months, after which they may re-activate their intrahepatic schizogony, and be ready to release into the bloodstream a new batch of merozoites. Every merozoite entering the bloodstream will try to invade an erythrocyte, as plasmodia are obligate intracellular parasites, in order to multiply inside the red blood cell and form what is known as erythrocytic schizonts. Following 48 hour waves for all plasmodia or 72 hours ones for *P. malariae*, these schizonts will burst, destroying their container cell, and liberating new merozoites to the bloodstream, responsible for perpetuating the blood stage of the cycle. Some of those merozoites will take a parallel pathway and differentiate into gametocytes, the sexual stage of the parasite. Once a mosquito will absorb them through a new bite, the gametocytes (male and female) will close down the life cycle undergoing sexual reproduction within the vector's gut. Only then, new sporozoites will be produced, ready to start a new life cycle within a new host.

Figure 1: Life cycle of Plasmodium falciparum

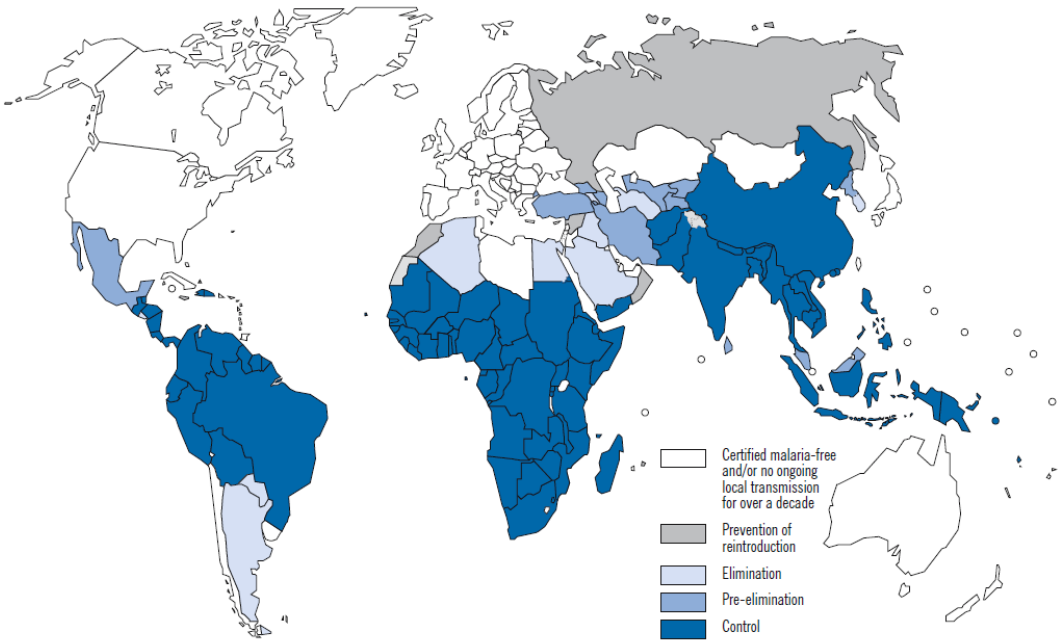


4.5 Geographic distribution

Malaria is currently endemic in 109 countries, scattered over Africa, Asia, Central and South America, and some Caribbean and Pacific islands. Figure 2 illustrates, according to WHO, the geographic distribution of malaria in the world. Of the more than 200 million annual cases in the African region only, the vast majority correspond to *P. falciparum*, followed by *P. ovale* and *P. malariae*. *P. vivax* seems extremely infrequent in Africa, but non-black people may acquire this infection in small foci of transmission located fundamentally around the East coast, in the area known as the horn of Africa. Among infections acquired in South America, *P. vivax* slightly prevails over other species, although *P. falciparum* is steadily increasing in recent years, especially in Brazil, and spreading to neighbouring countries. In Central America, *P. vivax* clearly remains the main species, but *P. falciparum* is also starting to increase. In Asia, *P. vivax* also dominates, with a high associated burden of disease in the Indian

Subcontinent, but in South East Asia, *P. falciparum* has grown and become a serious public health problem due to its association with multidrug resistance, especially in the "opium triangle" defined within the borders of Myanmar, Cambodia and Thailand. Malaria is also present in the Pacific Islands, where *P. falciparum* is the main parasite found, reaching as far as Vanuatu. It is important however to remember that mixed infections are also frequent.

Figure 2: Malaria-free countries and malaria-endemic countries in phases of control, pre-elimination and prevention of reintroduction, end 2007 (according to WHO[5])



**China, Indonesia, Philippines, Solomon Islands, Sudan, Vanuatu and Yemen have subnational elimination programmes*

4.6 The acquisition of Immunity to malaria: Relation between the endemicity and the epidemiology of malaria

Individuals born in malaria-endemic areas gradually acquire with age a partial immunity against malaria, starting from an initial protection against the most severe forms of disease (severe malaria, and death), followed by a lower incidence of clinical episodes (anti-disease immunity), and eventually reaching a suppression of parasitaemia (low or non-detectable parasitaemias, -anti-parasite immunity-). This naturally acquired immunity (NAI) requires a continuous booster effect, and never becomes sterilizing, as individuals may continue getting infected, despite not always developing clinical signs. Thus, typically, a newborn in a highly endemic area will suffer repeated malaria episodes in the first years of life, all contributing to the gradual development of a partial immunity that will eventually protect him in the long term against severe disease and death, but not from getting infected. The pace of acquisition of such protection will depend upon the age of the first episode and the intensity of transmission. For this reason, in areas of intense transmission, where the EIR is high, and transmission is perennial, NAI will develop very rapidly, and severe disease and deaths will concentrate in the first 5 years of life and pregnant women, with mild clinical expression of the infection in adults. In areas with lower transmission, NAI may take longer to appear, and clinical disease will extend to older age groups. This also occurs in areas were malaria transmission remains unstable, or epidemic, as transmission is highly variable and may last for short periods of the year. Under such circumstances, the acquisition of NAI may take longer or not even develop, and all parasitaemias may be associated to clinical symptoms. In these situations, clinical disease and mortality may involve all age groups, and there is a potential risk for epidemics with high associated mortality rates. Similarly, individuals living in areas where malaria is not transmitted and thus previously never exposed to infective bites, will therefore lack the acquired capacity for responding to the infection, becoming at risk of quickly progressing to severe disease and death, if not treated.

Mechanisms involved with the development of NAI are complex and relatively poorly known[15]. Despite the huge progress witnessed in recent years in the malaria immunology field, there is no yet clear understanding on how immunity is acquired, or why certain infected individuals develop severe disease while

others don't[16]. No clear surrogate marker of protection has been found either, and this remains a key underachievement that urgently needs to be addressed in malaria research.

4.7 Pathogenesis

It is nowadays accepted that the pathogenesis of malaria, and especially of *P. falciparum* severe malaria, is a complex multifactorial process, involving simultaneously multiple systems, organs and tissues in the infected host. Current opinions[17] suggest that, contrarily to what was previously believed, no clear correlation between pathogenic processes and clinical symptoms exists, as very different mechanisms may lead to identical clinical symptomatology. Different basic mechanisms interact jointly to determine severe disease in *P. falciparum* malaria: (1) The rapid expansion of the infected erythrocytic mass; (2) the invasion and subsequent destruction of the infected and uninfected erythrocytes; (3) the obstruction at the microvasculature level; and (4) immunological processes mediated by inflammatory cytokines that interact in a combined way to reduce tissue flow.

4.8 Clinical malaria

Five plasmodia species are known to infect humans, being *P. falciparum* the main responsible for the severe cases and almost the totality of deaths. Nevertheless, consistent evidence in the literature suggests that *P. vivax*, that was previously thought to be a 'non-severe, non-lethal' parasite, is also directly related to severe clinical complications[18, 19]. Past work and studies show a change in the paradigm of *P. vivax* as a non-virulent malaria parasite, but this specie's weight as a cause of severe disease remains insignificant when compared to that due to *P. falciparum*. Infections by the other plasmodia species cause a relatively mild and unspecific clinical picture, similar to the uncomplicated cases of *P. falciparum*.

The incubation period for the disease ranges normally between 8 and 30 days, being generally shortest for *P. falciparum* and longest for *P. malariae*. Incubation periods exceeding one month have been described for certain strains of *P. vivax* but remain exceptional.

The general clinical picture for any plasmodia species affecting humans is dominated by fever, which appears abruptly, and is accompanied by general malaise. The typical malaria episode normally begins with an intense feeling of coldness ("cold" phase) with shivers, lasting around one hour, followed by a rapid increase in body temperature ("hot" phase) that can quickly reach 40º C. This phase may last up to 6 hours, and is followed by a state of drowsiness, weakness and profuse sweating ("Sweating" phase). Paroxysms, which usually start during the day, are believed to coincide with the bursting of the blood schizonts, and last for approximately 9 to 10 hours, leaving the patient in a relatively acceptable state between crises. Historically, malaria was described according to the periodicity of these paroxysms, which coincided with the duration of the parasite's intraerythrocytic cycles. Hence, tertian fevers would correspond to the cycles of *P. vivax, P. ovale* and *P. falciparum*, which lasts 48 hours (re-starting every third day) and quartan fevers to the cycle in *P. malariae,* which lasts 72 hours. This nomenclature is nowadays considered obsolete, as the presence and course of fever in *P. falciparum* infections is frequently erratic, and has been artificially modified by the regular use of antipyretics.

These paroxysms can repeat themselves for a few cycles, with no further complications (uncomplicated malaria) or alternatively, and less frequently, progress to a more severe form of the disease (severe malaria).

Uncomplicated malaria

The vast majority of *P. falciparum* cases and almost the totality of infections secondary to other species are relatively benign and mild, showing the typical malarial paroxysms and unspecific symptomatology. Besides fever, the clinical picture includes malaise, dizziness, arthromyalgias, and headache. In children, gastrointestinal and respiratory symptoms are also frequent, and often an enlarged spleen can be found. The full blood cell count often reveals a mild decline in the three main cellular lines, with anaemia, low platelets and leucopoenia, but the expected compensatory increase in reticulocytes is often only seen after parasites have disappeared from the bloodstream. Biochemical changes include hyponatremia, hypo or hyperpotassemnia, and more often mild or moderately increased liver enzymes, which require the exclusion of alternative diagnoses such as viral hepatitis.

Both *P. vivax* and *P. ovale* are uniquely associated with clinical relapses secondary to the presence of intrahepatic hypnozoites. Such relapses are frequent in the first six months after the initial infection, unless the patient has been specifically treated with drugs targeting the hypnozoites. The average duration of an incorrectly treated *P. vivax* or *P. ovale* infection is 3-5 years, but spontaneous recovery is also frequent. *P. malaria* infections may be initially clinically identical to the other species, but normally behave less aggressively, and in a more chronic way. This species lacks a hypnozoitic stage, thus precluding it from relapsing, but reactivations have been described in patients up to 50 years after the original infection.

Severe malaria

Only 1-2% of the malaria infections with clinical repercussion occurring in children living in malaria-endemic areas qualify as severe and may be life-threatening[14, 20]. It is widely believed that increasing levels of peripheral parasitaemia associate worse prognoses, but severity can be present even with minimal parasitaemias. However, peripheral parasitaemias may underestimate the real parasite mass, as *P. falciparum* is capable of sequestering in the microvasculature, and may falsely "disappear" from the bloodstream.

Malaria complications develop very rapidly in children, and death may occur only a few hours after the first signs of disease. More than three quarters of the malaria deaths in Africa are thought to occur in the first 24 hours after hospital admission, and within the first 2 days of onset of symptoms[21]. Paediatric malaria differs in many senses from adult malaria. While multi organ failure is common in severe adult malaria, it is exceptional in children, where three main severe syndromes are typically described[21, 22]: (1) Cerebral malaria; (2) Severe malarial anaemia; and (3) Malaria with respiratory distress. These syndromes are not exclusive, and frequently overlap, appearing from the beginning or after an initial uncomplicated episode. Other signs or symptoms, such as hypoglycaemia, convulsions or prostration, have also been used to classify paediatric malaria as severe, and need to be addressed urgently when found in a malaria patient, as they are related to a worse prognosis[23-25].

Cerebral malaria

Several definitions have been used for what is known as cerebral malaria. For many years, a child was considered to have cerebral malaria when, in the presence of peripheral asexual parasitaemia of any density, unconsciousness was present, and this could not be attributed to other obvious causes (meningitis, hypoglycaemia or post-ictal state)[25]. Unconsciousness was defined as the inability to localize a painful stimulus in children older than 9 months of age, or the absence of response to a painful stimulus in younger infants. The Blantyre Coma Scale (BCS)[26] is a simplified coma grading scale (Table 1), developed in the Malawian city of Blantyre, and widely used because of its simplicity and practicality for grading consciousness in children in different malaria-endemic areas.

Table 1: Blantyre Coma Scale

	Score*
Best Motor Response	
Localizes painful stimulus	**2**
Withdraws limb from pain	**1**
Non-specific or absent response	**0**
Verbal Response	
Appropriate cry	**2**
Moan or inappropriate cry	**1**
None	**0**
Eye Movements	
Directed (e.g., follows mother's face)	**1**
Not directed	**0**

*Total score can range from 0 to 5; 2 or less indicating "unrousable coma"

Any score inferior or equal to 2 (out of a possible total of 5), in the presence of malaria parasites in the blood, is considered cerebral malaria. Such definitions, fundamentally clinical, are nowadays considered inadequate or at least imperfect, as clinical-pathological studies have shown that many of the deaths attributed to cerebral malaria, on the basis of such clinical definitions, corresponded in reality to other causes[27]. The recently recognized *P. falciparum* retinopathy[28, 29] (including retinal haemorrhages, macular whitening, retinal vessel abnormalities and papilledema), is an adequate living correlate to post-mortem pathological findings of cerebral malaria. The excellent diagnostic and

prognostic value of this finding[30] suggests that adding the results of a simple fundoscopic exam to other clinical signs, may greatly enhance the diagnostic precision of cerebral malaria.

Neurological involvement in cerebral malaria is dependent on age, and may be highly variable, ranging from a diffuse cortical encephalopathy to more specific brainstem abnormalities. Table 2 summarizes the main differences in the neurological features of cerebral malaria both in children and adults.

Table 2: Differential characteristics from cerebral malaria in children and adults (adapted from Idro et al[31])

Clinical characteristics	Children	Adults
Neurological signs and symptoms		
Convulsions	Referred history present in up to 80% of cases, and directly observed during admission in up to 60%. Recurrent convulsions include focal motor (>50%), generalized tonic-clonic (34%), partial with secondary generalization (14%), subtle, or purely electroencephalographic (15%). *Status epilepticus* is frequent[26, 32]	Present in up to 20% of patients, mainly generalized tonic-clonic. *Status epilepticus* is rare[33, 34]
Neurological abnormalities	Prostration. Brainstem changes in >30%, often associated with intracranial hypertension[35]. Malaria retinopathy present in >60%[30]. Cerebral oedema visible in CT scan in up to 40%[36]	Typically symmetric upper motor neuron signs. Brainstem abnormalities or malaria retinopathy rare[33, 37]
Coma	Appears rapidly, often following a convulsion[26]	Develops more gradually, often aften an insiduous 2-3 day-long phase of drowsiness, confusion and/or agitation. May be triggered by a tonic-clonic convulsion[33]
Evolution		
Conscience recovery	Fast, between 24-48 hours[25, 38]	More slowly, >48 hours[39]
Mortality	20-75% of all deaths within first 24 hours of admission[25, 40]. Many cases never reach the hospital	20-50% of all deaths within first 24 hours
Neurological sequelae	Frequent (10%)[41]. Most common include: ataxia (2.5%), hemiparesis (4.4%), tetraparesis (3.5%), deafness (1.9%), cortical blindness (2.3%) and aphasia (2.1%). Epilepsy [42, 43]. Neurocognitive abnormalities[44, 45]	Infrequent (<5%). Isolated cranial nerve abnormalities, multiple mononeuritis, polyneuropathies, extrapyramidal tremors and other cerebellar signs[33]

Patients may present on arrival or develop throughout their admission tone abnormalities, opisthotonus, descerebrate or decorticate posturing, abnormal respiratory patterns[46], absence of corneal reflexes or other cranial nerve abnormalities[25] (Figure 3). The impairment of conscience may be transient or long-lasting, but in general, patients showing a good evolution regain full consciousness within the first 48-72 hours. In children, convulsions are very frequent, being referred or directly observed in up to 60-80% of African children with malaria, and may trigger the beginning of coma. Prolonged convulsions, or those refractory to treatment or starting once antimalarial treatment has been initiated entail a worse prognosis and a higher risk of neurological sequelae or death[26, 32]. Lumbar puncture is needed for the differential diagnosis of bacterial meningitis, and in cerebral malaria cases shows an acellular and sterile cerebro-spinal fluid (CSF), with a slight increase in proteins and elevated opening pressure[47].

Case fatality rates for cerebral malaria, both for children and adults, approach 20%[31, 41], and neurological sequelae, of whichever severity, are present at discharge in 9 to 12% of patients[48, 49], half of which will recover completely in the following two months.

Figure 3: Mozambican infant with cerebral malaria showing signs of brainstem involvement and persistent convulsions (Photo by Quique Bassat)

Severe malarial anaemia

P. falciparum infections are very frequently associated with a certain degree of anaemia, of a multifactorial aetiology, and non regenerative in nature as long as the parasite is not eliminated from the bloodstream. Severe anaemia is considered when haemoglobin concentration is ≤ 5g/dl, or haematocrit (PCV) ≤ 15%. It has been estimated that severe malarial anaemia occurs 1.42-5.66 million times annually and kills 190,000-974,000 (> 13% CFR) children < 5 years of age every year[41]. Associated clinical findings may include pallor, respiratory distress, and a hyperdynamic circulation. This symptomatology is possibly the consequence of the combination of both an insufficient oxygenation at the tissue level and a potential cardio-vascular compromise, but the relative contribution of each of those two situations remains unclear and the subject of controversy[50-53]. Blood transfusions and iron supplementation continue to be the cornerstone of anaemia treatment in malaria-endemic areas[54]. Blood transfusions may be life-saving[55] (Figure 4) but also associate a high risk of transmission of other infections that should not be underestimated[56].

Figure 4: Blood transfusions may be life-saving interventions in children with severe malarial anaemia (photo by Quique Bassat)

Malaria with respiratory distress

Children with severe malaria often present respiratory distress which may be clinically very important, and which normally includes deep (acidotic) breathing, low chest wall indrawing, and other signs and symptoms such as increased respiratory rate, or nasal flaring. Many of those patients have normal oxyhaemoglobin concentrations, and non-pathological chest X-rays, which suggests that the respiratory distress present in such cases corresponds to metabolic disturbances rather than a pathological process at the lung level[57, 58]. In other cases, the malaria episode coexists with a bacterial infection, with both infections acting synergistically to produce respiratory distress. The real prevalence of this overlap is not well known, but has important implications in terms of establishing clear treatment policies in countries where malaria is endemic and no accurate diagnostic tools such as microscopy or radiography are present[59-61].

Good correlations have been established between the presence of clinical deep breathing and high plasmatic lactate[62], or low bicarbonate levels[22]. The role played by metabolic acidosis in the pathophysiology of severe malaria has already been clearly established[17, 22, 63-65], not only in its relation with clinical symptomatology, but also in relation to the other two major severity syndromes and linked to its prognosis. Indeed, the presence of metabolic acidosis in patients which already have either cerebral malaria or severe malarial anaemia is the best independent prognostic marker of a negative outcome[23, 58]. The case fatality rate of this syndrome is also high, varying between 14% and 25%[22] in endemic areas, but could dramatically improve if respiratory support (ventilator) was available in such settings.

4.9 Strategies to tackle malaria

Prompt and effective treatment of suspected or confirmed malaria cases remains the cornerstone of all malaria control strategies. It is however necessary to increase the uptake of other preventive tools to achieve a greater impact in terms of disease control. Four main strategies can be differentiated: (1) vector control tools; (2) strategies to reduce vector-human contact; (3) vaccines; and (4) drugs for the prevention and treatment of malaria cases.

Vector control

Vector control is based on the use of insecticides, larvicides and environmental procedures. These three mechanisms have altogether helped save many human lives. The widespread use of DDT targeting adult vectors, the exploitation of *Gambusia* fish designed to eat Anopheles larvae, and the different civil engineering works, envisaged to remove mosquito breeding areas, were all critical in the elimination of malaria transmission in vast Southern European areas during the 20th century. IRS with long-lasting insecticides remains a valid and widely used technique in many areas of the world. However, due to its complex implementation, high cost, environmental risks, and potential for triggering the development of resistance in mosquitoes, its use has nowadays been limited to very specific areas and ecological niches.

Strategies to reduce vector-human contact

Such strategies are based in the use of insecticide-impregnated bednets (ITNs), and other basic mosquito bite prevention measures. Randomized clinical trials have shown over the past decades that the use of ITNs not only reduces malaria-related deaths, but also all-cause paediatric mortality[66], and that this protection is maintained during time, even in areas of high endemicity[67]. By the end of 2006, nearly all of the 45 countries in the African WHO region had adopted the policy of providing ITNs free of charge to children and pregnant women, but only 16 aimed to cover all age groups at risk[5]. This addresses the hitherto overwhelming failure of international agencies, governments and the public health community when trying to guarantee widespread implementation of this tool, but still only about 10% of all Africans at risk sleep under an ITN[5]. For those who cannot get them for free, prices (~5 US$), remain prohibitive. Nowadays, the protection conferred by sleeping under LLINs impregnated with

pyrethroids may extend up to 4 years, with no need for annual re-impregnations to maintain their efficacy. Topical insect repellents, popular among travellers to malaria-endemic areas, have a very limited use outside this target group.

Vaccines

An effective and safe vaccine against malaria for children living in malaria-endemic areas would be a formidable tool to boost global malaria control. It is perhaps in this field where malaria research is producing its greatest breakthroughs. Tribulations related with funding have been temporarily overcome by different private initiatives working together with the public sector. However, the development of a malaria vaccine remains a formidable scientific challenge, due to the enormous complexity of the parasite and its life cycle. Its development also entails a long and expensive process, and several phases must occur before a candidate vaccine can be tried in children. Currently, several candidate vaccines have reached different development stages, although most of them are still in the preclinical phases. More than 50% of the more than 100 candidate vaccines in active development today are based on just three antigens cloned two decades ago: the circumsporozoite protein (CSP), the merozoite surface protein (MSP) and the apical membrane antigen 1 (AMA-1)[68]. The *Plasmodium falciparum* genome project has identified hundreds of parasite proteins that could form the basis for new vaccines[12].

The most advanced candidate vaccine, the RTS,S/AS02A, has been developed and jointly financed by GlaxoSmithKline and the Malaria Vaccine Initiative (MVI).[69] This pre-erythrocytic subunit vaccine is based on the fusion of the surface antigen from the circumsporozoite (CS) with the Hepatitis B surface antigen (HBsAg), formulated with the AS02A adjuvant. In a phase IIb clinical trial carried out in 2003 in children from 1 to 4 years of age in Mozambique, this vaccine showed to be safe, immunogenic and efficacious, reducing *P. falciparum* clinical malaria cases by 30% and episodes of severe disease by up to 58%.[70] Moreover, this efficacy seemed not to wane[71] after an 18 months follow-up, where the protection was maintained.[72] A proof of concept trial was undertaken in children less than one year of age, the ideal target population, and showed similar safety and efficacy results[73]. The promising findings in this age group imply that the vaccine, if finally registered, could be included in the

Expanded Programme of Immunization (EPI), one of the few existing effective mechanisms for the universal distribution of health measures in poor countries.

Drugs for the prevention and treatment

History of malaria treatment

Malaria has infected humans for over 50,000 years, and many treatments have historically been used to counteract its main symptoms. One of the first effective treatments for malaria was powder obtained from the bark of the cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru, and first reports of its use date to 1632. Cinchona bark was subsequently introduced by the Jesuits into Europe as a treatment for what was known as "the ague", and it took almost two centuries (1820) to isolate the alkaloid quinine from the cinchona bark. Quinine remained for many centuries the only available treatment for the disease.

The development of other antimalarial drugs runs in parallel with the military history of the 20th century. The First World War triggered research to find alternative treatments to quinine, the hitherto only available treatment. Quinacrine and several other compounds were developed, but the breakthrough came in 1934, with the synthesis of chloroquine, a new class of antimalarial of the 4-aminoquinoline family. This drug, first synthesized in Germany, was only recognized as a powerful antimalarial in the beginning of the 1940's, as part of the US World War II military effort. By the end of the war, in 1946, chloroquine was designated the drug of choice for the treatment of malaria, becoming the cornerstone treatment of this disease for the following four decades, as it was safe, highly effective and cheap. Other antimalarials such as proguanil (1946), amodiaquine and primaquine (1950's), the antifolate sulfadoxine-pyrimethamine (1967), halofantrine and mefloquine (developed by the US army as a result of the Vietnam War in the 1960's) were born to counter parasites that had become resistant to chloroquine. In China, piperaquine replaced chloroquine in 1978 as the first-line treatment, and was used extensively as monotherapy for over 15 years[74]. Lumefantrine was introduced in the 1980's as a response to the parasite's resistance to both chloroquine and the antifolates. During the last quarter of the 20th century almost no new malaria drugs were developed, a proof of the little interest that drugs targeted at neglected diseases originated in

the pharmaceutical industry. Among the circa 1400 drugs licensed in the world during this period, only four corresponded to drugs specifically designed to cure malaria[75].

The most recent addition to the global antimalarial arsenal (Artemisinin) is also one of the oldest. Infusions prepared from wormwood (*Artemisia annua*) were a component of Chinese herbal medicine for the treatment of fevers for more than 2000 years. However, the deployment on a large scale of this drug, was only started in the 1960's, as a result of an antimalarial research programme undertaken by the Chinese army. The benefits of this highly effective reborn drug were kept secret by the Chinese communist government for many years, and widespread availability of the semi synthetic derivatives of this plant was upheld until the last decades of the century. Atovaquone (1996) and various other drugs have emerged at the turn of the century, a period that is witnessing an unprecedented boom in malaria drug research.

The emergence of resistance to antimalarial drugs

Emergence of resistance has been observed for all clinical antimalarials with the exception of artemisinin-derived drugs, although resistance to these compounds is expected to appear soon. Of the 5 human malaria species, only two (*P. falciparum* and *P.vivax*) have developed resistance to antimalarial drugs. Chloroquine resistance in *P. malariae* has also been reported from Indonesia[76], but is possibly debatable[77].

Chloroquine resistance from *P. falciparum* was first observed in Thailand and in the Colombian-Venezuelan border in the late 1950's[78]. By the late 1970's it had spread to New Guinea and eastern Sub-Saharan Africa[79]. Resistance spread from the African coasts inland, and by 1989, chloroquine resistance was widespread in sub-Saharan Africa. Although the drug has lost its efficacy in most parts of the world, it remains effective in some areas of Central America and of South-Western Asia. Despite the widespread chloroquine use, *P. vivax* chloroquine resistance remains very limited to certain areas in Asia (Indonesia, Papua New Guinea and Myanmar). *P. vivax* remains chloroquine sensitive in Africa and the Americas. Except when stated, sensitivity patterns for the other drugs will refer to *P. falciparum*.

Resistance to SP and the other antifolates, unlike that to chloroquine, seemed to appear independently, fast and simultaneously in different parts of the world. Resistance to proguanil, a type-2 antifolate, appeared in 1947, only one year after its introduction, and SP resistance was detected in 1967 in Thailand, the very same year of being launched. This did not prevent it from becoming the standard second-line therapy against chloroquine-resistant *P. falciparum* malaria. The shorter lag from introduction to appearance of resistance for SP is probably related to the smaller number of genetic mutations needed when compared to those involved in chloroquine resistance, and to the longer half-life of the drugs. Currently, high-level of resistance is seen in most South-East Asia, southern China and the Amazon region. In Africa, SP sensitivity started declining in the late 1980's, and now high level resistance is found in large areas of East Africa, and in a lesser degree in some western African countries, with a predicted rapid spread to the rest of the continent. Because of its pharmacokinetic properties, SP has also been used for intermittent preventive treatment (IPT) both in pregnant women and infants. IPT is a malaria control strategy that consists in the administration of a full course of an antimalarial treatment to a population at risk at specified time points, regardless of whether or not they are known to be infected[80]. However, there is a need to develop alternative new regimens for IPT as increasing levels of parasite resistance may undermine this useful intervention[81].

Quinine has been extensively used for the treatment of malaria since the 17th century. The first reported evidence of resistance to this drug came from Brazil, in 1910. More recent evidence of quinine resistance has been regularly found in the Thai-Cambodian border, an area of intense drug pressure. Quinine efficacy remains therefore at risk in some countries in South-East Asia and Oceania, but seems uncompromised in Africa and South America. A poor compliance of the seven days regimen, undesirable side-effects, and the risk of losing a precious treatment that may be effectively used for the treatment of severe malaria, has discouraged its use as monotherapy for the treatment of *P. falciparum* infections.

The extensive use in China of piperaquine, as monotherapy, in mass treatment and mass prophylactic campaigns, triggered the development of resistances to

the drug. This drug has subsequently been combined with dihydroartemisinin, and the combination appears to be safe and highly effective[74].

Mefloquine resistance was first documented near the Thai-Cambodian border, 5 years after its introduction in 1977. Emergence of resistance was probably associated to the previous widespread use of quinine in the area, a compound to which Mefloquine is structurally related[82]. Resistance in that border area remains high, but decreases substantially in nearby regions, and the risk of therapeutic or prophylactic failure outside that particular region remains low for the rest of Asia. In Africa and South America, reports have been published of Mefloquine resistance, but its overall efficacy remains high.

Primaquine is a unique antimalarial showing a broad action against most of the parasite's different stages, including gametocytes. Moreover, it is the only licensed tissue-stage schizontocidal drug, and therefore is widely used for the prevention of relapses after infection in the two plasmodia species (*vivax* and *ovale*) that have such a characteristic. Resistance to this drug has been observed for *P.vivax*, but is yet not fully understood, and appears to be of no clinical consequence[83]. Resistance to the liver stages may already have developed, and if proven, could be disastrous due to the lack of licensed therapeutic alternatives.

Plant-derived drugs such as quinine or the artemisinin derivatives, used during centuries, seem to have outlived most of the synthetic antimalarial drugs that were developed subsequently. Confirmed resistance to the artemisinin derivatives has not been reported yet[84], although recrudescence among patients treated with short therapeutic courses has been observed[85], and probably is a consequence of the pharmacodynamic properties of these agents[86]. The development of resistance to these drugs has probably been delayed by its very short half life (4hours) and the drug's ability to reduce gametocytaemia, two important factors in the development of drug resistance. In this context, and to safeguard the effectiveness of these drugs, a consensus has emerged that they should never be used as monotherapy and should be combined with longer-acting antimalarials. Similarly, atovaquone resistance appeared as soon as it was introduced as monotherapy in 1996, but its use in combination with proguanil (Malarone®) has guaranteed high efficacy.

Other determinants of drug efficacy

Drug resistance *per se* is an important cause of treatment failure, but not all treatment failures are due to drug resistance. Several factors can be responsible for treatment failure, conditioning the effectiveness of malaria treatment, and it is possible that by causing treatment failure, such factors may all indirectly contribute to the development and intensification of true drug resistance by increasing the probability of exposing parasites to suboptimal drug levels.

The quality of drugs used to treat malaria episodes is clearly related to their efficacy. Poor manufacturing practices or deterioration due to inadequate handling or bad storage conditions can cause drugs to contain insufficient quantities of the active ingredients. Prescription of the correct drugs at incorrect dosages may have the same consequences. The lucrative and qualm-less industry of counterfeit drugs poses a serious threat in regions where the trade in pharmaceuticals is not rigorously regulated[87] and surely should be held responsible for not only the emergence of resistance but also for many avoidable deaths.

Incorrect adherence to the therapeutic regimens is another important determinant for treatment failure. Complex, inconvenient or poorly tolerated regimens carry a substantial risk of inadequate adherence. Often patients feel better after the first doses of treatment and abandon it, although they might have not yet cleared all parasites. Long regimens (such as quinine or primaquine) condition poor treatment compliance, and put at risk both the patient and the drug. User-friendly packaging and education of the patients have become important to improve adherence and with it increase treatment success. Young children may have difficulties swallowing tablets, and specific liquid or dispersible paediatric formulations, with friendlier taste may enhance efficacy by guaranteeing a better drug intake and hence compliance. Interactions with other drugs may modify absorption or metabolism of antimalarials, causing treatment failures which are not related to the efficacy of the drugs. Home-based treatment strategies, in which patients may treat fevers at a village-level with antimalarials, are increasingly considered in many developing settings, but may enhance drug-resistance if compliance is not adequate. Misdiagnosis is an extremely frequent problem, since symptoms of

malaria are not specific and other infections may present with identical clinical pictures. In this context, guidelines proposed by the WHO in their Integrated Management of Childhood Illnesses (IMCI) programme in malaria endemic countries suggest that all fevers should presumptively be treated with antimalarials. This strategy has surely saved many lives, but is probably leading to excessive over-diagnosis and unnecessary treatments, increasing drug pressure.

Drugs used for prophylaxis

Among populations living in malaria-endemic areas, it would seem reasonable to think that the use of widespread and maintained prophylaxis effect could play a role in reducing the burden of disease and death caused by malaria. However, this efficacious strategy to control malaria[88] has a very limited use as long-lasting tool to control malaria in areas where this disease is endemic, such as Sub-Saharan Africa. Several reasons support this, including the lack of adequate drugs, the evidence that, in children, continuous administration of an effective antimalarial drug jeopardizes the natural development of acquired immunity versus the disease, a prohibitive cost, a scarcity of functioning systems to guarantee the long-term adequate distribution to a wide proportion of the population, and finally the perceived risk of promoting drug resistance.

New and creative strategies, such as intermittent preventive treatment, have helped overcome a few of those hurdles. IPTi consists in the administration of three doses of an antimalarial with a long half-life (such as for instance SP) during the first year of life, taking advantage of the visits to health centres that infants make to receive the programmed vaccines framed within the EPI, which is universal[89]. IPTp uses the same rationale but applied to pregnant women attending clinics for the prenatal programmed visits[90]. The systematic use of IPTp is restricted to the African region, and 33 of the 45 African countries had adopted it as national policy by the end of 2006[5]. Research is being conducted at the moment in areas with different epidemiological characteristics, to ascertain whether these strategies should be globally recommended.

Current approaches to the treatment of malaria

Strategies to tackle malaria rely in a multidisciplinary synergistic approach. While an effective vaccine is an exciting perspective[91], a hypothetical

deployment is likely to occur only in a few years. Vector control strategies are currently being reconsidered, and the widespread mass distribution of insecticide treated bednets (ITNs) is being promoted in malaria endemic areas. However, since the World Health Organization's Global Malaria Eradication Campaign was abandoned in 1969, focus has been placed on treatment rather than in prevention. Effective case management remains therefore the cornerstone of malaria control strategies, and in the past decades, this has relied essentially in the use of inexpensive and widely available antimalarial drugs, such as chloroquine or more recently sulfadoxine-pyrimethamine (SP). An alarming waning efficacy has been observed for those two drugs in several parts of the world, and is now affecting Africa, where the major disease burden is observed, and where those two drugs had managed to constrain the pandemic. While in the recent years the death toll for other major child killers in developing countries (notably pneumonia, diarrheal diseases and measles) has fallen, deaths from malaria have increased. The main reason behind such an increase in malaria-related mortality, and for the global resurgence of malaria in the last three decades[92], lies in the continuous deployment of ineffective antimalarial drugs in the face of increasing resistance[93, 94]. Drug resistance has also been implicated in the comeback of malaria in areas where the disease had previously been eradicated, and in the occurrence and severity of epidemics in some parts of the world[95].

The inexorable spread of drug-resistant parasite strains has also threatened some of the existing limited alternative antimalarial drugs, particularly in South East Asia. Misuse and poor adherence to treatment regimens, inadequate absorption, intolerability, or the disturbing increase of counterfeit or inappropriate quality drugs have all contributed to the reduced effectiveness of antimalarial drugs. Parasites have managed to develop resistance against most of the existing drugs, but to date, there is no clear evidence of resistance to artemisinins, a family of highly effective antimalarial compounds. Combined therapies, including artemisinin-derived drugs have emerged as the best current approach against resistance and more than 40 countries have adopted them in their National treatment policies[96], despite their prohibitive cost. These same economic reasons have however constrained many other poor countries to maintain the older therapies, despite clear evidence of their weak efficacy.

4.10 Malaria in Manhiça

Data from the Mozambican national malaria control programme reported at the end of the 20th century a national malaria prevalence of 40%, and confirmed that as many as 44% of the country's outpatient visits, 57% of paediatric admissions and 29% of all hospital deaths were caused by malaria[97]. However, the accuracy of national malaria statistics such as these, is heavily dependent on access to health facilities and patterns of health seeking behaviour, and therefore may be heavily biased in countries such as Mozambique, where access to care is a major challenge. Indeed, according to Mozambique's Ministry of Health, up to 60% of the population has no access to curative facilities from the National Health Service. In such cases, epidemiological studies become necessary for obtaining reliable data to guide the planning and conduct of control strategies, as the silent burden of malaria is greatest in the rural areas, where people often live far from health facilities and in absence of adequate malaria surveillance systems or control tools.

Prior to the establishment of the Manhiça research centre, epidemiological studies describing the community incidence of malaria in Mozambique were scarce and normally restricted to urban or semi-urban settings[97].

Malariometric indexes

As part of a larger study of the epidemiology of malaria in southern Mozambique, comprehensive estimates of different malariometric indicators, including the prevalence and incidence of malaria in Manhiça district, were produced during the years 1996-99[97-99]. In a first study[98], almost 2000 children were followed in two different cohorts (including one birth cohort) on a weekly basis during a period ranging from 12 to 31 months, and used to ascertain the incidence of clinical malaria in the area. In this home-based active case detection, blood slides were taken whenever fever or a history of fever in the preceding 24 hours was referred. Albeit some seasonality, malaria occurred all year round, and was absent in the first two months of life. Incidence rates rose drastically in the first year of life, peaking at 0.9 episodes/100 person-weeks at risk in those aged 8-12 months, and children aged 6 months to 4 years showed the highest incidence ranging from 0.65 to 0.74 episodes/100 person-weeks at risk. The number of clinical malaria episodes ranged from 0 to 6 per child. No clinical malaria was detected in up to 70% of the children, and some spatial

clustering of cases was found in the proximity of swampy areas or the river bed. The prevalence of malaria in the community was assessed in a second study[97], in which more than 2000 children aged < 10 years were investigated in 4 different cross-sectional surveys performed during different seasons. *Plasmodium falciparum* accounted for 90% of all malaria infections, and the prevalence of asexual *P. falciparum* ranged from 13.7-21.7% at the end of the dry season to 30.5-34.0% at the end of the rainy season. The malaria attributable fraction (MAF) of fever was determined using 1021 hospital fever cases and corresponding afebrile controls from the cross-sectional surveys. The crude MAF was 36%, and showed a marked age-dependency, highlighting the importance of other non-malarial fever-causing conditions.

Drug resistance in the area

For many decades, and similarly to the rest of Sub-Saharan African countries, treatment of uncomplicated malaria in Mozambique relied entirely on chloroquine, a safe and inexpensive drug which remained highly effective against the parasite. The spread of resistance to this drug posed major challenges to malaria control programmes and policy makers, forcing countries to rapidly adapt to new and generally more costly first line therapies. First evidences of CQ resistance in Mozambique were reported in 1983[100], but despite the early understanding of the problem, first line treatment with CQ was only switched to AQ-SP in 2003, two decades later. Two consecutive trials performed in Manhiça during the first years of the millennium contributed to the assessment of drug efficacy in the area and provided evidence-based data to support the policy change[101]. The first trial, conducted in 272 children with uncomplicated malaria in the year 2001, assessed the clinical efficacy and parasitological response of *P. falciparum* to three drugs used in monotherapy, namely CQ, AQ and SP. Efficacy was high for AQ (91.6%) only, moderate for SP (82.7%), and low for CQ (47.1%). Parasitological resistance, measured at day 14, was detected for the three drugs, but was higher for CQ (69%) than for the other two (SP 21.4% and AQ 26%). The second trial conducted in 2002, investigated the safety and efficacy of different drug combinations, namely AQ+SP, AQ+AS and SP+AS. The three combinations showed 100% clinical efficacy when measured at day 14. These trials[101] confirmed that CQ resistance was unacceptably high for the drug to remain first-line treatment, and for this

reason Mozambique adopted the combination of AQ+SP in 2003 as the National policy for treating uncomplicated malaria. Since then, further changes in National policy have been made after WHO's recommendation for the introduction of ACTs throughout Africa[102].

5. Specific introduction to this thesis

This thesis is based on work undertaken through a partnership between Barcelona's centre for International Health Research (CRESIB) and CISM, Manhiça's Health Research Centre, in Mozambique. The partnership benefited from collaborations with the Mozambican Ministry of health, the director of Mozambique's "*Programa Nacional de control da Malária"*, the district and local health service authorities, and the director and personnel from Manhiça's district hospital. Some of the work presented in this thesis also involves other African research institutions, as some of these studies were carried out as inter-African multi-centre programmes. The studies received support from the Spanish Agency for International Cooperation (AECI), the Spanish Fondo de Investigaciones Sanitarias (FIS), and MMV (Medicines for Malaria Venture). The work in the two clinical trials presented in this thesis was performed in tight collaboration with two pharmaceutical companies, namely Sigma Tau Industrie Farmaceutiche Riunite, (Pomezia, Italy), and Novartis (Basel, Switzerland).

Both the first and second papers presented in this thesis report results from rigorously-conducted randomised non-inferiority clinical trials, assessing the efficacy and safety of different antimalarial drug combinations. These studies have been conducted in collaboration with research institutions from various other African countries, and reported according to internationally accepted CONSORT guidelines[103]. The third and fourth papers presented here report data obtained on admitted children with severe malaria to a rural district hospital in Southern Mozambique. These are two thorough analyses describing the malaria burden of disease in the area, the importance of coexisting infections, and factors involved with a poor prognosis. Despite these data coming from a single hospital in Mozambique, they may still be highly informative and relevant for other endemic areas.

5.1 First paper

The first paper documents the safety and efficacy of the co-formulated, GMP-produced, Dihydroartemisinin-piperaquine (DHA-PQP) combination for the treatment of malaria in African children, when compared to Artemether-lumefantrine (AL). This trial included 1553 children with uncomplicated malaria,

aged 6 months to 5 years, recruited from 5 different African countries. As the World Health Organization praises the use of combination therapies for the treatment of malaria, there is a paradoxical scarcity of data regarding valid alternative ACTs to the one most widely deployed in endemic areas, namely artemether-lumefantrine. This trial highlights the excellent safety profile of DHA-PQP, and its non-inferiority in terms of efficacy, as measured by standard, PCR corrected, day 28 ACPR. Interestingly, DHA-PQP seems to maintain its safety profile and efficacy among infants (children younger than 1 year of age), an age group particularly vulnerable to malaria and from which insufficient data are available in Africa. This study also provides robust data on DHA-PQP's additional superior efficacy in preventing new infections (post-treatment prophylactic effect). Finally, no concerns seem to arise from the trial's results regarding any potential cross resistance between piperaquine and the structurally related chloroquine, despite high chloroquine resistance levels documented previously in most of the participating sites.

5.2 Second paper

The second paper on this thesis reports the results of a randomised, single-blind, multicentre trial designed to assess the safety and non-inferiority of artemether-lumefantrine dispersible tablets (paediatric-friendly) compared with the widely available commercial tablets, crushed. It involved 899 children aged 6 months to 12 years, in 8 health facilities from 5 different African countries. The motivation for this study was the realisation that crushed tablets, the commonly available method of administering AL to children incapable due to their age of swallowing a full tablet, may have been a suboptimal way of delivering the drug to youngest children; perhaps the population group at most need of being correctly treated for malaria. The study shows that a six-dose regimen of artemether-lumefantrine with the new dispersible formulation is as efficacious as the currently used crushed tablet in infants and children, and has a similar safety profile. Moreover, by extending efficacy analysis to 42 days of follow-up, it also confirms that this efficacy was sustained, even under the pressure of moderate to high transmission intensities.

5.3 Third paper

The third paper describes features on admission to hospital and risk factors for death in African children with malaria in an area of intense *Plasmodium falciparum* transmission such as rural Mozambique. Detailed descriptions of the clinical presentation of malaria and of the risk factors associated with a bad prognosis are scarce in the literature, and were unavailable for a country such as Mozambique. These data provide invaluable guidance to help identify children who are at highest risk of death and tailor accordingly and most efficiently the limited available resources in areas where health systems are fragile. Particular attention is drawn to the description of the high burden of malaria-related severe disease detected at Manhiça's district hospital, particularly among the youngest children, and to the importance of terms of high incidence rates of severe malaria at the community level. Attention is also drawn to the importance of simple clinical signs and symptoms, which, when present, should forewarn the clinician as they have been found to associate a poor prognosis.

5.4 Fourth paper

The fourth article describes in detail the prevalence, aetiology and prognostic implications of coexisting invasive bacterial disease in children admitted with severe malaria in a rural Mozambican Hospital. Previous reports had explored this interaction, and this paper confirms that this relation is both frequent and life-threatening. Few assessments are available in the literature of such an interaction in countries where not only malaria, but also HIV is highly prevalent. The paper highlights the importance of *Streptococcus Pneumoniae* as the principal bacteria related to severe malaria cases, in comparison to Non-typhoid Salmonella, traditionally associated with severe malaria cases, and in particular with the development of severe malarial anaemia. The paper speculates that the high HIV-related immunosuppression present in Manhiça may be partly responsible for these findings, which challenge previous commonly held beliefs.

5.5 The way forward

The challenge now is to move forward from these four studies, and translate their conclusions into improvements in malaria control strategies. New drugs, and new drug formulations, better suited to tailor the needs of those populations at greater risk such as infants and younger children, need to be registered and deployed. Both clinical trials presented here are a critical part of the respective clinical development plans for either DHA-PQP or the paediatric friendly dispersible AL tablets, and their results will be pivotal for the registration plans of both products. If the spectrum of antimalarial drugs or specific formulations available becomes wider, clinicians facing the need to treat malaria in endemic areas will benefit from a much better choice range. Presumably, this will also lower the prices, as competition will drive market forces. This is likely to further benefit patients, as one of the underlying problems of achieving universal and prompt effective treatment of malaria are the prohibitive costs of the currently recommended drug combinations, as compared to traditionally cheaper, albeit less effective first generation anti-malarial drugs, such as chloroquine or SP.

A more precise understanding of the clinical presentation of malaria, and of which signs or symptoms are associated with a higher risk of dying will surely be useful to identify on arrival those cases that need special attention by clinicians. This will allow for specifically targeted training for clinicians and more critical approaches to clinical algorithms proposed in the context of the integrated management of childhood illnesses (IMCI) program. In this specific context, understanding the burden and detrimental prognostic implications of coexisting bacterial infections may prove of additional value to critically assess the overall performance of such strategies.

These studies make a contribution to the improvement of malaria control by highlighting areas in which enhanced case management may be possible, and by providing innovative and alternative pathways for the effective treatment of this infection. These studies need to be pursued by more research in different geographical areas, and especially by proactive efforts to translate their conclusions into policy. This may guarantee the sustainability of these approaches and a wider impact in terms of contributing to the reduction of the intolerable burden of this disease.

6. Hypotheses and objectives

6.1 General objectives

1. Evaluate the efficacy and safety of new antimalarial drug combinations in African children
2. Describe severe malaria at a rural hospital setting and its interactions with other diseases.

6.2 Specific objectives

1. Estimate the efficacy of DHA-PQP when compared to AL in African children <5 years of age.
2. Assess the safety of DHA-PQP when used as treatment for uncomplicated malaria in African children <5 years of age.

3. Estimate the efficacy of the new paediatric dispersible formulation of AL when compared to the conventional AL in African children <5years of age.
4. Assess the safety of the new paediatric dispersible formulation of AL in African children < 12 years of age

5. Describe the clinical presentation of clinical malaria in children presenting to Manhiça district hospital (MDH), in southern Mozambique
6. Describe the risk factors for death in children admitted with malaria to MDH
7. Describe the incidence rates (MCBIR) of severe malaria in the Manhiça community

8. Describe the prevalence and aetiologies of invasive bacterial disease in patients with malaria
9. Assess whether bacteraemia is a risk factor for death in severe malaria patients

7. Materials and methods

7.1 Origin of patients in the different studies

Children involved in the 4 studies were recruited or admitted, at least in part, by personnel working for CISM in Manhiça's district Hospital (MDH). The first two studies were part of two different multicentre trials. The first multicentre trial was possible thanks to the establishment in 2005, of a public-private partnership programme funded by the Medicines for Malaria Venture (MMV) and led by the Italian Company Sigma-Tau I.F.R. SpA (Rome) in collaboration with the University of Oxford, set up for the international registration of DHA-PQP. This included a phase III, randomized multicentre trial to test the non-inferiority of DHA-PQP compared with AL in treating non-complicated malaria in African children. Five different African countries (Burkina Faso, Uganda, Zambia, Kenya and Mozambique) participated in this study, which has been presented as study number 1. *Circa* 1500 children were recruited, two thirds of which were randomly allocated to the DHA-PQP group, and the remaining group to the comparator drug AL. Manhiça contributed with a third of all recruited patients.

Study number 2 was designed as a randomised, single-blind multicentre trial to assess the safety and efficacy of a new paediatric formulation of AL. It involved almost 900 children up to 12 years of age recruited from 8 different study sites in 5 African countries (Benin, Kenya, Mali, Mozambique, and Tanzania). It also involved a public-private partnership programme funded by the Medicines for Malaria Venture (MMV) and by the Swiss pharmaceutical company Novartis.

Studies number 3 and 4 were performed solely in MDH, by retrospectively analysing all paediatric admissions from 2003 to 2007.

7.2 Manhiça's Health Research Centre (CISM)

The Manhiça Health Research Centre (*Centro de Investigação em Saúde da Manhiça*, CISM) was developed with the mission of improving health and development through provision of health care, and carrying out research in priority health problems.

Figure 5: Map of Mozambique, and location of Manhiça District

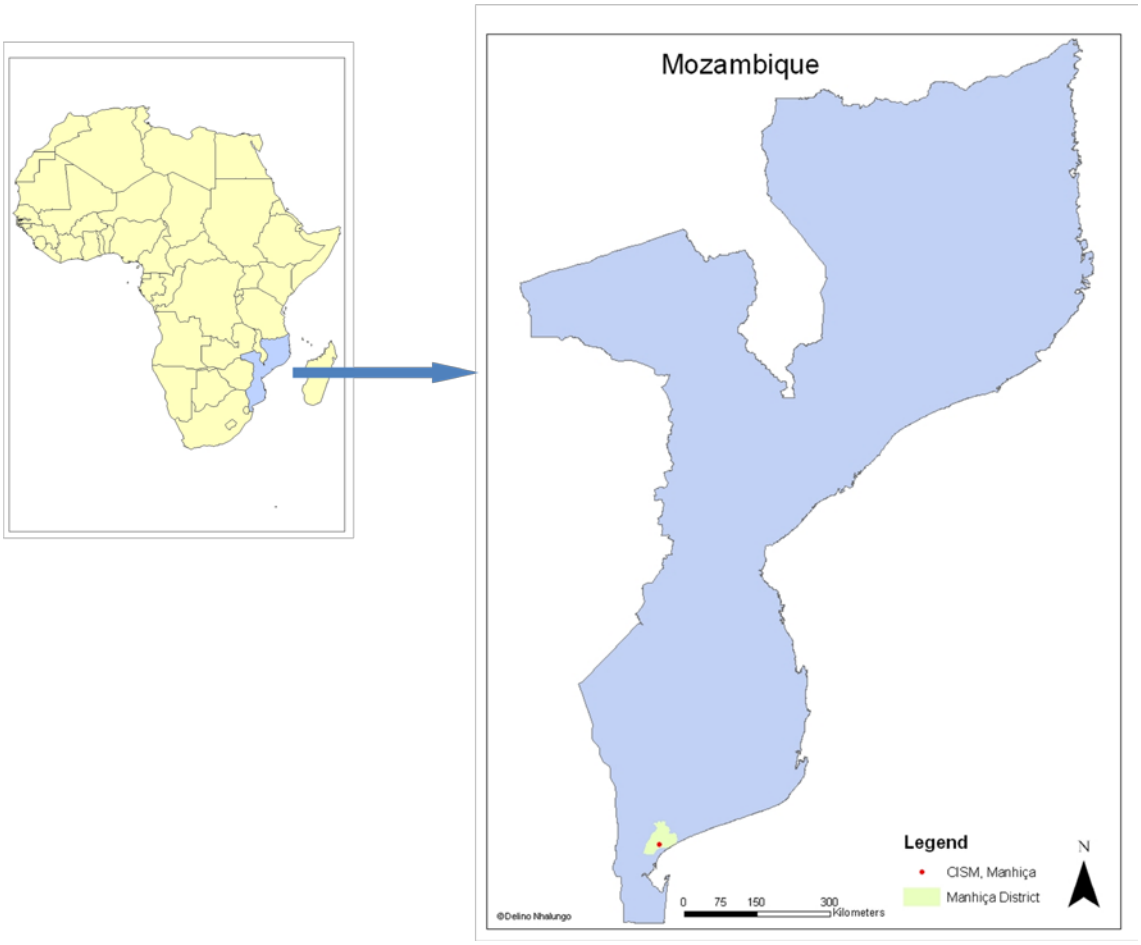
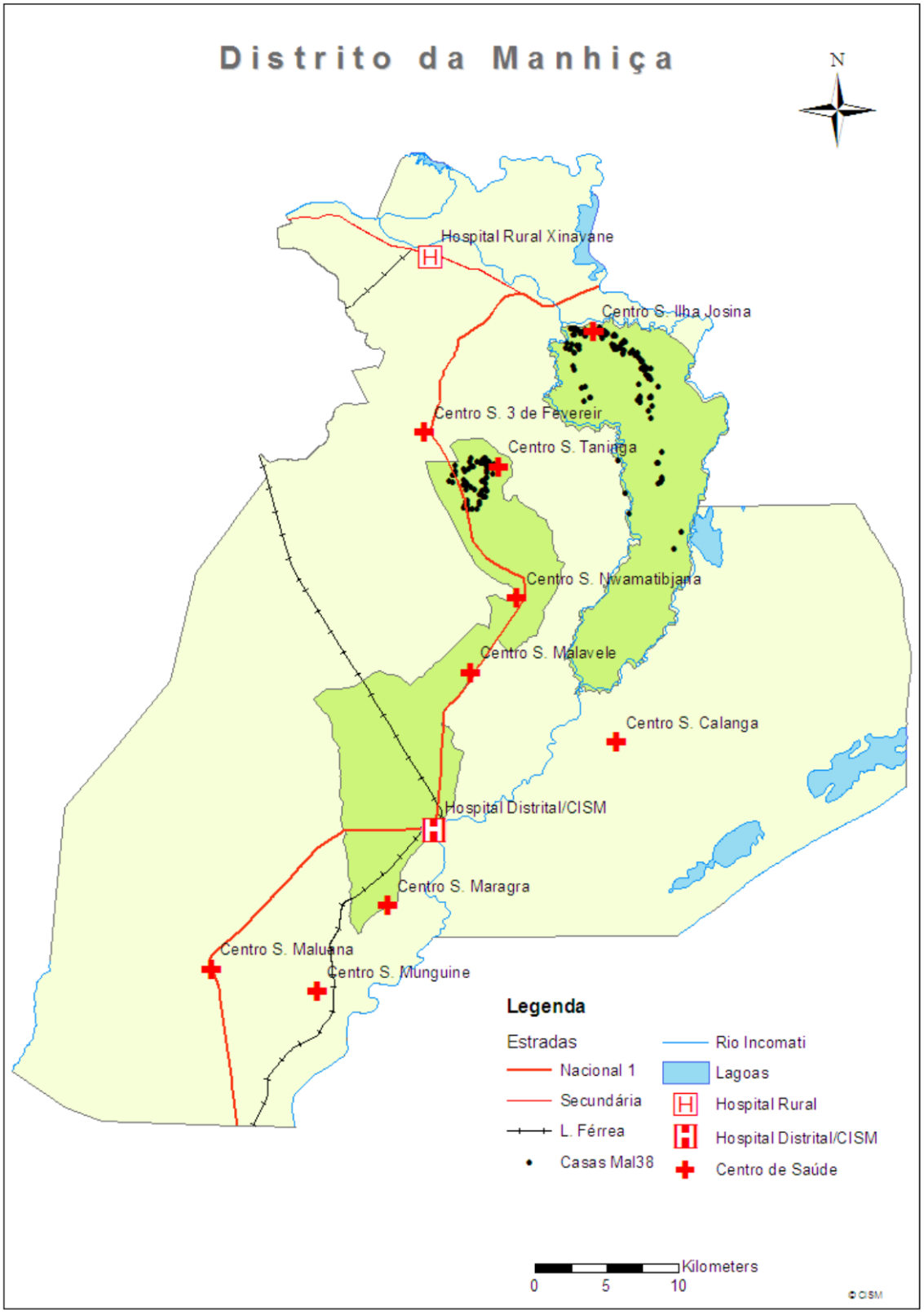


Figure 6: Map of Manhiça District



CISM has been running a Demographic Surveillance System in the area since 1996[104] and a morbidity surveillance system at Manhiça District Hospital and other peripheral health posts[105]. By linking the information obtained through its morbidity surveillance system to the demographic data available for the DSS area, CISM has provided in recent years detailed descriptions of the health status of the community.

7.3 Study area and population

The study area is located in Manhiça, Maputo Province, Southern Mozambique (Figures 5, and 6). A full description of the geographic and socio-demographic characteristics of the study community has been presented elsewhere[104]. There are two different seasons: a warm and rainy one between November and April and a cool dry period during the rest of the year. During the years 2006 and 2007, the mean temperature was 22.8ºC, ranging from a mean monthly temperature of 26.6ºC in March to 18.8ºC in July. During that same time period, the total mean annual rainfall was 872mm.

The district has an estimated population of 140000 inhabitants. The DSS covers Manhiça town and the surrounding villages, an area of around 100 km^2, with a total population under surveillance of around 82000 inhabitants, 17% of which are under 5 years of age. In 2005, the infant and under-five mortality rates for the study area were 77.5/1000 and 138.6/1000 respectively[106]. The HIV prevalence among pregnant women attending the antenatal clinic is high (23.6%)[107]. All children resident in the study area have a card with a permanent identification number issued by the DSS.

7.4 Health care facilities and morbidity surveillance

The Manhiça District Hospital (MDH), with a 110-bed inpatient ward, is the main health facility in the area, used for primary health care by the nearby population, and one of the two referral health centres for Manhiça District[108]. There are two other peripheral health posts in the area, used only for primary health care. Both the hospital and the health posts are easily accessible and all outpatient consultations are free, except for a standard subsidized fee for outpatient medication to be taken home.

A passive case detection system was established in 1996 to cover all paediatric (children aged 0-<15 years) outpatient and inpatient visits to MDH and all outpatient visits to the other 3 peripheral health posts within the DSS (Maragra, Taninga and Ilha Josina). A standardized questionnaire, which includes personal and demographic data (including the permanent identification number) and clinical signs and symptoms, is completed for each child seen at the outpatient clinic. The clinician on duty, usually a medical agent (with a minimum clinical training of at least 2 years after secondary school), records the physical signs found on examination and the symptoms and duration of those as referred by the child's guardian. The axillary temperature is recorded and a finger-prick blood sample is collected from children who present fever (axillary temperature ≥ 37.5 ºC) or report a history of fever in the preceding 24 hours. Blood is collected into heparinised capillaries to measure the packed cell volume (PCV), and thin and thick blood smears are prepared to determine parasitaemia. Final diagnosis/es are recorded on the questionnaire by the clinician upon discharge, transference to the Hospital or admission to the ward, after review of all signs, symptoms and laboratory results.

For all paediatric admissions, a second more detailed standardised questionnaire is also completed. A physician or experienced medical officer performs a physical exam of the child on admission and upon discharge or death up to four final diagnoses, based on the ICD classification of diseases, are recorded on the questionnaire after review of clinical evolution and laboratory results. As part of an ongoing bacterial disease surveillance programme, blood cultures are routinely collected from all children under 2 years of age, and among older children with hyperpyrexia (≥ 39ºC), neurological signs or suspected sepsis. HIV status is not routinely assessed in MDH.

For the two clinical trials, a specific study facility was set up at Manhiça's district hospital, and patients were admitted there for their initial observation (figure 7).

Figure 7: Antimalarial drug trial admission facility, at Manhiça's district hospital (photo by Quique Bassat)

7.5 Ethical considerations

The study protocol for the first randomised controlled trial was approved by the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, the Ethical Committee of the Antwerp University Hospital, the University of Heidelberg Ethics Committee and by the National Ethics Review Committee or Institutional Review Board at each trial site. For the second trial, ethical clearance was also sought for each individual IRB and National Ethics Committee at each site. Both trials were conducted under the provisions of the Declaration of Helsinki and in accordance with Good Clinical Practices

guidelines set up by the International Conference on Harmonization. For the other two studies, no specific Ethical approval was sought, as they consisted mainly in retrospective analysis of clinical data, although the invasive bacterial surveillance system described in study number 3 was included in several study protocols reviewed and approved by the Mozambican National Bioethics Committee and institutional review boards of Hospital Clinic of Barcelona, Spain, the U. S. Centres for Disease Control and Prevention and the University of Maryland School of Medicine.

7.6 Data management and statistical analysis

For the first and second studies, study questionnaires were transcribed into Case Report Forms (CRFs), which were then monitored and introduced into a centralised database together with the questionnaires from the other participant countries. For the last two studies, involving only the Manhiça site, study questionnaires were double entered using a programme written in FoxPro version 5.0 (Microsoft Corp., Seattle, WA, USA). In such cases, statistical analyses were done with Stata 10.0 (Stata Corp., College Station, TX, USA).

8. Articles

Study 1

Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for treating uncomplicated malaria in African children: A randomised open-label, non-inferiority trial.

Quique Bassat, Modest Mulenga, Halidou Tinto, Patrice Piola, Steffen Borrmann, Clara Menéndez, Michael Nambozi, Innocent Valéa, Carolyn Nabasumba, Philip Sasi, Antonella Bacchieri, Marco Corsi, David Ubben, Ambrose Talisuna, Umberto D'Alessandro

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Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for Treating Uncomplicated Malaria in African Children: a Randomised, Non-Inferiority Trial

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29

30

Abstract

Background

Artemisinin combination therapies (ACTs) are the best option for treating uncomplicated malaria. Dihydroartemisinin-piperaquine (DHA-PQP) is a promising fixed-dose ACT with limited information on its safety and efficacy in African children.

Methodology/Principal Findings

The non-inferiority of DHA-PQP versus artemether-lumefantrine (AL) in children 6–59 months old with uncomplicated *P. falciparum* malaria was tested in five African countries (Burkina Faso, Kenya, Mozambique, Uganda and Zambia). Patients were randomised (2:1) to receive either DHA-PQP or AL. Non-inferiority was assessed using a margin of -5% for the lower limit of the one-sided 97.5% confidence interval on the treatment difference (DHA-PQP vs. AL) of the polymerase chain reaction (PCR) corrected day 28 cure rate. Efficacy analysis was both intention-to-treat (ITT) and per-protocol (PP). 1553 children were randomised, 1039 receiving DHA-PQP and 514 AL. The PCR-corrected day 28 cure rate was 90.4% (ITT) and 95.7% (PP) in the DHA-PQP group, and 90.0% (ITT) and 95.7% (PP) in the AL group. The lower limits of the 97.5% CI of the difference between the two treatments were -2.80% and -2.24%, in the ITT and PP populations, respectively. The risk of recurrent infection at day 28 was significantly lower in the DHA-PQP (ITT 12.3%; PP 7.1%) than in the AL (ITT 23.3%; PP 18.6%) group.

Conclusions

Dihydroartemisinin-piperaquine is as efficacious as AL in treating uncomplicated malaria in African children from different endemicity settings, and shows a comparable

safety profile. It shows also a statistically superior efficacy in preventing new infections.

Name of registry: ISRCTN; registry number: ISRCTN 16263443

URL: http://www.controlled-trials.com/ISRCTN16263443/16263443

INTRODUCTION

Artemisinin-based combination therapies (ACTs) are highly efficacious and fast acting antimalarial medicines. The World Health Organization (WHO) recommends their use for treating uncomplicated malaria[1]. In Africa, their introduction on a wide scale began in 2003 and currently most African countries have adopted or are using ACTs as first or second line treatments, either artesunate-amodiaquine or artemether-lumefantrine (AL)[2], available as co-formulations produced under GMP, though the former is also used as a co-blistered or non-co-formulated product. The co-formulation of dihydroartemisinin (DHA), the active metabolite of artemisinin derivatives, with piperaquine (PQP), a bisquinoline structurally close to chloroquine, seems to be a promising combination and a good alternative to AL, whose optimal use in the public health system is challenged by the twice-daily dosing scheme and the need for co-administration with fatty food [3], necessary for improving the absorption of lumefantrine. DHA-PQP provides a simpler dosage scheme (a single daily dose over 3 days) than AL and can be given without food. Several trials [4-7] have assessed DHA-PQP safety, efficacy and effectiveness [8], mostly in Asia, reporting an efficacy of about 90% over 28–63 days [4]. There is little information on the safety and efficacy of DHA-PQP in African children, as only a few single-centre trials [9-12] have been done in Africa. DHA-PQP is registered in several countries in Africa and South-East Asia and has been widely used in Vietnam or Cambodia, though these formulations are not manufactured according to internationally recognised GMP. In 2005, a public-private partnership programme funded by the Medicines for Malaria Venture (MMV) and led by the Italian Company Sigma-Tau I.F.R. SpA (Rome) in collaboration with the University of Oxford was set up to fill the gaps needed for the international registration of DHA-PQP. This included a phase III, randomized multicentre trial to test the non-

inferiority of DHA-PQP compared with AL in treating uncomplicated malaria in African children.

METHODS

The protocol for this trial and supporting non-inferiority adapted[13] CONSORT checklist are available and annexed as supporting information.

Ethical considerations and patient safety

The study protocol was approved by the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, the Ethical Committee of the Antwerp University Hospital, the University of Heidelberg Ethics Committee and by the National Ethics Review Committee or Institutional Review Board at each trial site. The trial was conducted under the provisions of the Declaration of Helsinki and in accordance with Good Clinical Practices guidelines set up by the International Conference on Harmonization. A Study Steering Committee, a Data Monitoring Committee and a Clinical Development Committee were created prior to the beginning of the trial, and worked independently to harmonise and monitor the study. The trial was registered prior to the enrolment of the first patient in the International Standard Randomized Controlled Trials Register, number ISRCTN 16263443, at http://www.controlled-trials.com/isrctn.

Study design, sites and concealment of patient allocation

Between August 2005 and July 2006, a randomised, open-label, multicentre clinical trial was carried out in five African sites (Nanoro, Burkina Faso; Kilifi, Kenya;

Manhiça, Mozambique; Mbarara, Uganda; and Ndola, Zambia) Characteristics of the five sites are summarized in Table 1. [ONLINE PUBLICATION ONLY]

Children 6–59 months old attending the health facilities with uncomplicated malaria were included in the study if they fulfilled the following inclusion criteria: body weight >5 kg; microscopically confirmed *Plasmodium falciparum* mono-infection with asexual parasite densities between 2,000 and 200,000 /µl; fever (axillary temperature ≥37.5ºC) or history of fever in the preceding 24 h. Patients were not recruited if they met at least one of the following exclusion criteria: severe malaria [14], or other danger signs; acute malnutrition (weight for height <70% of the median National Center for Health Statistics/WHO reference) or any other concomitant illness or underlying disease; contra-indication to receive the trial drugs or history of treatment with any antimalarial drug or drug with antimalarial activity within the 14 days preceding enrolment. Patients satisfying the inclusion/exclusion criteria were enrolled if the parent/guardian signed a detailed written informed consent.

Patients were individually randomised according to a 2:1 (DHA-PQP:AL) scheme, so as to have more patients in the DHA-PQP arm to provide better estimates for its cure rates and more cases for the integrated safety data base. A randomisation list stratified by country was generated by an independent off site contract research organisation (CRO), with each treatment allocation concealed in opaque sealed envelopes that were opened only after the patient's recruitment.

Both drugs were administered under direct supervision during 3 consecutive days, according to the patient's body weight. AL (Coartem™, Novartis, Switzerland) was

administered twice a day (at enrolment and at 8, 24, 36, 48 and 60h) according to the following dosage: weight 5–14 kg: one tablet per dose; weight 15–24 kg: two tablets per dose; weight 25–34 kg: three tablets per dose. DHA-PQP (Eurartesim™, Sigma-Tau, Italy) was given once daily, at the standard dosage of 2.25 mg/kg and 18 mg/kg per dose of DHA and PQP, respectively, rounded up to the nearest half tablet. To facilitate the correct dosing of DHA-PQP, two formulations were used (DHA 20 mg + PQP 160 mg and DHA 40 mg + PQP 320 mg). In case of vomiting, a full dose was repeated if this occurred within the first half an hour, or half a dose if it occurred between 30 minutes and 1 h. AL was administered concomitantly with milk. For infants, drugs were crushed, mixed with water and administered as slurry. In order to minimise bias, treatment allocation was concealed until recruitment of the patient was completed. Both patient allocation to the different analysis populations and assessment of the primary end-point were made by staff blinded to the treatment assignment and before availability of the PCR results.

Treatment follow-up, clinical and laboratory procedures

All children were kept at the health facility for the 3-day dosing period. The mother/guardian was asked to return with the child for scheduled visits on days 7, 14, 21, 28, 35 and 42 post-treatment, or if any symptoms occurred. Field workers traced patients missing any visit. For each visit, a physical examination was performed by the study clinicians, vital signs were recorded, and axillary temperature measured with an electronic thermometer. Adverse events and serious adverse events were recorded and monitored throughout the study. A 12-lead electrocardiogram (ECG) was performed at enrolment and repeated on days 2 and 7 to assess any QT/QTc interval prolongation.

Any ECG abnormality detected at enrolment requiring urgent management was considered an exclusion criterion. All ECG records were transmitted daily online to a central cardiologist (Paris, France) who interpreted them in a blinded manner, and feedback was sent to the sites as soon as available. The QTc interval (ms) was evaluated after correcting for the heart rate with Bazett's or Fridericia's formulae and classified according to the following categories: Normal <430 ms; Borderline: 431-450 ms; Prolonged >450 ms.

The study was supervised by monthly monitoring visits. Rescue treatment for recurrent parasitaemia was according to local national guidelines. All participants, with the exception of those in Kilifi, received a free insecticide-treated bed net at recruitment.

Capillary or venous blood was taken at every visit. Thick and thin blood films were prepared, dried and Giemsa-stained, and parasite density estimated by counting the number of asexual parasites in 200 white blood cells (WBC), assuming a standard WBC count of 8,000 /µl. All slides were double read and discrepant ones were solved by a third reading. In addition, quality control was performed on 20% of all the slides at a central laboratory. Samples for haematology (full blood count) and biochemistry (liver and renal function) were taken at enrolment, at days 3, 28 and 42, and at any other visit if judged necessary by the clinician. For PCR analysis, three blood spots were collected on filter paper (Whatmann 3MM) at enrolment and at any visit after day 7. Each filter paper was dried and individually stored in a plastic bag containing silica gel. All filter papers were subsequently transferred to the Institute of Tropical Medicine (Antwerp, Belgium) where centralised genotyping was conducted. Purification of DNA was conducted as previously described [15]. Three polymorphic genetic markers MSP1,

MSP2 and GluRP were used to distinguish recrudescence from new infections [16,17]. Recrudescence was defined as at least one identical allele for each of the three markers in the pre-treatment and post-treatment samples. New infections were diagnosed when all alleles for at least one of the markers differed between the two samples. All gels were re-read under blinded conditions by an independent expert (National Museum of Natural History, Paris, France). In addition, 20% of the filter papers were re-analysed and assessed by an independent laboratory (Shoklo Malaria Research Unit, Mae Sot, Thailand).

Outcome classification

The primary endpoint was the PCR-corrected adequate clinical and parasitological response (ACPR) at day 28; secondary efficacy outcomes included PCR-corrected cure rates at days 14 and 42, PCR-uncorrected cure rates at days 14, 28 and 42; parasite and fever clearance times, presence and clearance of gametocytes, and haemoglobin (Hb) recovery from baseline to day 28. All standard safety outcomes such as incidence of adverse events, changes from baseline on haematology and clinical chemistry parameters, ECG findings and vital sign variation during the study were also evaluated.

Treatment outcome was analysed in two ways. The first was based purely on the standard definitions of early/late clinical and parasitological failure (World Health Organization)[2]. The second, based on a pre-defined (in the protocol) procedure further developed with the Data Monitoring and the Clinical Development Committees, complemented the WHO definitions with a set of rules allowing the evaluation of each individually randomised patient, e.g. patients having taken not-allowed anti-malarial drugs or with halfway missing data such as blood parasitaemia.

Statistical analysis

The intention-to-treat population (ITT) included all randomised patients having taken at least one dose of the study treatments. The per-protocol (PP) population included all randomised patients fulfilling the protocol eligibility criteria, having taken at least 80% of the study medication, completing the day 28 assessment and having an evaluable PCR in case of recurrent parasitaemia. All drop-outs and all patients with missing or non-interpretable PCR results were evaluated as failures in the ITT population and excluded from the PP population.

Efficacy analysis was based on a 97.5% (one-sided) confidence interval (CI) computed on the difference between the day 28 PCR-corrected cure rates of DHA-PQP and AL. To prove non-inferiority, the lower limit of this CI was to be within -5%, the non-inferiority margin. The Wald method (without continuity correction) was used to compute the CI, as this method was known to provide control of type I error around the nominal level for the 2:1 allocation, and also in the context of a hypothesis test of non-inferiority [18]. Secondary outcomes were assessed similarly. In addition, for sensitivity purposes, estimates of the failure rates were generated with the survival analysis, using the Kaplan-Meier method in which patients were censored when they were lost to follow-up, had a new infection or a non valid PCR result. When survival analysis was applied to the new infections, censoring of new infections was replaced by censoring of recrudescences. The Breslow-Day test, or logistic regression when the former was not applicable, was used to assess homogeneity across centres. For exploratory testing, categorical variables were compared using χ^2 or Fisher's exact test, and continuous variables using the Student t-test for independent samples. Cure rates

were stratified by age (age groups: ≤12 months; >12 months), though the study was not powered for proving efficacy within each age group.

Rates of person-gametocyte-weeks for measuring gametocyte carriage and transmission potential were calculated as the number of weeks in which blood slides were positive for gametocytes divided by the total number of follow-up weeks and expressed per 1,000 person-weeks.

Sample size calculation

This study was designed as a non-inferiority trial. According to previous studies, the PCR-corrected cure rate at day 28 for AL was estimated at around 90–92% [18] in the modified ITT population and 94–95% in the PP population. Assuming 80% statistical power, a one-sided α level of 2.5%, and adopting an unequal 2:1 randomisation ratio, 1,500 patients (1000 DHA-PQP, 500 AL) were needed to show that the difference of the day 28 PCR-corrected cure rates between DHA-PQP and AL was within -5%. This estimation accounted also for the rate of protocol violations (30% patient attrition rate in the PP).

RESULTS

Trial profile and baseline characteristics

Overall, 2,001 patients were screened, and 1,553 recruited and randomised to receive the study drugs (1,039 DHA-PQP and 514 AL) (Figure 1). Five patients were excluded from all analyses: one child in each treatment group who did not receive any treatment and three children in the AL group who were recruited twice (only data for the first recruitment were retained). A total of 1,548 patients were considered for the ITT population and the safety analysis, and the PP population consisted of 1,413 patients. The attrition rate of the PP population as compared to the ITT was approximately 9% and was due to lost-to-follow-up (~2%) or major protocol violations (~7%). These proportions were equally distributed between treatments (data not shown).

Randomisation generated comparable groups between countries and overall (Table 2).

Efficacy results

DHA-PQP was as efficacious as AL. The day 28 PCR-corrected cure rate was 90.4% (ITT) and 95.7% (PP) in the DHA-PQP group, and 90.0% (ITT) and 95.7% (PP) in the AL group (ITT: p=0.820; PP: p=0.988). The lower limits of the 97.5% CIs on the differences between the two treatments were -2.80% and -2.24%, in the ITT and PP populations, respectively (Table 3). All sensitivity analyses confirmed the robustness of the primary results. The day 42 PCR-corrected cure rates were lower than those at day 28 but similar for the two treatments, irrespective of the study population analysed (Table 3). The day 28 PCR-corrected cure rates in infants (6–11 months-old) were similar to those in older children and above 90% in both treatment groups (ITT: DHA-PQP 90.70%, AL 92.65%, p=0.643, 97.5% CI>-9.92%), though non-inferiority could not be confirmed as the study was not powered for this purpose.

277 The uncorrected cure rates were significantly higher in the DHA-PQP group, both at
278 day 28 (ITT: DHA-PQP 87.7% vs. AL 76.7%, p<0.001, 97.5% CI>6.82%; PP: DHA-
279 PQP 92.95% vs. AL 81.39%, p<0.001, 97.5% CI>7.67%) and at day 42 (ITT: DHA-
280 PQP 74.08% vs. AL 64.71%, p<0.001, 97.5% CI >4.45%; PP: DHA-PQP 78.44% vs.
281 AL 69.05%, p<0.001, 97.5% CI >4.44 %).
282
283 When the day 28 PCR-corrected cure rates were analysed by country, the heterogeneity
284 test was statistically significant at the 10% level in the PP population (p=0.058),
285 suggesting some differences among sites (Table 3). This applied also to the PCR-
286 uncorrected cure rates at all time points (data not shown). However, with the exception
287 of Kenya, such differences were of a quantitative type, i.e. rather in the size of the
288 treatment effect across countries, not in its direction, always favouring DHA-PQP.
289
290 When considering the WHO standard definitions of early/late clinical and
291 parasitological failure, the PCR-corrected treatment failure at day 28 was similar
292 between the two treatment groups (ITT: DHA-PQP 3.66% vs. AL 2.75%, p=0.347; PP:
293 2.73% vs 2.60%, p=0.882), while at day 42 it tended to be lower in the AL group
294 (6.26% vs. 3.92%, p=0.057). Instead, the PCR uncorrected treatment failure was
295 significantly lower in the DHA-PQP group than in the AL group at both day 28 (ITT:
296 7.13% vs. 17.06%, p<0.001; PP: 5.47% vs 16.02%, p<0.001) and day 42 (ITT: 19.56%
297 vs. 27.06%, p<0.001; PP: 18.72% vs 26.41%, p<0.001). This was mainly due to fewer
298 late failures, later shown to be new infections, in the DHA+PQP arm as compared to the
299 AL one. In the ITT population, new infections until day 42 occurred significantly less in
300 the DHA-PQP group (126 patients, 12.14%) than in the AL group (112 patients,

21.96%) (Figure 2). Similar results were obtained in the PP population (data not shown).

Parasite clearance was rapid in both treatment groups (Kaplan-Meier estimate of median time was 2 days in each group, in both populations). About 60% of patients had fever at baseline while at day 2 more than 97% of patients were afebrile in both treatment groups. Gametocyte prevalence at recruitment was similar in both study arms (DHA-PQP 11.75%; AL 12.94%, p=0.501). However, gametocyte carriage measured as rate of person-gametocyte-weeks was significantly higher in the DHA-PQP group than in the AL group, both for the ITT (DHA-PQP: 43.97/1,000; AL: 21.43/1,000; p=0.005) and the PP (DHA-PQP: 42.48/1,000; AL: 21.23/1,000; p=0.007) populations.

Haematological recovery, as measured by the increase in Hb values from recruitment to day 28, was adequate and comparable between treatment groups (data not shown). However, the change in Hb from baseline to the last available data was significantly higher in the DHA-PQP group (17.10 g/L) than in the AL group (14.91g/L) (p=0.036).

Safety results

Both DHA-PQP and AL were well tolerated with the majority of adverse events of mild or moderate severity, and consistent with symptoms attributable to malaria (Table 4). There were no significant differences in the occurrence of events, including serious adverse events. Gastrointestinal tolerability of both drugs was similar, with the majority of events being mild. Cutaneous adverse events were infrequent, and mainly involved minor dermatitis or rash. Three patients developed urticaria (one (0.1%) in the DHA-PQP group and two (0.4%) in the AL group) and three more developed mild

326 hypersensitivity (two (0.2%) in the DHA-PQP group and one (0.2%) in the AL group).
327 None of them required hospitalization.
328
329 In the DHA-PQP group, the proportion of patients with borderline (29.1%) and
330 prolonged (9.1%) QTc interval at day 2 corrected by the Bazett's method was higher
331 than in the AL group (19.8% and 6.9%) ($p<0.001$). However, this was not confirmed
332 when applying the Fridericia's correction as the corresponding proportions were 1.0%
333 and 0.2% in the DHA-PQP group and 1.2% and 0.2% in the AL group ($p=0.76$). In
334 addition, a ≥60ms increase of the QTc interval between day 0 and day 2 (Bazett's
335 correction) was observed in just 2.7% (DHA-PQP) and 2.0% (AL) patients; only 2
336 patients per group showed a QTc at day 2 higher than 500 ms. No other difference
337 between groups was observed during the follow up (data not shown).
338
339 Two deaths (one per group) occurred during the study. In Uganda, a 3 year-old girl died
340 24 h after commencing treatment with DHA-PQP. Sepsis or severe malaria was
341 considered by the investigating clinician as the most likely cause. In Mozambique, an
342 18 month-old girl died 7 h after the first dose of AL. Severe malaria was considered the
343 most likely cause of death, although other aetiologies such as sepsis, hypoglycaemia,
344 heart conditions or bronco-aspiration could not be ruled out. Death was considered as
345 possibly related to the study drug only in this case.
346

DISCUSSION

DHA-PQP was as efficacious as AL in treating African children aged 6–59 months with uncomplicated malaria, and the two treatments had similar safety profiles. Our study confirms the results of previous trials in Asia [4] and Africa [9-12] that found DHA-PQP to be as effective as other ACTs, including AL, though a recent study in Papua New Guinea (PNG) reports a significantly higher cure rate (adequate clinical and parasitological response) in children treated with AL as compared to DHA-PQP [7]. The reasons for such discordant results are unclear though the authors mention the cross-resistance between chloroquine and piperaquine. However, piperaquine, though structurally related to chloroquine, has been shown to be effective *in vitro* against chloroquine-resistant strains [4, 19]. In our study, DHA-PQP was equally efficacious in the 5 African countries despite the high chloroquine resistance previously reported from most study sites [20]. When taking into account all recurrent infections observed during the follow up period, i.e., without the PCR correction, the cure rates for DHA-PQP were significantly better than AL, indicating a better post-treatment prophylaxis (PTP) than AL [21] and confirming that chloroquine resistance did not interfere with DHA-PQP efficacy. The significantly higher Hb change from baseline to the last available data in the DHA-PQP group is in line with this observation. Therefore, the longer piperaquine's elimination half-life (about 20 days) as compared to lumefantrine (4–10 days), provides a longer PTP, prevents the emergence of new infections and improves the patient's haematological recovery, despite a significant chloroquine resistance background. While this is clearly an advantage for the individual, at the population level, it may increase the risk of selecting resistant parasites among the new infection [22] and stress the need of matching the large scale deployment of DHA-PQP with the careful monitoring of resistance [23].

372 One hundred twenty nine infants aged 6–11 months treated with DHA-PQ responded as
373 well to treatment as older children, though the study was not powered to confirm non-
374 inferiority between the two treatment groups. Infants represent a special group as they
375 are more at risk of malaria and of receiving inadequate doses of antimalarial treatments.
376 For DHA-PQP, a recently published study carried out in Papua, Indonesia, reported that
377 the PQP plasma concentration at day 7 was the major determinant of the therapeutic
378 response to DHA-PQP and that children had a higher risk of having lower levels [24].
379 Similarly, in PNG, a trend toward a lower risk of treatment failure (PCR uncorrected)
380 and plasma PQP levels at day 7 has been reported [7]. Two pharmacokinetics studies
381 have been carried out in adults (Thailand) and children (Burkina Faso) with
382 uncomplicated malaria within the program for the international registration of DHA-
383 PQP (manuscripts in preparation). The individual piperaquine's pharmacokinetic
384 profile, both in children and adults, was extremely variable so that variations within
385 individuals were larger than variations between age groups, though during treatment
386 always above the minimal inhibitory concentration (A. Evans, manuscript in
387 preparation), indicating that increasing DHA-PQP doses in children may not be needed.
388
389 Patients treated with DHA-PQP had a significantly higher rate of person-gametocyte-
390 weeks compared with those having received AL. This contrasts with a previous study in
391 Papua, Indonesia, which showed no difference in gametocyte carriage between DHA-
392 PQP and AL [24], but is in line with comparisons between DHA-PQP and mefloquine-
393 artesunate, where a higher production of gametocytes in patients treated with DHA-PQP
394 was observed [5,8]. Such an effect has been attributed to the lower dose of artemisinin
395 derivative used in the DHA-PQP. Gametocytaemia is a proxy measure of transmission
396 potential and the increased gametocyte production related to DHA-PQP use may be a

public health disadvantage that should be nevertheless balanced against a better PTP, particularly useful in areas of intense transmission.

Dihydroartemisinin-piperaquine was well tolerated, with few adverse events of clinical relevance. A higher frequency of abdominal pain and diarrhoea has previously been reported for DHA-PQP compared with mefloquine-artesunate [4] but this was not observed in this trial where the comparator treatment was AL. The statistical significant difference in the QTc interval at day 2 was observed only when applying Bazett's but not Fridericia's correction. This was not considered as clinical relevant because of the discrepant results obtained with the 2 methods and because both the proportions of patients with a QTc prolongation between day 0 and 2 higher than 60 ms or with an absolute QTc value greater than 500 ms were extremely low and balanced between groups. Therefore, considering that no cardiovascular AEs were reported, this study adds to the evidence [4] that, at therapeutic doses, DHA-PQP and AL do not have any clinically significant cardiotoxicity.

It has also been previously reported that the only potentially serious adverse effects of artemisinin derivatives are rare type 1 hypersensitivity reactions [4, 18]. However, no evidence of moderate or severe adverse reactions of this kind was observed in the current study and this was despite the larger sample size compared with other published studies in which the number of patients recruited for each arm did not exceed a few hundred. Indeed, it is reassuring that no major safety problem has been observed in more than 1,000 children treated with DHA-PQP. Nevertheless, such a sample size is unable to detect rare and unexpected serious adverse events and the development of a pharmacovigilance system should be a priority, not only for DHA-PQP but also for all

422 other ACTs. African countries should be encouraged, as the use of ACTs increases, to
423 establish pharmacovigilance systems [23] and drug developers and funding agencies
424 should contribute to their development.
425
426 In conclusion, DHA-PQP is a safe, efficacious, tolerable and affordable new
427 antimalarial treatment option in Africa. Its longer PTP period may be particularly useful
428 in areas where transmission is intense, though it may exert an important drug pressure
429 on the parasite populations, possibly selecting resistant strains. The deployment of
430 several ACTs as multiple first line treatments may overcome this problem. DHA-PQP
431 can definitely play an essential role in our effort to reduce the currently high malaria
432 burden.
433

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CONTRIBUTORS

All authors contributed to the design of the study and assisted with data interpretation. A Talisuna, U D'Alessandro, Q Bassat, M Mulenga, H Tinto, P Piola and S Borrmann coordinated the study and supervised the enrolment and follow-up of patients. A Bacchieri and M Corsi participated in data entry, collection and analysis of data. All authors participated in the preparation of the manuscript and approved the final version.

CONFLICT OF INTEREST STATEMENT

David Ubben is an employee of Medicines for Malaria Venture. Antonella Bacchieri and Marco Corsi are employees of Sigma-Tau. Umberto D'Alessandro has received additional research funds from Sigma Tau.

FIGURE LEGENDS

Figure 1

Trial profile

Figure 2

Kaplan Meier curve showing the cumulative proportion until day 42 of children with new infections (ITT population)

Table 1: Basic characteristics of the 5 African sites (online publication only)

	Nanoro, Centre Muraz (Burkina Faso)	Kilifi, KEMRI (Kenya)	CISM, Manhiça (Mozambique)	Epicentre, Mbarara (Uganda)	TDRC, Ndola (Zambia)
Characteristics of the area	Rural	Rural	Rural	Rural	Periurban
Malaria endemicity	Mesoendemic	Mesoendemic	Mesoendemic	Mesoendemic	Mesoendemic
Seasonality	High with transmission between June and December	Perennial, with two peak seasons: Jul-Sep; Dec-Jan	Perennial with marked seasonality (Oct-April)	Perennial with two peaks: April and October	High transmission between November and May
Entomological Inoculation rate (EIR)	100 to 160 (2003)	22 to 53[1]	38 (2002)[2]	Not available	Not available
Site area under Demographic surveillance system (DSS)	No	Yes	Yes	No	No
ITNs coverage	<10%	Subsidised available	<10%	11,4%	Approximately 30%
First line treatment at the time of the study	Amodiaquine-artesunate or AL	SP, and then AL	Amodiaquine-SP	AL	AL
Documented resistance to chloroquine	35%	60%	69%[3]	81%[4]	60%

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Table 2: Baseline characteristics ITT population (%)

Variable	Total	
	DHA-PQP (N=1038)	AL (N=510)
Gender M/F (%M/%F)	525/513 (50.1/49.4)	281/229 (55.1/44.9)
Age in years (mean±SD)	2.42±1.14	2.43±1.16
Weight in kg (mean±SD)	11.19±2.55	11.28±2.67
Fever	624 (60.12)	307 (60.20)
Temperature in ºC (mean±SD)	37.89±1.22	37.86±1.18
Parasite density (geometric mean)	24557	25884
Presence of Gametocytes	122 (11.75)	66 (12.94)
Hb in g/L (mean±SD)	89.23±18.15	90.59±18.20
Anaemia (=Hb<7g/dL)	141 (13.58)	63 (12.35)
Leucocytes in 10^9/L (mean±SD)	9.62±4.15	9.59±3.94
Platelets in 10^9/L(mean±SD)	182.84±108.70	181.59±106.74
Splenomegaly	41 (3.95)	19 (3.73)
Hepatomegaly	6 (0.58)	3 (0.59)
ALAT in IU/L (mean±SD)	34.08±61.34	31.08±36.23
Bilirubin in mg/dl (mean±SD)	0.97±1.04	0.94±0.81
Creatinine in U/L (mean±SD)	40.96±17.91	41.16±19.17

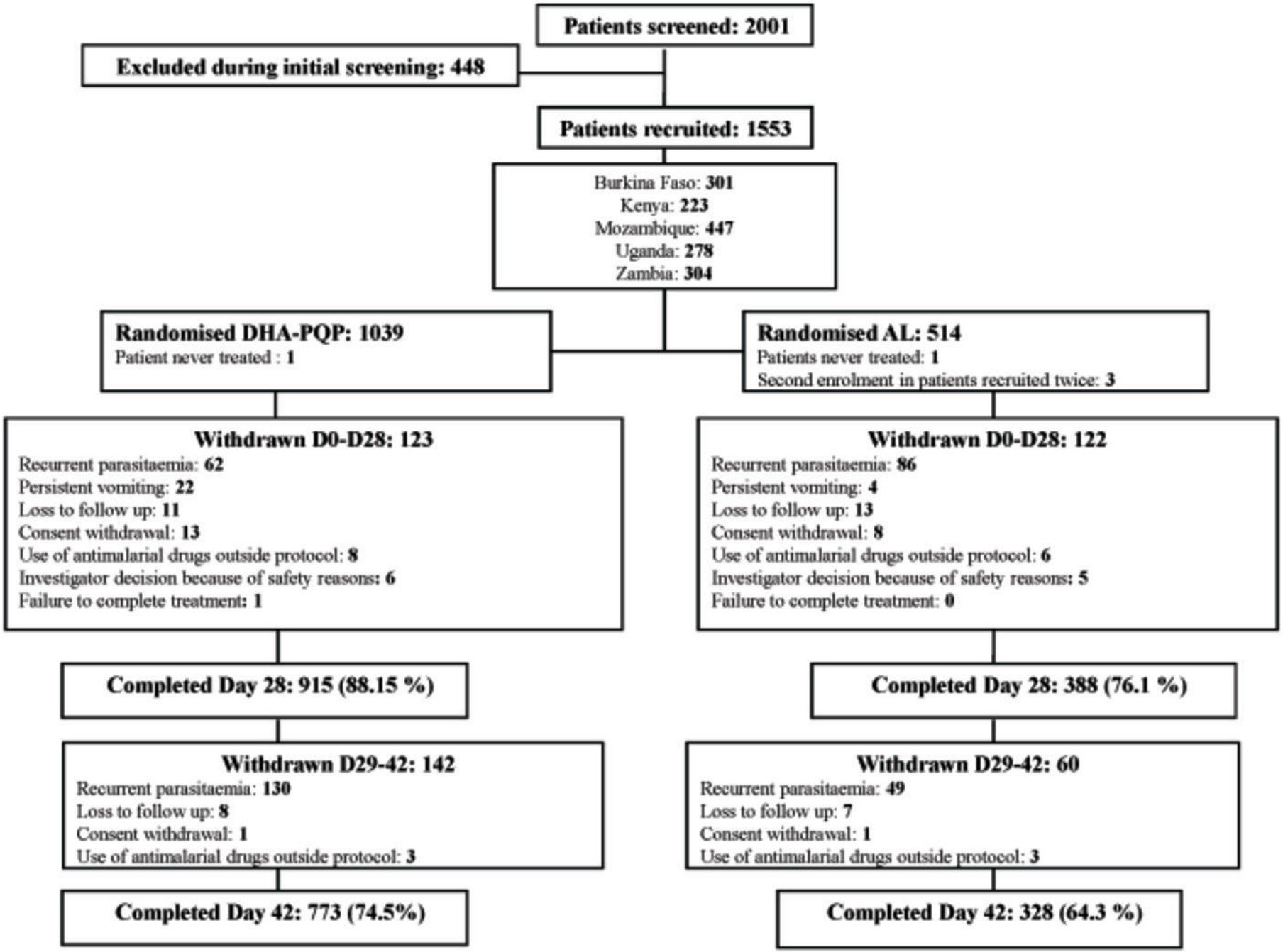
Table 3: PCR-corrected Adequate Clinical and Parasitological Response (ACPR) (PP population) by country and by day 28 and 42 (n/N)

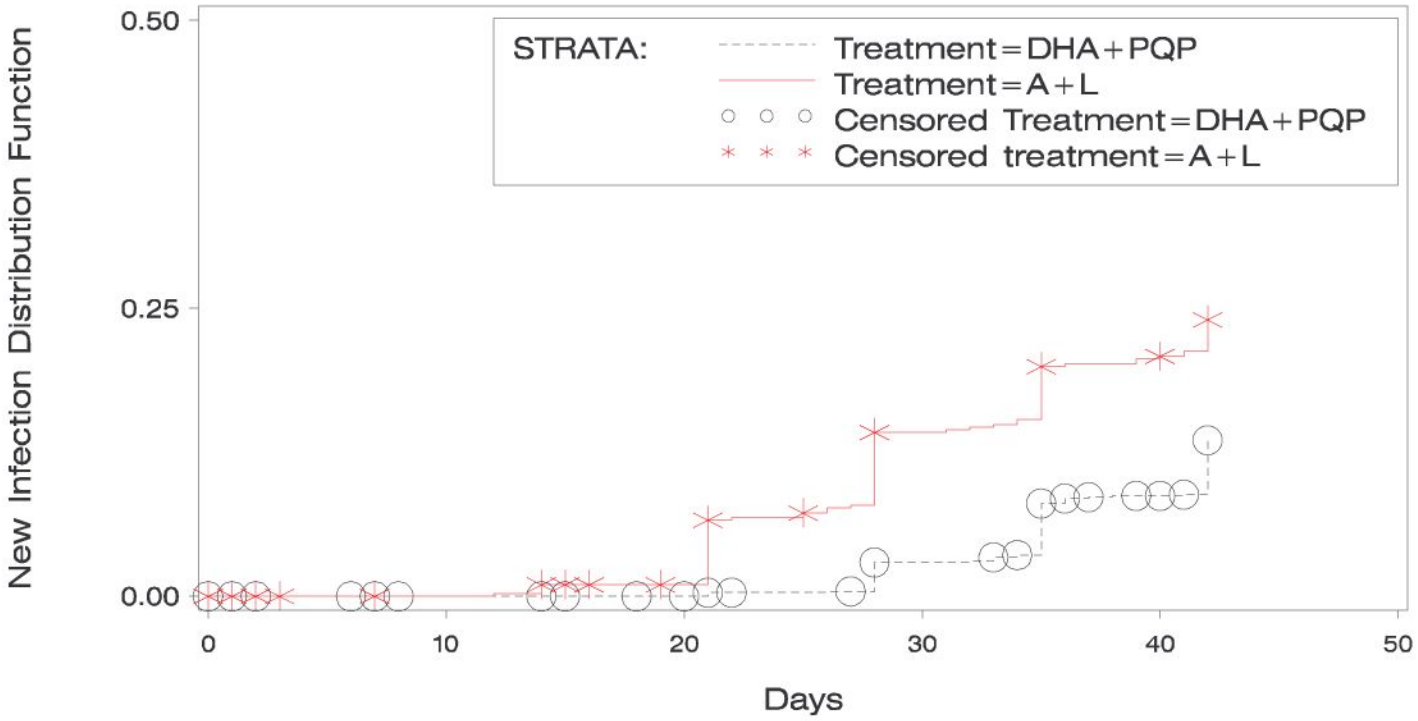
	Day 28			Day 42		
	DHA-PQP	AL	Lower limit 97.5% CI on difference	DHA-PQP	AL	Lower limit 97.5% CI on difference
Burkina Faso	98.48 (195/198)	94.85 (92/97)	-1.08	90.40 (179/198)	91.75 (89/97)	-8.19
Kenya	93.13 (122/131)	96.92 (63/65)	-9.83	90.84 (119/131)	95.38 (62/65)	-11.65
Mozambique	93.07 (255/274)	96.92 (126/130)	-8.08	90.88 (249/274)	95.38 (124/130)	-9.47
Uganda	99.36 (155/156)	96.25 (77/80)	-1.24	98.72 (154/156)	96.25 (77/80)	-2.05
Zambia	95.31 (183/192)	93.33 (84/90)	-3.98	92.71 (178/192)	93.33 (84/90)	-6.96
Total	95.69 (910/951)	95.67 (442/462)	-2.24	92.43 (879/951)	94.37 (436/462)	-4.63

Table 4: Summary of adverse events (ITT population)

Safety/ ITT Population (N)	DHA-PQP (N=1038)	AL (N=510)	p-value
At Least one AE (n,%)	823 (79.29%)	411 (80.59%)	0.55
At least one related AE (n,%)	737 (71.00%)	368 (72.16%)	0.637
At least one SAE (n,%)	18 (1.73%)	5 (0.98%)	0.249
At least one related SAE (n,%)	15 (1.45%)	4 (0.78%)	0.332
At Least one AE which caused discontinuation (n,%)	5 (0.48%)	0	0.178
At Least one SAE which caused death (n,%)	1 (0.10%)	1 (0.20%)	0.551

AE=Adverse event; SAE=Serious adverse event; Related SAE=Serious adverse event for which the investigator classifies as unlikely, possible, probable, definitely related to the study drug or whose classification is missing


Patients screened: 2001
Excluded during initial screening: 448
Patients recruited: 1553
Burkina Faso: 301
Kenya: 223
Mozambique: 447
Uganda: 278
Zambia: 304
Randomised DHA-PQP: 1039
Patient never treated : 1
Randomised AL: 514
Patients never treated: 1
Second enrolment in patients recruited twice: 3
Withdrawn D0-D28: 123
Recurrent parasitaemia: 62
Persistent vomiting: 22
Loss to follow up: 11
Consent withdrawal: 13
Use of antimalarial drugs outside protocol: 8
Investigator decision because of safety reasons: 6
Failure to complete treatment: 1
Withdrawn D0-D28: 122
Recurrent parasitaemia: 86
Persistent vomiting: 4
Loss to follow up: 13
Consent withdrawal: 8
Use of antimalarial drugs outside protocol: 6
Investigator decision because of safety reasons: 5
Failure to complete treatment: 0
Completed Day 28: 915 (88.15 %)
Completed Day 28: 388 (76.1 %)
Withdrawn D29-42: 142
Recurrent parasitaemia: 130
Loss to follow up: 8
Consent withdrawal: 1
Use of antimalarial drugs outside protocol: 3
Withdrawn D29-42: 60
Recurrent parasitaemia: 49
Loss to follow up: 7
Consent withdrawal: 1
Use of antimalarial drugs outside protocol: 3
Completed Day 42: 773 (74.5%)
Completed Day 42: 328 (64.3 %)


New Infection Distribution Function
0.50
0.25
0.00
0
10
20
30
40
50
Days
STRATA:
Treatment=DHA+PQP
Treatment=A+L
Censored Treatment=DHA+PQP
Censored treatment=A+L

Efficacy and safety of artemether-lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial

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Efficacy and safety of artemether-lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial

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Summary

Background **Combination treatments, preferably containing an artemisinin derivative, are recommended to improve efficacy and prevent *Plasmodium falciparum* drug resistance. Our aim was to show non-inferiority of a new dispersible formulation of artemether-lumefantrine to the conventional crushed tablet in the treatment of young children with uncomplicated malaria.**

Methods **We did a randomised non-inferiority study on children weighing 5–35 kg with uncomplicated *P falciparum* malaria in Benin, Kenya, Mali, Mozambique, and Tanzania. The primary outcome measure was PCR-corrected 28-day parasitological cure rate. We aimed to show non-inferiority (with a margin of –5%) of dispersible versus crushed tablet. We constructed an asymptotic one-sided 97·5% CI on the difference in cure rates. A computer-generated randomisation list was kept centrally and investigators were unaware of the study medication administered. We used a modified intention-to-treat analysis. This trial is registered with ClinicalTrials.gov, number NCT00386763.**

Findings **899 children aged 12 years or younger were randomly assigned to either dispersible (n=447) or crushed tablets (n=452). More than 85% of patients in each treatment group completed the study. 812 children qualified for the modified intention-to-treat analysis (n=403 *vs* n=409). The PCR-corrected day-28 cure rate was 97·8% (95% CI 96·3–99·2) in the group on dispersible formulation and 98·5% (97·4–99·7) in the group on crushed formulation. The lower bound of the one-sided 97·5% CI was –2·7%. The most common drug-related adverse event was vomiting (n=33 [7%] and n=42 [9%], respectively). No signs of ototoxicity or relevant cardiotoxicity were seen.**

Interpretation **A six-dose regimen of artemether-lumefantrine with the new dispersible formulation is as efficacious as the currently used crushed tablet in infants and children, and has a similar safety profile.**

Funding **Novartis Pharma, Basel, Switzerland, and Medicines for Malaria Venture (MMV), Geneva, Switzerland.**

Introduction

Plasmodium falciparum malaria is one of the commonest causes of morbidity and mortality in large areas of the world. Most deaths happen in children in sub-Saharan Africa.[1–3] Efforts to fight this disease are based on the combined use of preventive and therapeutic measures, including the adequate use of available antimalarial drugs, which are under constant threat from the emergence and spread of drug resistance of *P falciparum*. WHO now recommends combination treatments, preferably including an artemisinin derivative, to improve efficacy and prevent the emergence of parasite drug resistance.[4] Artemisinin-based combination therapy is highly effective against multidrug-resistant *P falciparum* malaria in Asia and Africa,[3,5] and has been used in southeast Asia for more than 10 years. Sub-Saharan African countries are currently implementing artemisinin-based combination therapies as first-line or second-line treatments.[5,6]

Artemether-lumefantrine (Coartem, Novartis Pharma AG, Basel, Switzerland) became the first fixed-dose combination therapy that was prequalified by WHO in April, 2004. A tablet consists of 20 mg artemether and 120 mg lumefantrine. A 3-day, six-dose regimen of artemether-lumefantrine is efficacious and safe in both adults and children,[7,8] and is recommended for infants and children weighing 5–35 kg, and adults weighing more than 35 kg. Many countries in sub-Saharan Africa have already adopted artemether-lumefantrine as first-line treatment for uncomplicated malaria, or have initiated the implementation process.[6] Studies in animals[5] suggested that artemisinin derivatives might have neurotoxic effects, although these findings have never been reproduced in people. Artemether-lumefantrine was reported to cause audiometric changes; however, that study has been much criticised.[9] Furthermore, the chemical similarity of lumefantrine and halofantrine, which prolongs corrected QT interval (QTc), has generated concern that artemether-lumefantrine might have cardiac adverse effects. To date, no clinically significant cardiovascular toxic effects have been seen with the combination.[10]

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In African children, efficacy of artemether-lumefantrine at day 28 after treatment is consistently more than 90%,[11–16] even with unsupervised administration.[17,18] However, many young children cannot swallow whole tablets, and the bitter taste of the crushed commercial tablet added to water could compromise tolerability. Also, crushing tablets is an inefficient procedure, which could result in loss of drug and reduced dose ingested. To overcome these problems, a sweetened cherry-flavoured dispersible formulation has been developed for young children. Dispersible tablets contain the same amounts of artemether and lumefantrine as crushed tablets. We compared the efficacy and safety of the new dispersible formulation of artemether-lumefantrine with the crushed one in children with uncomplicated *P falciparum* malaria. Our primary aim was to show non-inferiority of the new formulation compared with the conventional one on day-28 PCR-corrected cure rates. Major secondary and exploratory outcomes included time to fever clearance and the day-42 PCR-corrected cure rate. Additionally, safety and tolerability profiles (including QTc changes) were compared between the two groups.

Methods

Patients

We recruited infants and children with microscopically confirmed acute uncomplicated *P falciparum* malaria from eight health-care facilities in five malaria-endemic countries: one centre each in Benin, Mali, Mozambique, Tanzania mainland, and Zanzibar, and three in Kenya. Except one site in Kenya, study centres were located in rural and periurban areas. At these locations, malaria transmission is intense and perennial, with the exception of the two study sites in Mozambique and Zanzibar, where malaria is mesoendemic with transmission peaks during the rainy season. Patients were not systematically provided with insecticide-treated bednets.

Male or female infants or children presenting with symptoms of malaria were screened for study eligibility. Inclusion criteria were age 12 years or younger, bodyweight between 5 kg and less than 35 kg, fever (temperature ≥37·5°C axillary or ≥38°C rectally) or history of fever in the preceding 24 h, *P falciparum* malaria (single or mixed infection) with a density between 2000/μL and 200 000/μL blood, negative pregnancy test for patients of childbearing potential, ability to take drugs by mouth and to attend the study centre on stipulated days for follow-up, provision of written informed consent by parent or guardian, and no severe and complicated malaria as defined by WHO.[19] Exclusion criteria were haemoglobin less than 50 g/L, history of serious side-effects related to artemether-lumefantrine or similar drugs, use of antimalarial drugs other than chloroquine within the previous 2 weeks, ingestion of co-trimoxazole or any other agent with antimalarial activity within the previous 2 weeks, use of any drug known to affect cardiac function in the preceding 4 weeks, presence of QTc prolongation (>450 and >470 ms for male and female patients, respectively) or any condition known to prolong QTc, serious underlying disease, and artemether-lumefantrine treatment within the previous 30 days.

The trial protocol was approved by local institutional review boards and those of the University of Heidelberg School of Medicine (Germany); Centers for Disease Control and Prevention (Atlanta, GA, USA); Walter Reed Army Institute of Research (Silver Spring, MD, USA); Hospital Clínic i Provincial de Barcelona (Spain); Regional Ethics Committee, Stockholm (Sweden); and the Universitair Ziekenhuis Antwerp (Belgium). Before enrolment, written informed consent was obtained from the parents or legal guardians of the children. Children capable of writing were also asked to give assent.

Study design, treatment, and procedures

We did a randomised, investigator-masked, multicentre, parallel-group study in sub-Saharan Africa between August, 2006, and March, 2007, according to WHO guidelines that were applicable at the time the study was set up. Patients were admitted to hospital during the 3-day treatment phase and then followed up until day 42 after treatment. A follow-up period of 42 days complied with a WHO guideline from 2003, which recommended this observation period for patients treated with artemether-lumefantrine to avoid underestimation of recrudescence rates.[20] Between August, 2006 and September, 2006, about 20% of patients were recruited at four sites for a protocol-mandated interim analysis. From these patients, data up to day 7 after treatment were reviewed by an independent data monitoring board to stop the study if there was insufficient efficacy (based on prespecified futility criteria) or potential early safety signals.

Eligible patients were randomly assigned to artemether-lumefantrine either in dispersible (intervention group) or in crushed form (control group) on a one to one basis. Children were stratified into three dosing groups, and treatment was administered twice daily over 3 days. The treatment was given according to bodyweight: one tablet per dose for patients weighing 5–14 kg, two per dose for those weighing 15–24 kg, and three per dose for those weighing 25–34 kg. For each weight group, an independent computer-generated randomisation list was used. Randomisation lists were kept centrally and were not communicated to the sites. Both dispersible and crushed tablets were administered under supervised conditions with a cup, beaker, or syringe in suspension in 10 mL water. Immediately afterwards, another 10 mL water was given with the same device. The consumption of some food or drink (eg, breastmilk, broth, or sweetened condensed milk) was recommended after the intake of medication to increase absorption. Patients who vomited a dose within 1 h of treatment received a full replacement dose. During the entire treatment phase, no more than two doses were to be replaced. Antipyretic drugs were used to control high fever. Investigators were unaware of

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the study medication administered. Both treatments were identically packaged, prepared, and administered by a member of the staff not directly involved in clinical assessments.

After discharge, parents or guardians were asked to bring their children back to the clinic on days 3, 7, 14, 28, and 42 of follow-up or on any other day if the child was ill. Patients who developed severe malaria or danger signs of malaria were admitted and received rescue therapy (intravenous quinine), which, according to local treatment guidelines, was also given to children with early or late treatment failures,[20] and in case of vomiting the study replacement dose within 2 h of intake. Patients who discontinued study treatment before completing the study and those who prematurely withdrew from the study for any reason were scheduled for a final visit before or on day 42. We contacted those who did not return to the study site to find out the final outcome of the malaria episode.

Assessments of vital signs, body temperature, and neurological status were done twice daily during the first 3 days and at every follow-up visit. Neurological examination included tests for coordination (ie, heel–toe ataxia), fine finger dexterity (ie, ability to pick up a tablet), hearing (with a tuning fork), and an assessment for nystagmus, balance, and behaviour abnormality according to age groups. A 12-lead electrocardiogram was recorded at baseline and on day 3 (6–10 h after last dose). Two formulae (Bazett's and Fridericia's) were used to calculate QTc. Haematological and biochemical measurements were done at baseline and on days 3, 7, 28, and 42. During the hospital stay and at every follow-up visit, adverse events were assessed for severity and association with study medication.

Giemsa-stained thick and thin blood films were examined before every dose of study medication during the hospital stay and at every follow-up visit. Readings were done locally; two qualified microscopists independently read all the slides. Parasite densities were calculated by averaging the two counts. Blood smears with non-concordant results were re-examined independently by a third microscopist. Quality control was done on a percentage of randomly selected slides. Parasite density was calculated by counting the number of parasites per 200 leucocytes (or per 1000 leucocytes for gametocytes). Parasite density per µL was computed according to the actual leucocyte count or assuming an average of 8000 leucocytes per µL blood. Blood films were judged as negative if no parasites were seen in 200 oil-immersion fields in a thick blood film. The thin film was used for species identification.

To distinguish between recrudescence and new infection, blood samples were taken from every patient at baseline and from those with recurrent parasitaemia for PCR analysis. PCR experiments were processed centrally on all recurrent parasitaemias after day 7. Paired samples were genotyped by use of a standard protocol in a stepwise way on the basis of the *P falciparum* genes merozoite surface protein 2 (*msp2*), merozoite surface protein 1 (*msp1*), and genetic variation of the glutamate-rich protein (*glurp*).[21,22] A new infection was defined as the absence of any matching allelic band at baseline and on the day of parasitaemia recurrence in at least one of the markers. Recrudescence was defined as at least one matching allelic band in all markers.

In all patients enrolled before the interim analysis, two blood samples were taken at 1 h and 2 h after the first dose of dispersible or crushed tablet for measurement of artemether and its active metabolite dihydroartemisinin in the plasma. To reconstitute a full lumefantrine pharmacokinetic profile for the population studied, a blood sample was taken from every patient enrolled after the interim analysis at six different timepoints across individuals. Pooled samples allowed an estimate of the area under the curve from time 0 to the last quantifiable lumefantrine plasma concentration, and samples collected at 6 h after the sixth (last) dose of study medication, an estimate of the highest plasma concentration.

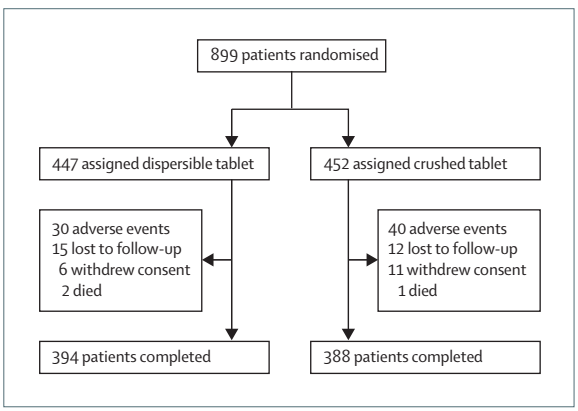

Figure 1: **Trial profile**

	Dispersible tablet (N=447)	Crushed tablet (N=452)
Female patients	215 (48%)	205 (45%)
Median age (IQR)	3·0 (2·0–5·0)	3·0 (2·0–5·0)
0–<6 months	7 (2%)	8 (2%)
6–<12 months	23 (5%)	28 (6%)
1–<6 years	318 (71%)	311 (69%)
6–12 years	99 (22%)	105 (23%)
Bodyweight (kg)	14·4 (6%)	14·5 (6%)
5–<15	274 (61%)	273 (60%)
15–<25	144 (32%)	145 (32%)
25–<35	29 (7%)	34 (8%)
Temperature (°C)	38·0 (1·1)	38·0 (1·1)
Median parasite density per µL (IQR)	26 364 (11 040–59 532)	32 288 (10 050–71 274)
Haemoglobin (g/L)	93 (17)	94 (17)
Chloroquine use before the study	27 (6%)	22 (5%)

Data are mean (SD) or number (%), unless otherwise indicated. IQR=interquartile range. *For the safety population.

Table 1: **Baseline characteristics of patients***

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	Dispersible tablet	Crushed tablet
Modified ITT (primary analysis)		
N	403	409
Cured	394	403
Cure rate (95% CI)	97·8% (96·3–99·2)	98·5% (97·4–99·7)
Treatment group difference*	–0·8%	..
Lower limit of one-sided 97·5% CI	–2·7	..
ITT		
N	418	423
Cured†	397	407
Cure rate (95% CI)	95·0% (92·9–97·1)	96·2% (94·4–98·0)
Treatment group difference*	–1·2%	..
Lower limit of one-sided 97·5% CI	–4·0	..
PP		
N	398	406
Cured	391	400
Cure rate (95% CI)	98·2% (96·9–99·5)	98·5% (97·3–99·7)
Treatment group difference*	–0·3%	..
Lower limit of one-sided 97·5% CI	–2·2	..

Data are n (%), unless otherwise indicated. ITT=intention-to-treat. PP=per protocol. *Dispersible minus crushed tablet group. †Patients with unclear or missing PCR results were considered not cured.

***Table 2*: PCR-corrected day-28 cure rate by analysis population**

	Dispersible tablet	Crushed tablet
5–<15 kg		
N	236	241
Cured	230	239
Cure rate (95% CI)	97·5% (95·4–99·5)	99·2% (98·0–100·0)
15–<25 kg		
N	139	138
Cured	137	134
Cure rate (95% CI)	98·6% (96·6–100·0)	97·1% (94·3–99·9)
25–<35 kg		
N	28	30
Cured	27	30
Cure rate (95% CI)	96·4% (89·6–100·0)	100·0% (100·0–100·0)

Data are n (%), unless otherwise indicated. ITT=intention-to-treat.

***Table 3*: PCR-corrected day-28 cure rate by bodyweight group in the modified ITT population**

Outcome measures

The day-28 PCR-corrected parasitological cure rate was the primary efficacy outcome—ie, proportion of patients with clearance of asexual parasitaemia within 7 days of initiation of treatment, without recrudescence within 28 days after initiation of treatment, and without use of rescue medication for clinical signs of malaria. Secondary efficacy outcomes included day-7 parasitological cure rate, day-14 PCR-corrected parasitological cure rate, time to fever clearance, parasite-clearance time, and gametocyte clearance. Exploratory efficacy variables included day-42 PCR-corrected cure rate, early treatment failure, late clinical failure, late parasitological failure, adequate clinical and parasitological response, and development of danger signs of malaria or severe malaria.[23] Parasitaemia in these definitions was PCR-corrected. Adequate clinical and parasitological response was defined as absence of parasitaemia from day 28 to day 42 irrespective of axillary temperature, without any previous occurrence of early treatment failure, late clinical failure, or late parasitological failure. Safety endpoints included adverse-event rates, laboratory assessments, and electrocardiographic data.

Statistical analysis

The non-inferiority hypothesis of the dispersible form of artemether-lumefantrine to the crushed form on the day-28 PCR-corrected cure rates was assessed by construction of a one-sided, lower limit, asymptotic 97·5% CI on the difference in cure rates between the two formulations. Non-inferiority was proven if the lower limit of CI was greater than −5% (for dispersible minus crushed). We used standard asymptotic methods to construct two-sample, one-sided 95% CI for cure rate proportions. We assessed parasite-clearance time and time to fever clearance by survival analysis (Kaplan-Meier estimation, log-rank test). We chose the non-inferiority margin of 5% on the basis of WHO guidelines[24] applicable at the time the study protocol was written, suggesting that a failure rate exceeding 15% is a threshold for a change in policy on the treatment of acute uncomplicated malaria. Furthermore, WHO guidelines recommended that any artemisinin-based combination therapy has cure rates of at least 90%. On the basis of expected day-28 PCR-corrected cure rates of at least 95%, the margin of 5% was chosen to meet the recommended 90% threshold. With an estimated cure rate of 95% for both treatments, we calculated that 800 patients (400 per group) would be needed to show non-inferiority of the dispersible formulation to the crushed one with about 90% power. Assuming a 10% non-evaluability rate (eg, loss of follow-up), we planned to enroll 445 patients per group. We did not do any adjustment for type I error (ie, the result is claimed positive when that is not the case) because the planned interim analysis meant that the study was not going to be stopped for positive efficacy. The study would not be stopped early unless the study drug was shown to lack efficacy (ie, futility with respect to efficacy).

Data from all sites were pooled and analysed on the basis of different populations. The intention-to-treat population included all randomised patients with confirmed *P falciparum* malaria who took at least one full dose of study medication and had at least one post-baseline efficacy assessment. Missing cure-rate data were imputed[25,26] to assess the robustness of results based on patients who provided observable data. The modified intention-to-treat population consisted of all patients in the intention-to-treat population who completed 28 days with a valid parasitological assessment (PCR if para-

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sitaemia was present at day 28). All patients who were classified as treatment failures before day 28 (ie, discontinuation of study drug and administration of rescue treatment) were also included. This analysis excluded patients if they: used other antimalarial drugs or antibiotics with antimalarial activity before day 28 for reasons other than rescue medication; had two replacement doses and vomited a subsequent dose within 1 h; vomited the replacement dose within 2 h; switched to rescue medication during the 3-day treatment period with study medication for withdrawal of consent or for other reasons (eg, overdose); experienced a new infection (confirmed by PCR) before day 28; or had unclear or missing PCR results at day 28. The per-protocol population included all patients of the modified intention-to-treat population who took at least 80% of study drug, had parasite counts between 2000 per µL and 200 000 per µL at baseline, and had a bodyweight between 5 kg and 35 kg. The safety population included all patients who received at least one dose of study drug and had at least one relevant post-baseline safety assessment. The primary analysis was based on the modified intention-to-treat population as specified in the study protocol. Analyses of all secondary and exploratory efficacy objectives were based mainly on the intention-to-treat population. The safety population was used for safety data analyses. This study is registered with ClinicalTrials.gov as NCT00386763.

Role of the funding source

The sponsors were responsible for collection and analysis of data. The authors and the sponsors were involved in study design, interpretation of data, and writing of the report. All authors had full access to all data in the study and they held final responsibility for the decision to submit for publication.

Results

Overall, 899 patients were randomly assigned to the two treatment groups (figure 1; 447 to dispersible tablets and 452 to crushed tablets; 110 in Benin, 193 in Kenya, 225 in Mali, 102 in Mozambique, 240 in Tanzania mainland, and 29 in Zanzibar). More than 85% of patients in each treatment group completed the study. 4% of patients withdrew during the 3-day treatment period (16 of 447 in the dispersible formulation group and 17 of 452 in the crushed formulation group). 886 (99%) and 804 (89%) of patients were included in the intention-to-treat and per-protocol populations, respectively. 812 (90%) participants qualified for the modified intention-to-treat analysis. The main reason for exclusion from the modified intention-to-treat population was a missing day-28 parasite count, without having treatment failure before that day (7·6% and 7·5% for the dispersible and the crushed formulation groups, respectively). Table 1 shows the baseline characteristics of all participants. The data monitoring board recommended continuing the study after an interim analysis on 166 patients showing 100% cure rates by day 7.

	Dispersible tablet	Crushed tablet
Modified ITT		
Day 14		
N	403	409
Cured	401	408
Cure rate (95% CI)	99·5% (98·8–100·0)	99·8% (99·3–100·0)
Day 42		
N	354	355
Cured	340	344
Cure rate (95% CI)	96·0% (94·0–98·1)	96·9% (95·1–98·7)
ITT*		
Day 14		
N	429	433
Cured	417	424
Cure rate (95% CI)	97·2% (95·6–98·8)	97·9% (96·6–99·3)
Day 42		
N	377	372
Cured	343	347
Cure rate (95% CI)	91·0% (88·1–93·9)	93·3% (90·7–95·8)
PP		
Day 14		
N	398	406
Cured	398	405
Cure rate (95% CI)	100·0% (100·0–100·0)	99·8% (99·3–100·0)
Day 42		
N	349	352
Cured	337	341
Cure rate (95% CI)	96·6% (94·6–98·5)	96·9% (95·1–98·7)

Data are n (%), unless otherwise indicated. ITT=intent-to-treat. PP=per protocol.
*Patients with unclear or missing PCR results were considered not cured.

Table 4: **PCR-corrected day-14 and day-42 cure rates by analysis population**

Cure rates were high in both treatment groups in the modified intention-to-treat population (table 2). The lower bound of the one-sided 97·5% CI calculated around the difference between the day-28 cure-rate point estimates in both groups was –2·7%, and thus within the prespecified –5% non-inferiority limit. The uncorrected day-28 cure rates were 92·1% for the dispersible formulation and 90·5% for the crushed formulation for the modified intention-to-treat population, and 87·6% and 87·0% in the intention-to-treat population. Cure rates were generally similar across bodyweight groups for both treatments (table 3). Overall, no difference between study centres for the primary efficacy variable was discernable (data not shown).

PCR-corrected cure rates at days 14 and 42 were also similar for the two formulations, irrespective of the study population analysed (table 4). Day-7 cure rate was 97·2% in the dispersible formulation group and 98·4% in the crushed formulation group. Similarly, uncorrected

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day-14 cure rates were similar for both formulations (data not shown). Non-PCR-adjusted day-42 cure rates were 77·7% for dispersible tablets and 74·5% crushed tablets in the intention-to-treat population. Moreover, median time to fever clearance (7·9 *vs* 7·8 h) and median parasite-clearance time (34·3 h *vs* 34·9 h) did not differ significantly between groups (95% CIs overlapped). Similar proportions of patients in the dispersible and crushed tablet groups achieved parasite clearance within 24 h (170 of 442 [38·5%] *vs* 166 of 444 [37·4%]) and within 48 h (391 of 442 [88·5%] *vs* 397 of 444 [89·4%]). Only three patients in each treatment group had parasite presence after 72 h. Gametocyte clearance could not be assessed because too few patients had gametocytes at baseline (less than 5%). Only a small proportion of patients had gametocytes after day 8 (≤1% in both groups). The comparison of time to fever clearance between the two groups was not confounded by the use of paracetamol because intake was almost identical in both groups (263 of 447 [58·8%] *vs* 243 of 452 [53·8%]).Two patients in the dispersible formulation group had early treatment failure: one had low haemoglobin concentration (48 g/L) at baseline and was erroneously included in the study. He needed immediate treatment for severe malaria anaemia. The other patient developed severe malaria during the first 3 days of the study. Late clinical failure occurred in a patient in the dispersible formulation group and in four patients in the crushed formulation group. The proportion of patients with late parasitological failure was similar between treatments (44 of 387 [11·4%] *vs* 52 of 389 [13·4%]). Furthermore, the proportion of patients with adequate clinical and parasitological response was also similar between the two groups (328 of 415 [79·0%] *vs* 318 of 420 [75·7%]). Few patients developed danger signs of malaria or severe malaria (three patients on dispersible tablets and four on crushed tablets).

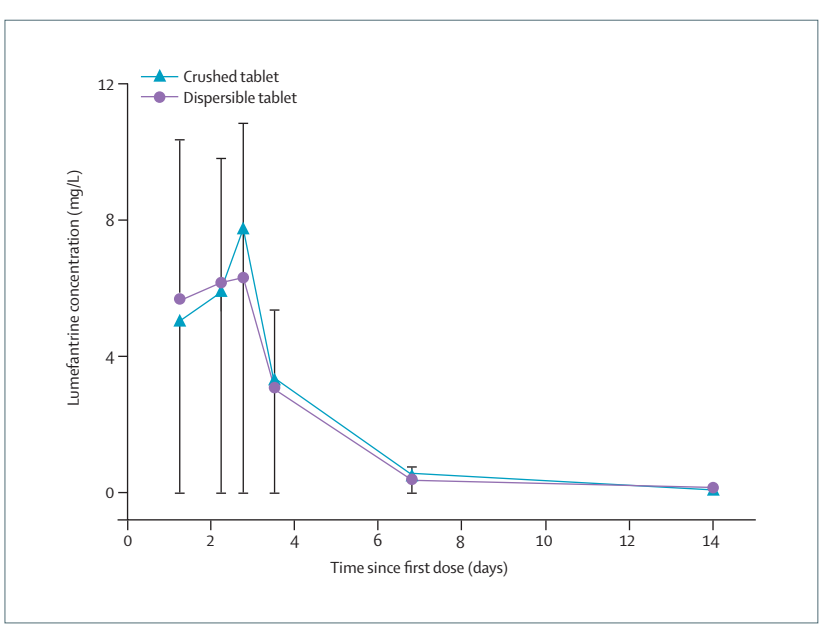

Figure 2: **Lumefantrine plasma concentration during treatment with dispersible and crushed artemether-lumefantrine tablets**

Tolerability was adequate in both treatment groups, with no difference in pattern and overall frequency of adverse events (dispersible tablets 307 of 447 [68·7%] *vs* crushed tablets 318 of 452 [70·4%]). No new or unexpected adverse event was seen. Most commonly reported adverse events were also symptoms or signs of malaria (eg, pyrexia). The most frequent drug-related adverse event was vomiting (33 of 447 [7·4%] patients receiving the intervention treatment and 42 of 452 [9·3%] receiving the control treatment), but very few patients needed rescue medication because of vomiting the study drug (six *vs* 11). Vomiting was more frequently reported in the lowest bodyweight category. Other drug-related adverse events occurred in less than 1% of patients in either group. No clinically relevant neurotoxic effects were seen during the study. In particular, no adverse event related to the auditory system was reported. On systematic neurological examination, only five individuals in the dispersible tablet group and two in the crushed tablet group had baseline abnormalities that persisted on day 1 (mainly abnormal gait and tandem walk). Isolated cases of somnolence (three), convulsion (three), dyskinesia (one), epilepsy (one), dizziness (one), and tremor (one) were reported as unrelated adverse events. No patient in either group had hearing loss. Three individuals died during the study. In the group receiving the dispersible tablet, one patient died of haemorrhage after scarification by a witch doctor, and one from an unspecified infection accompanied by severe dehydration. One patient in the crushed tablet group died of severe *P falciparum* malaria (new infection). The proportion of patients with serious adverse events was low (between 1% and 2% in both groups), most being infections.

No clinically relevant findings or differences between study groups were found in vital signs or laboratory and electrocardiographic assessments. Haemoglobin concentration decreased from baseline to days 3 and 7, and thereafter recovered in both treatment groups. At day 42, haemoglobin concentration increased from baseline by a mean of 11 g/L (SD 17) in both groups. No cases of adverse events or serious adverse events were reported as haemolysis. Neither of the formulations had an effect on renal function, as assessed by serum creatinine measurements. Aspartate and alanine aminotransferase concentrations, as markers of hepatocyte damage, decreased or returned to normal during the course of the study with no difference between treatments. About 36% of patients had high aspartate aminotransferase concentrations (>62 U/L) and 47% had high alanine aminotransferase concentrations (>45 U/L) at baseline. Almost all returned to normal by day 3.

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The QTc interval (by Bazett's formula) increased by a mean of 7·6 ms (SD 24·9) in the dispersible tablet group and 7·1 ms (24·3) in the crushed tablet group from baseline to day 3. 161 of 429 (37·5%) and 168 of 436 (38·5%) patients, respectively, did not have any QTc prolongation. The most frequent QTc change was an increase from baseline of less than 30 ms (183 of 429 [42·7%] and 196 of 436 [45·0%], respectively). The proportion with increases of more than 60 ms in QTc was low in both treatment groups (three of 429 *vs* ten of 436). No patient had a QTc interval of more than 500 ms after treatment, by either Bazett's or Fridericia's formula, and no clinical symptoms attributable to QTc prolongation (eg, syncope and sudden death) were reported. Overall, seven cases of arrhythmia (mostly tachycardia) were described as adverse events (four in the intervention group and three in the control group). All were mild, resolved without intervention, and did not lead to study drug discontinuation. In one patient of each group, this adverse event happened after day 25 of follow-up.

The maximum concentration of artemether plus dihydroartemisinin did not differ substantially across bodyweight subgroups, and the overall mean for artemether was 175 µg/L (SD 168; n=91) in the dispersible tablet group and 211 µg/L (262; n=93) in the crushed tablet group. The maximum concentrations for dihydroartemisinin were 68·0 µg/L (64·4) and 63·7 µg/L (64·9), respectively. Lumefantrine plasma concentrations were available from 310 patients assigned dispersible tablets and 315 assigned crushed tablets. No difference in lumefantrine pharmacokinetics between treatment groups was apparent (figure 2). The mean maximum lumefantrine concentrations were 6·3 mg/L (4·6) and 7·7 mg/L (5·9) after treatment with dispersed or crushed tablets, respectively. The areas under the concentration–time curve were 574 and 636 mg·h/L, respectively.

Discussion

The dispersible formulation of artemether-lumefantrine was not inferior in efficacy to crushed tablets in children with acute uncomplicated *P falciparum* malaria, and had a similar safety profile. PCR-corrected day-28 cure rates were high for both formulations in children. No differences were seen in the response to treatment across the various bodyweight groups, or between the two formulations in terms of clearance of asexual parasites and fever, which was rapid for both treatments.

Multiple imputation methods accounting for the patients excluded from primary intention-to-treat analysis showed that the day-28 cure rates for the modified intention-to-treat population (primary analysis population) were robust. The percentage of excluded patients (8%) was below the prespecified value of 10%. Results from intention-to-treat and per-protocol analyses were in agreement with those from the modified intention-to-treat analysis.

The PCR-corrected day-14 and day-28 cure rates in the control group were higher than or similar to those in previously published investigations on the efficacy of artemether-lumefantrine in African children, when administered as crushed or uncrushed tablets in a six-dose regimen.[11–16] For uncorrected day-7 cure rates, our findings confirm the results from Falade and co-workers[11] who used a non-comparative study design.

Follow-up periods longer than 28 days have been recommended for antimalarial drugs with a long half-life (eg, lumefantrine or mefloquine) to allow drug concentrations in the blood to fall below the minimum therapeutic threshold.[20] Short observation periods can yield an underestimation of recrudescence rates. Hence, the long follow-up and the large sample size reinforce the findings in this study. Because few day-42 cure-rate data with artemether-lumefantrine have been published, this efficacy variable deserves special attention. Conservative analysis of corrected day-42 cure rates that counted patients with unclear or missing PCR results as having recrudescence (91% and 93% for the two groups, respectively) showed they were high and suggested a sustained efficacy with both formulations. These results were in accordance with those of a smaller study by Martensson and colleagues,[13] who reported a day-42 PCR-adjusted success rate of 92% in children from Zanzibar receiving the combined treatment, with a similarly conservative approach for analysis. Furthermore, in our study we did not see any substantial difference between the two formulations in non-PCR-adjusted day-42 cure rates. Because uncorrected cure rates are mainly affected by new infections in high-transmission areas, this finding suggests a similar prophylactic effect by the two formulations.

The dispersible formulation was well tolerated, and comparable with the crushed tablets. No new safety issues arose and our findings are in line with former ones.[8,27] Most commonly reported adverse events were symptoms of malaria. Similarly, the pattern of changes in clinical laboratory variables was consistent with acute malaria and its resolution, with no difference between the treatment groups. The commonest drug-related adverse event was vomiting, and the frequency was similar to that previously reported in African children receiving crushed or uncrushed artemether-lumefantrine tablets.[11] Neurotoxic effects caused by artemisinin derivatives in animals and hearing loss by artemether-lumefantrine in people have raised concerns.[9,28]

In our study, no adverse event indicating a possible neurotoxic effect of this drug combination (based on specific neurological examinations) and no adverse events related to the auditory system were reported, thereby confirming, in a large group of patients, previous findings.[29,30] The chemical similarity between lumefantrine and halofantrine, which causes QTc prolongation, led to scrutiny of electrocardiographic findings in our study, which is thus the largest study

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addressing this issue prospectively. We noticed a slight increase in QTc interval, which was similar for both treatments and not associated with clinical symptoms. Similar findings have been previously reported in both adults and children, and were judged as not relevant cardiac risks for patients treated with artemether-lumefantrine.[7,8,31] In this context, we point out that recovery from malaria is associated with a consistent reduction in heart rate and lengthening of the QT interval as a result of decreased autonomic tone, which seems to be independent of antimalarial treatment.[10] Overall, our safety data did not show any increased risk of adverse events in the treatment with the dispersible formulation.

Our study was done under supervised conditions and may therefore not entirely represent normal outpatient practice, which could be a limitation of the trial. Additionally, the relative acceptability of the dispersible versus the crushed formulation was not assessed because it was not an objective of this study. Finally, the trial was done in one continent, which carries the largest burden of *P falciparum* malaria. No formal meta-analysis of previous trial data could be undertaken because this is the first therapeutic study comparing these two formulations.

Artemisinin-based combination treatments have the potential to lower the emergence and spread of drug resistance, and delay in decisions to adopt them as treatment is likely to increase morbidity and mortality.[32] Implementation of artemether-lumefantrine in Africa can reduce total expenditure on malaria treatment.[33] A recent cost-effectiveness analysis of artemether-lumefantrine for treatment of uncomplicated malaria in an area of Africa with high drug resistance showed that the use of this combination is clearly justifiable on both economic and public-health grounds. The high treatment success rate, less demand for second-line treatment, and reduction in the prevalence of severe malaria, associated with a decreased need for hospital care, have led to cost savings.[34]

Since we found that the dispersible formulation was similar in efficacy and safety to the standard formulation, cost savings are also likely with its use, with the potential benefit of improving acceptability of the combination once on the market. The price of the dispersible tablets has not yet been decided but the manufacturers have indicated that it is likely to be similar to the existing formulation. Most countries in sub-Saharan Africa that have adopted artemisinin combination therapies have chosen artemether-lumefantrine as first-line treatment for uncomplicated *P falciparum* malaria because it has recently become more affordable and, according to the manufacturer, 120 million of 160 million courses of treatment provided so far through the public health-care system for children. The dispersible formulation is easy to administer, gives compliance and effective treatment; and hence facilitates adoption in malaria control programmes.

Contributors

All authors contributed to the design of the study and assisted with data interpretation. IS, SB, UDA, QB, RG, MH, BO, AB, and SA coordinated the study and supervised the enrolment and follow-up of patients. KA, MC, and GL participated in data entry, collection, and analysis of data. All authors participated in the preparation of the report and approved the final version.

Conflict of interest statement

SA, IS, SB, RG, MH, BO, AM, JL, HM, PS, AN, QB, EJ, AB, HPB, and ZP received payments to attend meetings related to the trial. IS, ZP, UDA, and BO received payments from Novartis Pharma to attend meetings related to malaria. SA, UDA, BO, and PS received payments for travel expenses or speaking engagements. KA, MC, and GL are employees of Novartis Pharma and hold stock ownership with Novartis. DU is an employee of Medicines for Malaria Venture.

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Malaria in rural Mozambique. Part II: children admitted to hospital

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Abstract

Background: Characterization of severe malaria cases on arrival to hospital may lead to early recognition and improved management. Minimum community based-incidence rates (MCBIRs) complement hospital data, describing the malaria burden in the community.

Methods: A retrospective analysis of all admitted malaria cases to a Mozambican rural hospital between June 2003 and May 2005 was conducted. Prevalence and case fatality rates (CFR) for each sign and symptom were calculated. Logistic regression was used to identify variables which were independent risk factors for death. MCBIRs for malaria and severe malaria were calculated using data from the Demographic Surveillance System.

Results: Almost half of the 8,311 patients admitted during the study period had malaria and 13,2% had severe malaria. Children under two years accounted for almost 60% of all malaria cases. CFR for malaria was 1.6% and for severe malaria 4.4%. Almost 19% of all paediatric hospital deaths were due to malaria. Prostration (55.0%), respiratory distress (41.1%) and severe anaemia (17.3%) were the most prevalent signs among severe malaria cases. Severe anaemia and inability to look for mother's breast were independent risk factors for death in infants younger than eight months. For children aged eight months to four years, the risk factors were malnutrition, hypoglycaemia, chest indrawing, inability to sit and a history of vomiting.

MCBIRs for severe malaria cases were highest in children aged six months to two years of age. MCBIRs for severe malaria per 1,000 child years at risk for the whole study period were 27 in infants, 23 in children aged 1 to <5 years and two in children aged ≥5 years.

Conclusion: Malaria remains the number one cause of admission in this area of rural Mozambique, predominantly affecting young children, which are also at higher risk of dying. Measures envisaged to protect children during their first two years of life are likely to have a greater impact than at any other age.

Background

Out of the 350–550 million malaria cases that are estimated to occur in the world every year [1,2], only around 1–2% are severe or life threatening [3-5]. However, this small proportion represents an enormous malaria death toll per year, especially in sub-Saharan Africa, where more than 90% of the malaria deaths are thought to take place every year, affecting mainly children and pregnant women [1,2,6]. Incidence rates of severe malaria among populations in endemic areas are difficult to estimate as the demographic information required is often unavailable, and morbidity data can often only be inferred from hospital records. Characterization of severe malaria syndromes among hospitalized African children has been previously done in different settings [7-12], and prognostic significance to the different clinical presentations has been attributed. Nevertheless, severe malaria features may change according to a number of factors including the genetic characteristics of the population, malaria epidemiology, health-seeking behaviour, non-malaria co-morbidity, clinical assessment and the local case management.

In Mozambique, as in other sub-Saharan African countries, malaria represents the main cause of paediatric outpatient consultations and admissions to hospital. However, no detailed characterization of the different malarial clinical syndromes on admission exists in the country. A comprehensive picture of the clinical and epidemiological characteristics of severe malaria is necessary to prioritize public health interventions and to guide national policies. This paper presents information on the clinical features, outcome and community incidences of malaria and severe malaria in children admitted to a rural hospital in Mozambique. Data on children with malaria who attend the outpatient clinic of the same hospital are presented in a companion article [13].

Methods

Study site and population

The study area is located in Manhiça, Maputo Province, southern Mozambique. The Manhiça Health Research Center (CISM) runs a Demographic Surveillance System in the area [14] and a morbidity surveillance system at Manhiça District Hospital. A detailed description of these and of the study area can be found in the companion article [13].

Study design

Retrospective study of the data collected through the Manhiça morbidity surveillance system. This paper presents data from children younger than 15 years who were admitted to Manhiça District Hospital during a period of two years (1st of June 2003 to 31st of May 2005).

Hospital surveillance system

A standardized admission questionnaire, which includes demographic, clinical and outcome data, was filled-in for all paediatric admissions (children less than 15 years of age) to the hospital. A physician or experienced medical officer performed a physical exam of the children on admission and completed the questionnaire. An open clinical process was also filled, where the daily clinical evolution was recorded during admission.

Laboratory data was also recorded on the admission questionnaire. Upon arrival a finger prick blood sample was collected into heparinized capillaries to measure packed cell volume (PCV) and blood glucose concentration, and thick and thin blood films were prepared to quantify *Plasmodium falciparum* parasitaemia.

HIV status information was not routinely collected. Admission criteria for children with malaria included any sign of severe disease (see definitions below), inability to take oral medication, or moderate anaemia with a risk of cardio-respiratory decompensation. Hyperparasitaemia, although mentioned as an admission criterion in the national guidelines, was rarely a cause of admission at Manhiça hospital unless accompanied by moderate anaemia or other severity symptoms.

Upon discharge or death up to four final diagnoses, based on the ICD classification of diseases, were recorded on the questionnaire by a project physician after review of clinical signs and symptoms and laboratory results.

Case management

Children admitted with the diagnosis of malaria were managed according to Mozambican national guidelines, which, at the time of the study, included parenteral treatment with quinine (with an initial loading dose of 20 mg/kg plus subsequent 10 mg/kg doses, three times a day) for a minimum of six doses if completed with treatment with sulphadoxine-pyrimethamine (SP), or 21 doses when used as monotherapy. Treatment was switched to oral as soon as the child was able to tolerate. Blood transfusions were restricted to children with a PCV < 12% or to children with higher PCVs but with clinical signs of decompensation (respiratory distress or signs of heart failure) or neurological impairment. Hypoglycaemia was handled with intra-venous 30% dextrose solution, repeated as necessary, and convulsions treated with up to two doses of rectal or intra-venous diazepam, followed by intramuscular or intravenous phenobarbitone if seizures could not be controlled. Lumbar punctures were normally performed in children with a witnessed convulsion or a history of convulsions in the previous 24 hours, with positive meningeal signs, focal neurological signs, suspected clinical sepsis or a Blantyre Coma Score <5. Facilities for inten-

sive care do not exist. All clinical assistance and treatment of admitted children is free of charge. Children requiring specialized care were transferred to Maputo Central Hospital.

Laboratory methods

PCV was measured using a microcentrifuge and a Hawksley haematocrit reader card (Hawksley & Sons Ltd, Lancing, UK). Thick and thin blood films were air-dried, Giemsa-stained, and examined using a light microscope fitted with a 100× oil immersion lens. Slides were declared negative only after 2000 leukocytes have been counted. Parasite numbers were converted to a count/μL by assuming a standard leukocyte count of 8000/μL. Glycaemia was determined using Accu-Chek® (Roche Inc., Manheim, Germany) at the bedside. All cerebrospinal fluids obtained from lumbar punctures were Gram-stained and cultured.

Definitions

All case definitions were based on admission data from the admission questionnaires. An uncomplicated malaria case was defined as a child admitted with a clinical diagnosis of malaria with a *P. falciparum* asexual parasitaemia > 0 parasites/μL and not fulfilling the criteria for severe malaria.

Severe malaria was defined as a malaria case with at least one of the following criteria: PCV < 15%, deep coma (Blantyre coma score ≤ 2), prostration (inability to sit unaided or to look for mother's breast/feed in children who cannot yet sit), hypoglycaemia (< 2.2 mmol/L), convulsions (≥ 2 reported episodes in the 24 hours prior to admission) or respiratory distress (deep breathing or indrawing). Patients who met this definition but had a diagnosis of meningitis were excluded.

Impaired consciousness was defined as a child having a Blantyre coma score of less than 5. Severe anaemia was defined as a PCV < 15% on admission and cerebral malaria was defined as children with malaria and deep coma.

An increased respiratory rate (tachypnoea) was defined according to age: respiratory rate ≥ 60 breaths/min for children < 2 months; ≥ 50 for children 2 to < 12 months and ≥ 40 for children 1–5 years. Weight-for-age Z scores (WAZ) were calculated using the LMS method and the 2000 CDC Growth Reference.

The clinical processes of all children who had been classified as a malaria death based on the admission questionnaire were reviewed by a paediatrician and reclassified according to the clinical evolution and other concomitant diagnoses. A malaria death was defined as a death in an admitted child in which malaria was considered the only or main cause of death. Children with malaria parasitaemia for whom the cause of death was not malaria, were not considered severe malaria cases.

Data management and statistical methods

All admission questionnaires were double entered using a programme written in FoxPro version 5.0 (Microsoft Corp., Seattle, WA, USA). Statistical analyses were done with Stata 9.0 (Stata Corp., College Station, TX, USA).

Minimum community-based incidence rates (MCBIRs) for malaria and severe malaria were calculated referring cases to population denominators establishing time at risk (child years at risk (CYAR)) inferred from the DSS census information.

Children did not contribute to the numerator or denominator for a period of 28 days after each episode of malaria or when they were outside the study area.

Plasmodium falciparum parasitaemia results were missing in 10.7% of admitted children and PCV in 9.8%. Almost half of the parasitaemia and PCV missing results were in children younger than one month or older than five years. Children with missing PCV or parasitaemia results had a significantly higher case fatality rate compared to children with non-missing results. Outcome was missing in 0.1% of children. Children with missing age were dropped from the analysis.

Case fatality rates (CFRs) were calculated for individual signs and symptoms and combinations of these as the number of patients who died having that clinical presentation divided by the total number of patients with known outcome admitted with that clinical presentation. These CFRs represent in-hospital mortality and do not include patients who absconded or were transferred.

The seasonality of malaria and severe malaria admissions was assessed, using November to April as the defined rainy season.

Qualitative variables were compared using a χ^2 test or Fisher's exact test. Means of normally distributed variables were compared using the Student's t-test or ANOVA. Geometric means of parasitaemia were compared using the Wilcoxon Rank sum test.

A multivariate regression analysis was done to assess risk factors for malaria death in children aged one month to five years with malaria, the age group that concentrates most malaria deaths. The analysis was done separately for children aged one to seven months and for children aged eight months to five years, given that the ability to locate

a painful stimulus, strongly associated with the risk of death [9], is unreliable in the younger age group. Given that the dependent variable was the final outcome (dead/alive), only children with a known outcome were included in the analysis (those that absconded or were transferred and had no final outcome were excluded). A multivariate logistic regression was performed with malaria death or survival as the outcome, using an automated backward and forward stepwise estimation. All variables that were associated with death at a significance level of $p < 0.10$ in the univariate analysis were included in the initial model. The significance level for removal from the model was set at $p = 0.06$ and that for addition to the model at $p = 0.05$.

Thus, three different subgroups of data were used for the different analyses. The analysis of the **clinical presentation and case fatality rates of children admitted with malaria** includes all children who were admitted to the hospital during the study period (n = 8311). However, CFRs refer only to children with a known outcome. The multiple logistic regression analysis of the **risk factors for death in children admitted with malaria** was done for all children who were admitted with malaria during the study period and for which the final outcome (dead/alive) was known (n = 3859). Finally, the analysis of the **minimum community-based incidence rates** was restricted to study area children who were admitted with malaria (n = 1678).

Results

Clinical presentation and case fatality rates of children with malaria admitted to the hospital

During the two year study period, a total of 8,311 children younger than 15 years were admitted to Manhiça District Hospital. Almost half of them (4,080 – 49.1%) had a diagnosis of malaria, and for 2.8% the diagnosis was missing. 27.0% of all admitted malaria cases fulfilled the definition of severe malaria, which corresponds to 1,100 (13.2%) of all admitted patients.

Males accounted for 52.1% of the malaria (2,126/4,078, $p = 0.007$) and 53.5% of the severe malaria admissions (589/1,100, $p = 0.02$).

The age distribution of uncomplicated malaria cases, severe malaria cases and malaria deaths among children admitted to the hospital is shown in Figure 1. The highest burden of malaria cases occurred among children younger than five years of age (91.4%), with more than half (58.1%) of the cases occurring in the first two years of life. Infants carried the brunt of severe malaria, accounting for almost 30% of the cases. Malaria was very rare in the neonatal period, with only a few cases diagnosed, but was fairly frequent in the first six months of life. Figure 2 shows the relative contribution of malaria (severe and uncomplicated) to hospital admissions in children younger than 15 years of age at Manhiça District Hospital, according to age group.

The CFR for all admitted children was 4.0% (332/8,311). During the study period, there were 62 deaths attributable

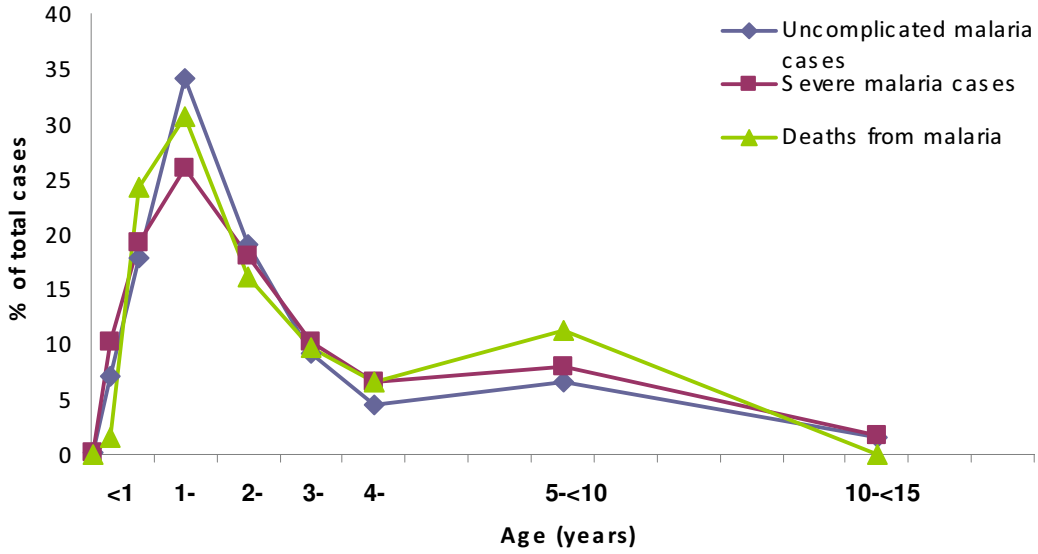

Figure 1
Age distribution of uncomplicated malaria, severe malaria and malaria deaths in children admitted to hospital.

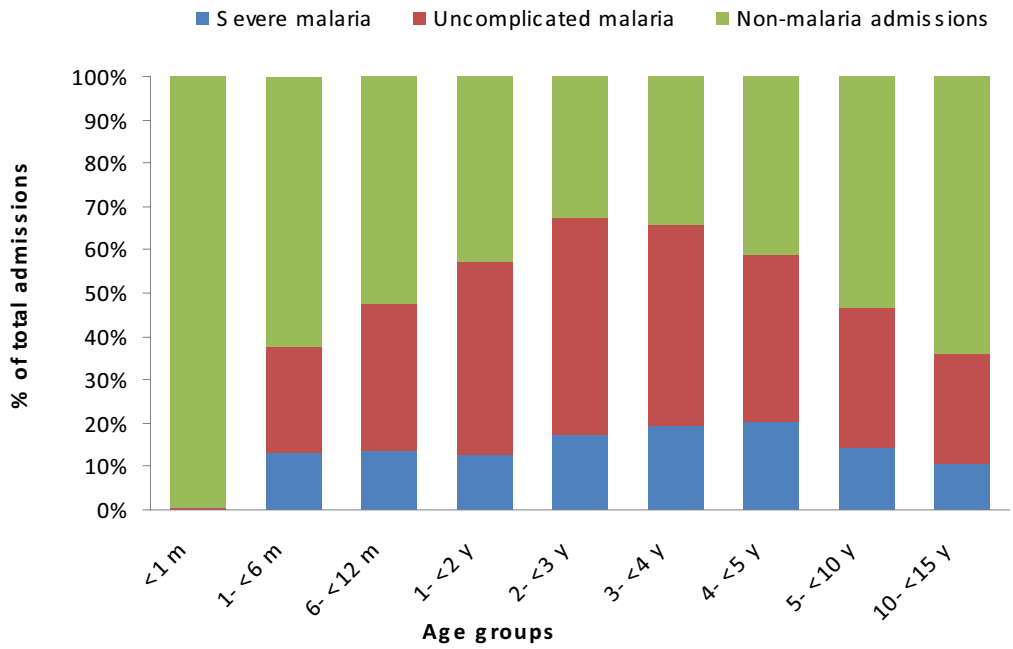

Figure 2
Relative contribution of uncomplicated malaria and severe malaria to hospital admissions according to age group.

to malaria, representing 18.7% of all paediatric in-hospital deaths. The age distribution of malaria deaths was similar to that of malaria cases (Figure 1), although very few of those deaths occurred in the first six months of life. While the majority of malaria deaths were clustered in the first two years of life, one in ten deaths occurred in children five to ten years of age. The case fatality rate for malaria (1.6%) was significantly lower than that for other diagnoses (7.3%, p < 0,0001). The CFR for severe malaria cases was 4.4%. There were no statistically significant differences in malaria CFRs by age group (data not shown).

Table 1 summarizes some key characteristics among malaria admissions. Although nearly all children had a history of fever, one third of all malaria cases requiring admission did not have fever on arrival. The median of reported fever days prior to admission was of two days, with no significant differences between uncomplicated malaria and severe malaria cases or between non-fatal and fatal malaria cases. The median length of stay in hospital for malaria cases was three days, but among fatal cases, more than 50% died within the first 48 hours.

Mean PCVs among malaria patients significantly increased with increasing age (data not shown) and were significantly lower for severe malaria cases (23.4%, SD 8.3%) than for uncomplicated cases (25.2%, SD 6.3%; p < 0.0001). Severe malaria cases had a significantly lower geometric mean parasitaemia (14,755.7 parasites/μL) than non-severe malaria cases (20,615.8 parasites/μL, p < 0.0001). The geometric mean parasitaemias among malaria deaths was also similarly lower when compared to non-lethal cases. Case fatality rates by parasitaemia level are shown in Table 2. Overall, case fatality rates tended to be higher for lower parasitaemias, although differences were not significant.

62.8% of the malaria and 59.5% of the severe malaria cases (p < 0.0001), 59.4% of severe malarial anaemia episodes (p = 0.006) and 64.5% of deaths attributable to malaria occurred during the rainy season (p = 0.03) (Figure 3). No clear-cut seasonal pattern was found for the different syndromic presentations.

Characterization of severe malaria cases

Table 2 shows the prevalence and case fatality rates of the commonly used criteria for defining severe malaria, as well as of other signs and symptoms. Among severe cases, prostration was the most frequently observed severe malaria criterion (55.0% of total severe malaria cases), followed by respiratory distress (41.1%) and severe anaemia (17.3%). Deep coma (BCS ≤ 2), which was infrequent, had the highest associated CFR (18.2%), followed by hypoglycaemia (16.2%) and respiratory distress

Table 1: Anaemia, fever, parasitaemia and duration of admission among hospitalized malaria cases

	Total malaria cases	Uncomplicated malaria cases	Severe malaria cases	Signif.#
1. PCV* on admission in children with malaria	**% (n/N)**	**% (n/N)**	**% (n/N)**	
25–< 33%	32.3% (1302/4027)	33.5% (985/2945)	29.3% (317/1082)	p = 0.01&
15–< 25%	48% (1931/4027)	52.0% (1532/2945)	36.9% (399/1082)	p < 0.0001&
< 15%	4.6% (187/4027)	0.0% (0/2945)	17.3% (187/1082)	
Mean (SD) PCV	24.7% (6,9)	25.2% (6,3)	23.4% (8,3)	p < 0.0001§
2. Fever (on admission)	**% (n/N)**	**% (n/N)**	**% (n/N)**	
History of fever in the past 24 h	99.4% (4056/4079)	99.5% (2965/2979)	99.2% (1091/1100)	p = 0.19&
Axillary temperature ≥ 37,5°C	66.1% (2692/4074)	65.0% (1933/2975)	69.1% (759/1099)	p = 0.01&
Axillary temperature ≥ 39°C	34.7% (1413/4074)	35.1% (1045/2975)	33.5% (368/1099)	p = 0.33&
Median (IQR$) length of reported fever before admission (days)	2 (1;3)	2 (1;3)	2 (1;3)	
3. Parasitaemia on admission				
Geometric mean parasitaemia (95% CI) (parasites/μL)	18838.3 (17732.6–20013.0)	20615.8 (19250.0–22078.5)	14755.7 (13022.5–16719.5)	p < 0.0001##
4. Duration of admission				
Median (IQR$) length of stay (days)	3 (2;4)	3 (2;4)	3 (2;4)	

*PCV: Packed Cell Volume
Significance: p-value of the statistical test comparing uncomplicated with severe cases
&χ^2 test
§t-test
$ Inter quartile range
Wilcoxon rank sum test

(6.2%). Among other signs and symptoms, impaired consciousness (BCS < 5) and moderate to severe dehydration had CFRs of 12.3% and 10.3% respectively. These CFRs were lower when considering total malaria patients.

Geometric mean parasitaemias in malaria cases on admission were higher for deep coma patients (26,241.5 parasites/μL) than for severely anaemic children (11,924.1 parasites/μL) or children with respiratory distress (11,425.6 parasites/μL). Figure 4 represents the age distribution of these three classical syndromic presentations of severe malaria.

78.4% of children with severe malaria presented on admission with a single severe malaria criterion, 16.6% had two criteria and only 5.1% had three or more criteria. The case fatality rates significantly increased with increasing number of criteria fulfilled (p < 0.0001; Table 2).

Risk factors for death in children admitted with malaria

During the study period there were 3,859 children admitted with malaria and with complete survival data, of which 62 died (CFR of 1.6%). None of these deaths occurred in the neonatal period and only seven in children above five years of age. The analysis of risk factors for death in malaria cases was restricted to children aged one month to five years (55 deaths), the age group where most deaths occur. Results are presented separately for children aged one to seven months and eight months to five years. Additional file 1 shows by age group the prevalence and CFRs of signs and symptoms significantly associated with death in the univariate analysis in children admitted with malaria. Table 3 presents the risk factors independently associated with death in children with malaria admitted to the hospital. In children aged one to seven months, severe anaemia and inability to look for mother's breast were independently associated with an increased risk of death. In children aged eight months to five years two logistic regression models were obtained. Hypoglycaemia, inability to sit, malnutrition (WAZ) and history of vomiting were clear independent risk factors for death in both models. There was collinearity between nasal flaring and indrawing. The model with indrawing had a better fit according to the Akaike's information criterion (AIC) than the model with nasal flaring and is thus the one presented.

Minimum community-based incidence rates

The age-specific minimum community-based incidence rates (MCBIRs) for malaria and severe malaria in the study area are shown in Table 4. Malaria incidence in the neonatal period was negligible, but then increased rapidly with age and children from six to 24 months of age had the highest MCBIRs for malaria and severe malaria. The number of cases does not coincide with the numbers given above, as only study area children were included to calculate the MCBIRs.

MCBIRs for the total paediatric population admitted to the hospital were of 50 and 26 episodes per 1,000 CYAR

Table 2: Prevalence and associated case fatality rates of different signs on admission in malaria patients

	Prevalence among total malaria patients		CFR in total malaria patients		Prevalence among severe malaria patients		CFR in severe malaria patients	
	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
Commonly used criteria for severe malaria								
Severe Anaemia (PCV < 15%)	4.6% (187/4027)	4.0–5.3	5.7% (9/159)	2.6–10.5	17.3% (187/1082)	15.1–19.7	5.7% (9/159)	2.6–10.5
Deep Coma (BCS ≤ 2)	0.6% (24/4078)	0.4–0.9	18.2% (4/22)	5.2–40.3	2.2% (24/1099)	1.4–3.2	18.2% (4/22)	5.2–40.3
Repeated (≥2/24 h) convulsions	2.4% (98/4076)	2.0–2.9	4.4% (4/91)	1.2–10.9	8.9% (98/1099)	7.3–10.8	4.4% (4/91)	1.2–10.9
Hypoglycaemia (<2.2 mmol/L)	1.0% (40/3904)	0.7–1.4	16.2% (6/37)	6.2–32.0	3.7% (39/1046)	2.7–5.1	16.2% (6/37)	6.2–32.0
Prostration	14.9% (607/4080)	13.8–16.0	5.1% (29/564)	3.5–7.3	55.0% (605/1100)	52.0–58.0	5.2% (29/563)	3.5–7.3
Respiratory distress	11.1% (454/4076)	10.2–12.1	6.1% (25/407)	4.0–8.9	41.1% (452/1100)	38.2–44.1	6.2% (25/405)	4.0–9.0
Other Signs								
Impaired consciousness (BCS < 5)	2.1% (86/4078)	1.7–2.6	10.0% (8/80)	4.4–18.8	6.5% (71/1099)	5.1–8.1	12.3% (8/65)	5.5–22.8
Jaundice	1.2% (47/4076)	0.8–1.5	0% (0/43)	-	1.9% (21/1100)	1.2–2.9	0% (0/20)	-
Transfusion recipients	14.4% (586/4065)	13.3–15.5	2.8% (16/568)	1.6–4.5	29.1% (320/1099)	26.4–31.9	4.5% (14/310)	2.5–7.5
Dehydration (mod./sev.)	3.4% (139/4076)	2.9–4.0	6.2% (8/129)	2.7–11.9	5.8% (64/1100)	4.5–7.4	10.3% (6/58)	3.9–21.2
Number of severity criteria*								
Only one					78.4% (808/1031)	75.7–80.8	3.0% (23/762)	1.9–4.5
Two					16.6% (171/1031)	14.4–19.0	8.3% (12/145)	4.3–14.0
Three					4.2% (43/1031)	3.0–5.6	13.2% (5/38)	4.4–28.1
Four or more					0.9% (9/1031)	0.4–1.7	42.9% (3/7)	9.9–81.6
Parasitaemia on admission (parasites/μL)								
1 to 4,999	20.8% (848/4080)	19.5–22.1	2.2% (17/788)	1.3–3.4	25.7% (283/1100)	23.2–28.4	4.2% (11/259)	2.1–7.5
5,000 to 19,999	17.5% (713/4080)	16.3–18.7	2.0% (13/666)	1.0–3.3	17.7% (195/1100)	15.5–20.1	6.2% (11/177)	3.1–10.8
20,000 to 99,999	43.7% (1783/4080)	42.2–45.2	1.3% (22/1695)	0.8–2.0	40.3% (443/1100)	37.4–43.2	3.9% (16/411)	2.2–6.2
≥ 100,000	18.0% (736/4080)	16.9–19.3	1.4% (10/710)	0.7–2.6	16.3% (179/1100)	14.1–18.6	4.1% (7/169)	1.7–8.3

* Any of the following: Severe anaemia, deep coma, repeated convulsions, hypoglycaemia, prostration or respiratory distress

for clinical malaria and 14 and seven cases per 1,000 CYAR for severe malaria in the first and second study year respectively. MCBIRs for clinical malaria per 1,000 child years at risk for the whole study period were 90 in infants, 86 in children aged 1 to <5 years and 6 in children aged ≥ 5 years, whereas for severe malaria, the numbers were 27 in infants, 23 in children aged 1 to <5 years and two in children aged ≥5 years.

The overall MCBIRs for malaria and severe malaria, as detected through hospital passive case detection, were twice as high during the first year of study than during the second year and the age patterns were different. In the first year, incidences were highest in infants and decreased with age, whereas in the second year the burden was slightly shifted to the right and children aged one to four years carried the highest burden of malaria and severe malaria.

Discussion

This is a retrospective study of paediatric malaria cases admitted to a rural hospital in southern Mozambique. The sex and age-pattern of admitted children was similar to that reported in other countries in the region [9,15]. Data for very young infants and children between 5 and 15 years of age, seldom available in the malaria literature, are also presented in this paper. Males accounted for a significantly higher number of admissions due to malaria and severe malaria, but also for admissions other than malaria, reflecting either a local gender-bias in treatment-seeking behaviour, exposure or an increased susceptibility to severe disease. Uncomplicated malaria and severe malaria cases and deaths attributable to malaria decreased with age in the first five years of life. Indeed, more than 50% of all admitted malaria cases, including life-threatening and fatal ones, occurred in the first two years of life. This confirms the importance of malaria among children ≤ 2 years of age in this area, despite being considered mesoendemic on the basis of the entomological data (estimated entomological inoculation rate of 38 infective bites per person per year [16]). The proportion of severe cases among infants and young children is not very different from that found in a high malaria transmission area in Tanzania [9], suggesting that the burden of malaria morbidity and mortality is borne mainly by infants and young children and this may be relatively independent of the

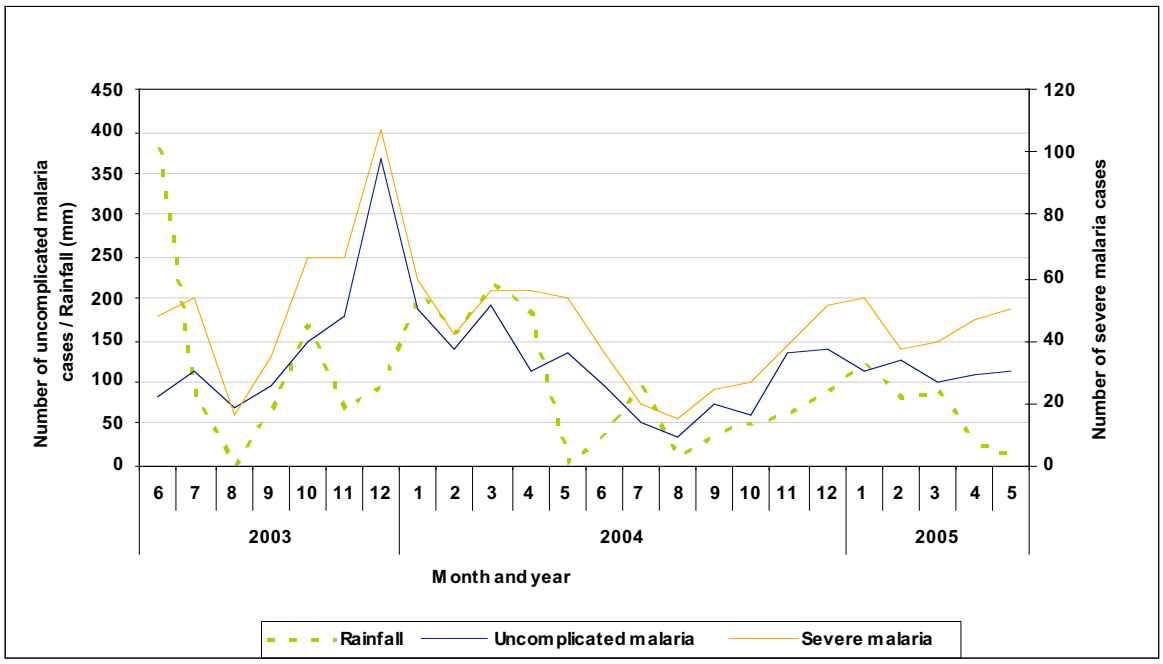

Figure 3
Seasonality of cases of malaria (severe and uncomplicated) admitted to the hospital and rainfall pattern.

intensity of transmission. This also suggests the potential significant impact in reducing the burden of malaria by malaria control interventions targeted at early infancy, such as intermittent preventive treatment in infants (IPTi) [17], and reinforces the need to implement malaria control measures specifically for this age group. Moreover, the real contribution of malaria may possibly be underestimated in both the neonatal period and the oldest children, as half of the missing slide data corresponded to these two age groups, and efforts should be made in the future to strengthen their slide collection.

The age distribution and prevalence of the three classical severe malaria clinical syndromes (respiratory distress, severe anaemia and coma) is also concordant with a pattern of moderate transmission [18]. Respiratory distress and anaemia, together with the clinical sign prostration, were the most prevalent presentations of severe malaria, with the majority of cases occurring in younger children, as opposed to coma, infrequent and slightly shifted to older children. Nevertheless, this should be interpreted with caution, as the assessment of neurological status in children younger than 8 months, and therefore the percentage of those with low Blantyre coma scores, is unreliable in this age group [9,19].

The case fatality rate of hospitalized malaria cases in this study (1.6%) was slightly lower than those recently published for six other African sites in the largest severe malaria study performed so far in Africa (ranging from 2.0 to 9.7%) [20]. This may reflect either a lower admission threshold for malaria patients in this site, implying a potential over-admission of patients, or different accessibility to hospital, leading to children arriving in less severe conditions. Alternatively, free access to health services in the country may also explain a wider and earlier use of health facilities.

Sixty-two malaria-attributable deaths occurred during the two year study period. Among children who died with malaria, a quarter died the same day of admission and more than half within the first 48 h of arriving to hospital. Most of those cases (73%) were already characterized as severe on admission, and the rest (27%) worsened during their stay in hospital before actually dying. Most of these early deaths occurred in children arriving to hospital in a much evolved and often irreversible clinical condition, raising the concern of how rapid access to hospital may determine the prognosis. In such cases, only aggressive resuscitation interventions, including artificial ventilation, may have had an impact on the outcome, but such measures are unavailable in most African settings. Early recognition of severe malaria cases at home or at the community level [21] and prompt pre-referral treatment, with effective and easy to administer antimalarials, such as rectal artesunate [22], may improve their survival likelihood.

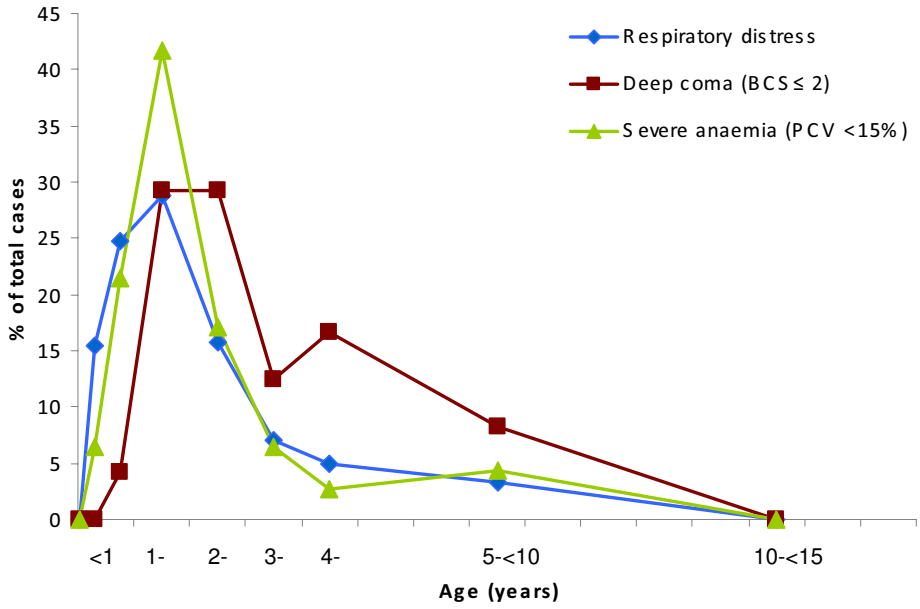

Figure 4
Age distribution of severe malaria cases according to syndromic presentation.

Little has been published concerning severe malaria in Mozambique [23,24]. The results show the association between several characteristics on admission and a high risk of dying or a higher case fatality rate. However, caution should be taken when interpreting these type of data and it is essential to distinguish between the likelihood of a fatal outcome in a patient with certain symptomatology and the real contribution of malaria to such death. Hypoglycaemia and respiratory distress (including whichever presentation) have already been associated in previous studies in other African countries with a higher risk of death in malaria cases [7-10,12,23,25]. These results corroborate their importance, as they were found to be independent risk factors for death and presented high associated case fatality rates.

Alongside malaria, malnutrition is a recurrent underlying problem in all major child mortality causes [12,26], and this is reflected by the higher risk of death as the weight-for-age z-score decreases, also seen in other studies [12,27]. Co-infection with HIV/AIDS, not analysed in this study, may also play an important role in the outcome of malaria illness or in the vulnerability to develop a more severe presentation of the disease [28,29]. The magnitude of the overlapping malnutrition-immunosuppression syndrome, and the relevance of malaria infection in the progression of HIV infection towards AIDS in children, needs also to be specifically assessed.

Neither deep coma nor impaired consciousness were associated in this model with a higher risk of dying, contrary

Table 3: Risk factors independently related to mortality in children < 5 years with malaria, according to a multivariate logistic regression model

Risk factor	Adjusted OR	95% CI	p (LR X^2 test 1 d.f.)
Age 1–7 months (n = 524)			
Severe anaemia (PCV < 15%)	9.5	2.0–45.5	0.01
Inability to look for mother's breast	4.6	1.1–19.1	0.04
Age 8 months-<5 years (n = 2667)			
Hypoglycaemia	8.2	2.2–31.0	0.005
Indrawing	5.8	2.8–12.1	<0.0001
Weight-for-age z-score (per unit increase)	0.66	0.53–0.82	0.0003
Inability to sit	4.5	2.1–9.3	0.0001
History of vomiting	2.4	1.1–5.0	0.03

Table 4: Minimum community-based incidence rates of admitted malaria (total and severe) cases per 1,000 child years at risk, by year of study

		1st June 2003–31th may 2004			1st June 2004–31th may 2005		
	Age group	**N° of cases/ CYAR**	**Rate per 1000 CYAR**	**95% CI**	**N° of cases/ CYAR**	**Rate per 1000 CYAR**	**95% CI**
Total malaria cases	<1 month	0/155	0	-	1/153	6.5	0.9–46.4
	1–<6 m	65/800	81.2	63.7–103.6	14/764	18.1	10.7–30.5
	6–<12 m	184/889	207.0	179.2–239.2	66/890	74.1	58.2–94.4
	1–<2 y	304/1626	187.0	167.1–209.2	169/1731	97.6	84.0–113.5
	2–<3 y	199/1486	133.9	116.6–153.9	134/1605	83.5	70.5–98.9
	3–<4 y	98/1504	65.2	53.5–79.4	67/1494	44.8	35.3–57.0
	4–<5 y	70/1499	46.7	36.9–59.0	34/1521	22.3	16.0–31.3
	5–<10 y	80/6812	11.7	9.4–14.6	51/7100	7.2	5.5–9.5
	10–<15 y	12/5342	2.2	1.3–4.0	12/5666	2.1	1.2–3.7
Severe malaria cases	<1 month	0/155	0	-	0/153	0	-
	1–<6 m	22/803	27.4	18.0–41.6	6/774	7.7	3.5–17.2
	6–<12 m	51/898	56.8	43.1–74.7	19/894	21.3	13.6–33.3
	1–<2 y	60/1644	36.5	28.3–47.0	42/1741	24.1	17.8–32.6
	2–<3 y	55/1497	36.8	28.2–47.9	30/1614	18.6	13.0–26.6
	3–<4 y	30/1509	19.9	14.9–28.4	22/1498	14.7	9.7–22.3
	4–<5 y	32/1502	21.3	15.1–30.1	13/1523	8.5	5.0–14.7
	5–<10 y	27/6816	4.0	2.7–5.8	19/7102	2.7	1.7–4.2
	10–<15 y	2/5343	0.4	0.1–1.5	6/5667	1.1	0.5–2.4

CYAR: child years at risk
95% CI: 95% confidence interval

to what most studies have shown [7-10,12,25,27,30,31]. However, the low prevalence of those two conditions (0.6% and 2.1% respectively) found among malaria patients in Manhiça, when compared to other sites [20], suggests that this may reflect a problem of low numbers rather than of a true lack of significance. Despite being infrequent, coma still carries the highest case fatality rate.

The identification of the real cause of the respiratory distress in severe malaria patients remains unclear. Neither peripheral lactate determinations, nor acid/base statuses were performed during the two years of the study, and acidosis was only diagnosed on clinical grounds. The real importance of metabolic acidosis in this setting, as well as additional assessment of the incidence of overlapping respiratory infections (viral, bacterial or parasitic) need to be further investigated.

Prostration (as defined above), present in more than half of the severe malaria cases, was also identified as a major risk factor for death in both age groups. Identifying prostrated children at the outpatient clinic may be challenging, as it may be difficult to assess and depends on age, but it is likely to have a great impact on their survival.

A history of vomiting was also an independent risk factor for death, probably indicating an underlying dehydration, as vomiting often leads to clinical dehydration, implying an urgent need for parenteral treatment. In Tanzanian children [9] dehydration was associated with a higher risk of death, possibly reflecting the same mechanism. The hypothetical volume depletion among severe malaria patients, and the need for urgent and vigorous re-hydration measures, has become in recent years a subject of discussion [32,33]. Although these results can in no way shed light on this topic (due to lack of analytical data, electrolytes and subjectivity of the clinical diagnosis), the importance of rapidly diagnosing and treating clinical dehydration in such patients needs to be stressed, as it may improve their likelihood of surviving.

Anaemia, from mild to life-threatening, is highly prevalent among severe malaria patients. The real contribution of malaria to the burden of anaemia, the role of iron and other micronutrient deficiencies in anaemia, as well as the prevalence of haemoglobinopathies, still remains unclear in the Manhiça area. Preliminary data show that haemoglobinopathies, especially sickle cell disease, are relatively uncommon as compared to other African countries (Menéndez C, personal communication) and the effect on the risk of severe malaria of this low prevalence of a tradi-

tionally considered "protective" genetic trait, such as haemoglobin AS, is uncertain.

Despite the existence of a life-saving intervention for severe anaemia, this highly prevalent presentation of severe malaria is the most important risk factor for death (adjusted OR 9.5) in children younger than eight months. Interventions focused on this age group, and targeted at preventing anaemia could be highly beneficial.

These data show that the level of parasitaemia is not directly related to a higher risk of death. On the contrary, children with lower parasitaemias showed a tendency to have higher CFRs than children with higher parasite numbers, although differences were not significant. Parasitaemia level was not a significant risk factor in either the univariate or multivariate analysis. Poor outcome associated with low peripheral parasitaemias, has been previously associated to sequestration in the microvasculature [9,34], but among the deep coma cases in this setting, traditionally considered to suffer sequestration, higher geometric mean parasitaemias were found when compared with other malaria syndromes, such as anaemia or respiratory distress. Correlation between severity of disease and peripheral parasitaemia is not clear-cut and the latter may only play a small role in the overall complexity of severe malaria syndromes [4,35].

Fever on admission was absent in a third of the severe malaria patients, but a referred history of fever was almost omnipresent. Relying on the presence of fever or on the parasite count for identifying severe cases on admission may, therefore, be dangerous. Considering the high mortality associated to the first hours of admission, it would seem advisable that all ill children hospitalized with malaria parasitaemia receive prompt parenteral treatment, regardless of the parasitaemia level.

This paper describes MCBIRs for severe malaria ranging from seven to 14 cases per 1,000 CYAR for the whole paediatric population, but rising up to 17–29 cases per 1,000 CYAR in children between one and five years of age. These findings are concordant with MCBIRs found in children of similar ages in other settings [15,36]. These MCBIR findings are slightly lower than the 38 episodes per CYAR that can be inferred from a cohort of around 1,000 children (aged one to four years at enrolment) participating in the control group of a randomized controlled trial of the RTS,S malaria vaccine candidate within the same time period in the Manhiça study area [16,37]. The malaria vaccine study participants were closely followed-up for 18 months, and offered transport whenever they were found with fever, possibly leading to a higher rate of malaria (and severe malaria) diagnosis. Moreover, and as opposed to this series, where severe malaria was only diagnosed according to admission criteria, children in the malaria vaccine study were assessed throughout the whole hospitalization period, thus capturing severe malaria cases which may have not been classified as such on arrival.

Limitations arise when trying to estimate MCBIRs for both the neonates and the oldest children. Neonates are often admitted before their permanent identification number has been issued by the DSS, and the oldest children are more prone to present to hospital without reliable identification. Incidence rates can only be calculated among patients identified as belonging to the study area and may thus not include many malaria cases in those children. The substantial malaria burden among older children seen at the outpatient department [13], and the potential underestimation of the disease among the older admitted children suggests that malaria control programmes cannot disregard this specific age group.

MCBIRs for hospitalized malaria were in general twice as high during the first year of study follow-up. The incidence of outpatient malaria was also higher for all paediatric age groups during the first year [13]. Among hospitalized children with malaria, these differences were highest in infants, who were admitted with malaria or severe malaria three times less frequently in the second than in the first year of the study. These yearly differences could illustrate the high interannual variability that can occur within the same malaria endemic area or more likely reflect a true decline in the malaria burden possibly explained by a series of different coincident factors. The superior rainfall in the first 12 months of data collection (June 2003–May 2004), the introduction in June 2003 of a new more effective first-line antimalarial treatment (amodiaquine and sulphadoxine-pyrimethamine), and a better and more qualified triage and initial assessment of the children arriving to hospital, may all have played a key role in reducing malaria transmission. The concomitant progress of different intervention trials of malaria control tools (RTS,S malaria vaccine and intermittent preventive treatment in infants and pregnant women), targeted to this same paediatric population, and ongoing during the study period, were probably also influential.

Hospital-based results underestimate the real burden of disease and are greatly influenced by health-seeking behaviour. In a verbal autopsy study in the Manhiça DSS area, 53% of all paediatric deaths occurred at home (Sacarlal J, personal communication), thus cases seen at the hospital may only be a fraction of the total paediatric community deaths [38]. Nevertheless, hospital-based data are often the only available data and are useful indicators of the health status of a population.

Conclusion

This paper reviewed all malaria admissions during two years in a rural hospital of Mozambique, in which malaria still represents the principal cause of admission and an important cause of in-hospital deaths in the paediatric ward. Measures targeted at encouraging families to promptly take their sick children to hospital, at recognizing the risk factors for severity at presentation, and improving the initial care and management during admission, would be helpful to reduce malaria-attributable deaths. In populations with difficult access to hospitals, adequate pre-referral effective antimalarials should otherwise made available.

Authors' contributions

The hospital surveillance system and the DSS in Manhiça were designed by JJA, CM and PLA. They were set up in 1996 and have received numerous contributions to its design and implementation since then. During the study period QB, EM, PA, BS, AB, JS and TN were involved in the diagnosis and management of malaria patients and collection of data. AN coordinates the DSS in Manhiça. QB and CG led the analysis, interpretation and write up of this data set, and received input from all authors. All authors read and approved the final manuscript.

Additional material

Additional file 1

Prevalence and CFRs of signs and symptoms significantly associated with death in the univariate analysis in children admitted with malaria, according to age.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1475-2875-7-37-S1.doc]

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Severe malaria and concomitant bacteraemia in children admitted to a rural Moçambican hospital

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Summary

OBJECTIVES To describe the prevalence, aetiology and prognostic implications of coexisting invasive bacterial disease in children admitted with severe malaria in a rural Moçambican Hospital.

METHODS Retrospective study of data systematically collected from June 2003 to May 2007 in a rural Moçambican hospital, from all children younger than 5 years admitted with severe malaria.

RESULTS Seven thousand and forty-three children were admitted with a diagnosis of malaria. 25.2% fulfilled the criteria for severe malaria. 5.4% of the children with severe malaria and valid blood culture results had a concomitant bacteraemia. Case fatality rates of severe malaria cases rose steeply when bacteraemia was also present (from 4.0% to 22.0%, $P < 0.0001$), and bacteraemia was an independent risk factor for death among severe malaria patients (adjusted OR 6.2, 95% CI 2.8–13.7, $P = 0.0001$). *Streptococcus pneumoniae*, Gram-negative bacteria, *Staphilococcus aureus* and non-typhoid *Salmonella* (NTS) were the most frequently isolated microorganisms among severe malaria cases. Their frequency and associated case fatality rates (CFR) varied according to age and to syndromic presentation. *Streptococcus pneumoniae* had a relatively low CFR, but was consistently associated with severe malaria syndromes, or anaemia severity groups. No clear-cut relationship between malarial anaemia and NTS bacteraemia was found.

CONCLUSIONS The coexistence of malaria and invasive bacterial infections is a frequent and life-threatening condition in many endemic African settings. In Moçambique, *S. pneumoniae* is the leading pathogen in this interaction, possibly as a consequence of the high HIV prevalence in the area. Measures directed at reducing the burden of both those infections are urgently needed to reduce child mortality in Africa.

keywords severe malaria, bacteraemia, Africa, risk factors, co-morbidity

Introduction

Invasive bacterial disease and malaria remain the two leading causes of paediatric mortality and morbidity in Africa (Berkley *et al.* 2005; Bryce *et al.* 2005). Malaria causes one of the estimated 4.4 million annual child deaths in this continent (Snow *et al.* 2005; WHO 2005), and invasive bacterial disease is nowadays, despite the difficulties in confirming its diagnosis (Mulholland & Adegbola 2005), clearly recognised as one of the principal killers of children *per se*. *Streptococcus pneumoniae* and *Salmonella* species, have been consistently reported (Walsh *et al.* 2000; Enwere *et al.* 2006; Roca *et al.* 2006; Sigauque *et al.* 2009) as the bacterial isolates causing major morbi-mortality in African children.

Both malaria and bacterial infections constitute an enormous burden for the under-resourced African health facilities. They represent the principal causes of admission and together account for more than half of all paediatric in-hospital deaths (Berkley *et al.* 2005; Bassat *et al.* 2008). Both infections predominantly affect young children, which also have the highest risk of dying.

The extent to which those two diseases overlap may vary according to several factors, including the endemicity of malaria and the prevalence and aetiology of bacteraemia in different countries, and this interaction has been addressed

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in various ways. Firstly, data on selected series of severe malaria patients (Prada *et al.* 1993; Enwere *et al.* 1998; Berkley *et al.* 1999; Bronzan *et al.* 2007) illustrate the possible association of malaria or of certain severe malaria syndromes with specific bacterial isolates. Conversely, series of children with bacteraemia have been also used to describe potential associations with malaria infection (Graham *et al.* 2000; Berkley *et al.* 2005). Although the literature suggests that malaria may predispose to certain specific bacterial infections (Mabey *et al.* 1987), the physiopathological mechanisms, and the temporality of this causal relationship remains not yet clearly understood. Moreover, concomitant bacteraemia may trigger in malaria patients a more severe form of the disease (Berkley *et al.* 1999). This study aims to describe the relationship between severe malaria and bacterial invasive disease in children younger than 5 years admitted to a district hospital in Moçambique.

Methods

Study site and population

The study is a retrospective analysis of data systematically collected over 4 years from 1st of June 2003 to 31st of May 2007, from all children younger than 5 years admitted to the Manhiça District Hospital (MDH), in southern Moçambique. Most of the analysis includes only children with severe malaria.

The area has been described in detail elsewhere (Alonso *et al.* 2002). The Manhiça Health Research Centre (CISM) runs a Demographic Surveillance System in the area (Nhacolo *et al.* 2006) and a morbidity surveillance system at MDH (Guinovart *et al.* 2008). Malaria transmission, mainly caused by *Plasmodium falciparum*, is perennial with substantial seasonality and of moderate intensity.

MDH, with its 110-bed inpatient ward, is the main health facility in the area, used for primary health care by the nearby population, and one of the two referral health centres for Manhiça District (Loscertales *et al.* 2002).

Morbidity surveillance system

A standardised admission questionnaire is completed for all paediatric admissions. Upon arrival a finger prick blood sample is collected into heparinised capillaries to measure packed cell volume (PCV) and thick and thin blood films are prepared to quantify *P. falciparum* parasitaemia. A single venous blood culture is performed routinely for all children younger than 2 years of age and for children older than 2 years with confirmed hyperpyrexia (axillary temperature ≥ 39 °C), neurological signs or signs/symptoms of severity, including respiratory distress, circulatory collapse, or the clinician's suspicion of sepsis (Sigauque *et al.* 2009).

Upon discharge or death, a physician revises the clinical history and records the final diagnoses, using the coding system proposed by the International Classification of Diseases.

Antiretroviral therapy only became available at MDH at the end of 2005, and before that, screening for HIV was limited to a selected number of suspected AIDS cases. For this reason, no HIV data are available for the patients included in this analysis.

Case management

Malaria patients are managed according to Moçambican national guidelines, which at the time of the study included parenteral treatment with quinine combined with Sulphadoxine–Pyrimethamine. Specific treatments for the different malaria syndromes have been described elsewhere (Bassat *et al.* 2008). Following national recommendations, the first line antibiotic used presumptively for suspected invasive bacterial disease is parenteral cloramphenicol or penicillin with or without gentamicin, or ampicillin plus gentamicin for severely malnourished children. Antibiotic therapy is reassessed according to culture results and sensitivity patterns and parenteral ceftriaxone is available as second-line therapy. Children requiring specialised care are transferred to Maputo Central Hospital.

Laboratory methods

PCV is measured using a microcentrifuge and a Hawksley haematocrit reader (Hawksley & Sons Ltd, Lancing, UK). Thick and thin blood films are processed and examined according to standard methods (Guinovart *et al.* 2008).

For blood cultures, a volume of 1–3 ml of whole blood is inoculated in a paediatric blood culture bottle (Pedibact®; Becton-Dickinson, Franklin Lakes, NJ, USA) and incubated in an automated system (BACTEC® 9050; Becton-Dickinson) for 4 days. Positive samples are examined by GRAM stain and subcultured on blood, chocolate or MacConkey agar plates as required. Bacterial isolates are identified following standard microbiologic procedures (Roca *et al.* 2006; Valles *et al.* 2006) and tested by disk-diffusion for antibiotic susceptibility (CLSI 2006).

Definitions

Bacteraemia was defined as the isolation of at least one non-contaminant bacteria from the admission blood culture. Coagulase-negative *staphylococci*, *Bacillus* species

or *micrococci* were classified as contaminants. *S. viridans* isolates were not included in either the bacteraemia or the contamination group, due to the difficulty in attributing pathogenia. Non-typhoid *Salmonellae* (NTS) bacteraemia included all *Salmonellae* species, excluding *Salmonella typhi* cases. All other remaining gram negative bacteria were grouped for the analysis.

A severe malaria case was defined as a child admitted with a clinical diagnosis of malaria with *P. falciparum* asexual parasitaemia > 0 parasites/μl, and at least one of the following criteria: PCV < 15%, deep coma (Blantyre coma score ≤ 2), prostration (inability to sit unaided or to look for mother's breast/feed in children who cannot yet sit), hypoglycaemia (<2.2 mmol/l), convulsions (≥2 reported episodes in the 24 hours prior to admission) or respiratory distress (deep breathing or indrawing). Patients meeting this definition but with a clinical diagnosis of meningitis were excluded.

Unconsciousness was defined as a Blantyre coma score on admission ≤3 in children below 8 months of age or ≤4 in older children. This age-specific difference is related to the difficulties in assessing the ability to locate a painful stimulus in younger children (Schellenberg *et al.* 1999). Mild anaemia was defined as a PCV 25 to <33%, moderate anaemia as a PCV 15 to <25% and severe anaemia as a PCV < 15% on admission. Malarial anaemia was defined as anaemia of any degree present in a child with severe malaria.

Nutritional status was assessed using weight for age *Z*-scores (WAZ), calculated using the LMS method and the 2000 CDC Growth Reference.

Data management and statistical methods

All admission questionnaires were double entered in FoxPro version 5.0 (Microsoft Corp., Seattle, WA, USA). Statistical analyses were done with Stata 9.0 (Stata Corp., College Station, TX, USA).

Case fatality rates (CFRs), calculated as the number of patients who died having a specific clinical presentation divided by the total number of patients with known outcome admitted with that clinical presentation, represent in-hospital mortality and do not include patients who absconded or were transferred.

Qualitative variables were compared using a chi-square test or Fisher's exact test. Means of normally distributed variables were compared using the Student's *t*-test or ANOVA. Geometric means of parasitaemia were compared using the Wilcoxon rank sum test.

A multivariate logistic regression analysis was performed to assess whether bacteraemia was a risk factor for death among severe malaria cases, using automated backward and forward stepwise estimations (both gave the same results). Given that the dependent variable was the final outcome (dead/alive), only children with a known outcome were included in the analysis (absconded or transferred excluded). All variables that were associated with death at a significance level of $P < 0.10$ in the univariate analysis were included in the model. The significance level for removal from the model was set at $P = 0.06$ and that for addition to the model at $P = 0.05$. Newborns were excluded from these analyses.

Plasmodium falciparum parasitaemia results were missing in 6.3% of admitted children and PCV in 7.1%. Blood cultures were not performed on admission or missing for 12.1% of the children. 2.0% of the children were transferred and 5.5% absconded. A potential bias could have been introduced when attempting to perform a pooled analysis of children younger and older than 2 years of age, as blood cultures are not routinely performed in children older than two and having one performed implies the presence of a certain degree of clinical severity. Thus, in patients older than two years of age, many malaria cases not showing signs of severity may not have had their blood cultured, and therefore some bacteraemia cases may have remained undetected. To limit this potential methodological problem, we restricted the analysis to severe malaria cases only, assuming therefore the presence of severe disease on admission, in both age groups.

Results

During the 4-year study period, 14 337 children younger than 5 years were admitted to MDH. 7043 (49.1%) children had malaria, and a quarter of these ($n = 1780$) fulfilled the severe malaria definition. A flow chart (Figure 1) summarises the number of study children according to diagnosis, age group (<2 years and >2 years), availability and validity of blood culture results. 12.8% (207/1616) of severe malaria patients with a blood culture performed grew contaminant microorganisms, and 0.3% (5/1616) grew *S. viridans*. 5.4% (76/1404) of severe malaria patients with a valid culture result (excluding contaminations or *S. viridans*), had bacteraemia.

Among severe malaria patients with bacteraemia, *S. pneumoniae* was the most frequent isolate (26%, $n = 20$), followed by Gram negative bacteria (21.1%, $n = 16$), *S. aureus* (17.1%, $n = 13$) and NTS (15.8%, $n = 12$).

Figure 2 shows the aetiology of bacteraemia in patients with severe malaria, as compared to the aetiology of bacteraemia for all other non-malaria admissions. Distribution of microorganisms varied according to admission diagnosis, but in both cases *S. pneumoniae* was the most

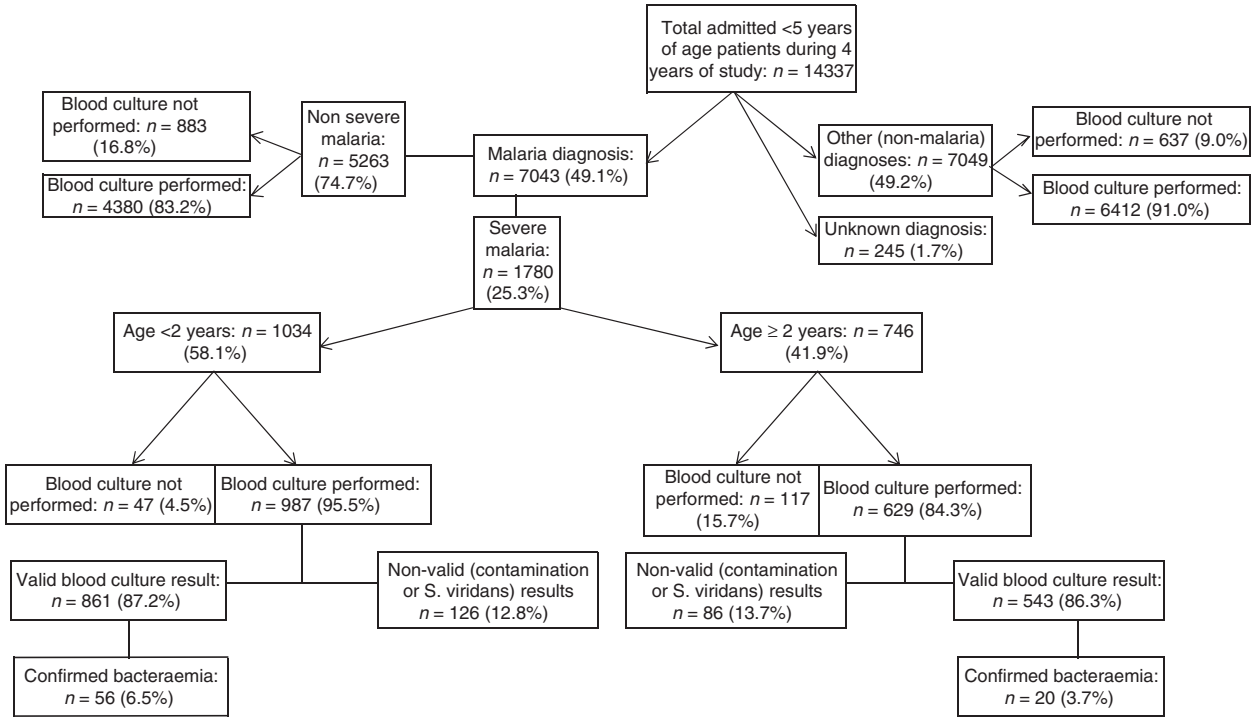

Figure 1 Flow chart summarizing the number of study children according to diagnosis age group (<2 years and ≥2 years) availability and validity of blood culture results.

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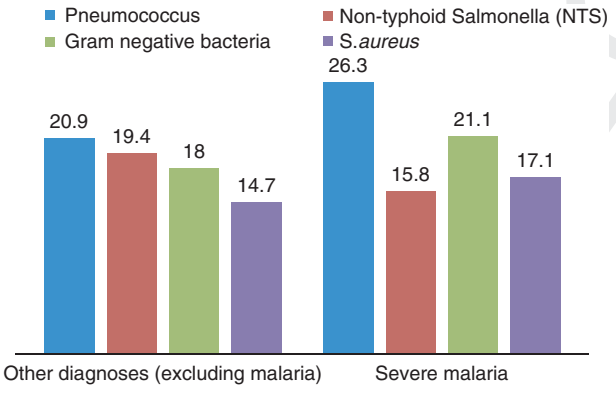

Figure 2 Relative contribution (in %) of the main 4 bacterial isolates as causes of bacteraemia among severe malaria cases compared to all other non-malaria diagnoses.

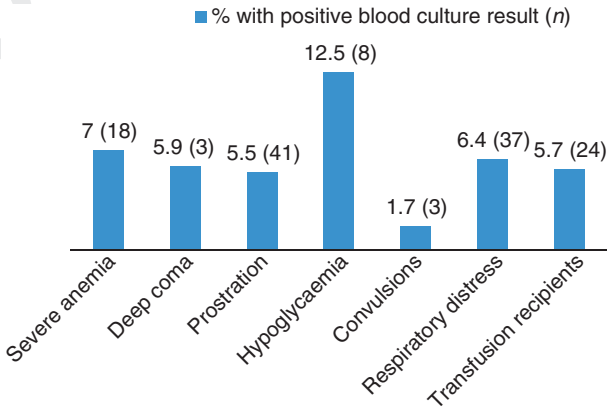

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Figure 3 Prevalence of bacteraemia among different severe malaria syndromes in children with valid blood culture results.

frequent isolate, although differences were non-significant. Figure 3 shows the prevalence of bacteraemia according to the different severe malaria syndromes. While the prevalence in the severe malaria cases with hypoglycaemia was very high (12.5%), it decreased substantially in the few severe malaria cases with convulsions (1.7%). Figure 4 shows the relative contribution of the main bacteria within each syndromic group. *S. pneumoniae* was consistently the most important isolate in each of these groups, except for patients with convulsions or prostration.

Table 1 shows clinical and laboratory parameters of children with severe malaria according to blood culture result, and Table 2 refers them to different bacterial

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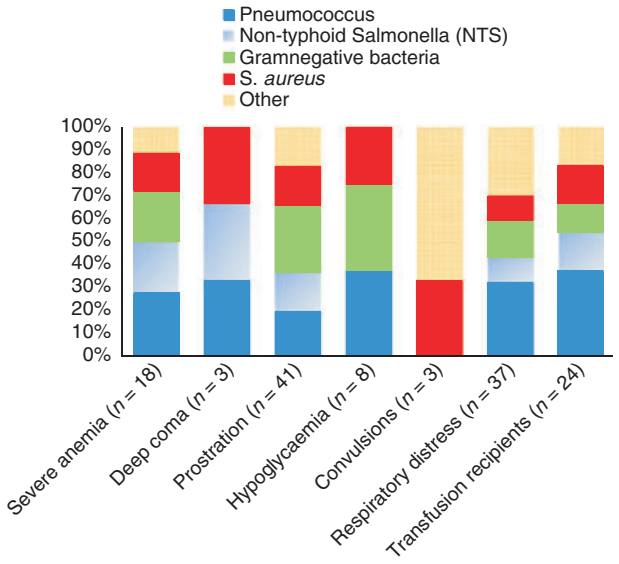

Figure 4 Relative contribution (in %) of different bacterial isolates according to different severe malaria syndromes/symptoms.

isolates. Bacteraemic children with severe malaria had a higher CFR, were more severely malnourished and had lower mean parasitaemias than severe malaria patients with a negative blood culture.

The prevalence of NTS bacteraemia was not significantly different between severe malaria and non-malaria cases (data not shown). Respiratory bacteria (*S. pneumoniae* and *Hib*) were isolated more frequently from patients with severe malaria who had respiratory distress (43.2%) than from patients without it (20.5%; $P = 0.03$).

When assessing specific signs and symptoms among severe malaria patients with valid blood culture results, bacteraemia was significantly more frequent in patients who presented with hypoglycaemia on admission (12.5% *vs.* 5.1% in those that did not, $P = 0.01$), hepatomegaly (16.1% *vs.* 5.0%, $P < 0.0001$) or oral candidiasis (30.8% *vs.* 5.2%, $P < 0.0001$), and significantly less frequent among those with convulsions (1.7% *vs.* 6.0%, $P = 0.02$). No significant differences were observed between children presenting and not presenting other signs or symptoms.

Table 1 Clinical and laboratory parameters of children with severe malaria according to blood culture result

	Negative ($N = 1328$)	Positive ($N = 76$)	P (+ *vs.* −)
Age (mean (SD)) (months)	22.0 (14.7)	19.1 (13.9)	0.09
Case fatality rate† (% (*n*))	4.0 (50)	22.0 (13)	<0.0001
Respiratory distress (% (*n*))	40.8 (542)	48.7 (37)	0.175
Haematocrit‡ (mean (SD))	23.4 (8.4)	22.7 (8.4)	0.440
Mean parasitaemia (parasite/μl) (geometric mean (95% CI))	16 639 (14 914–18 562)	6537 (3718–11 494)	0.002
Weight for age *Z*-score§ (median (IQR))	−1.6 (−2.7/−0.6)	−2.2 (−3.5/−1.2)	0.001

95% CI, 95% confidence interval; IQR, inter-quartile range.
†Negative: $N = 1244$; positive: $N = 59$.
‡Negative: $N = 1304$; positive: $N = 76$.
§Negative: $N = 1307$; positive: $N = 73$.

Table 2 Clinical and laboratory parameters of children with severe malaria and bacteraemia according to blood culture isolate

	S. pneumoniae ($n = 20$)	Non-typhoid *Salmonella* NTS ($n = 12$)	Gram negative bacteria ($n = 16$)	*S. aureus* ($n = 13$)
Age (mean (SD)) (months)	17.2 (10.8)	16.4 (6.1)	23.3 (19.9)	22.0 (14.8)
Case fatality rate (% (*n*/N))	13.3 (2/15)	25.0 (2/8)	25.0 (3/12)	30.0 (3/10)
Respiratory distress (% (*n*))	60.0 (12)	33 (4)	37.5 (6)	30.8 (4)
Haematocrit† (mean (SD))	20.8 (6.9)	22.1 (9.3)	22.3 (8.8)	23.2 (7.6)
Mean parasitaemia (parasite/μl) (geometric mean (95% CI))	1753 (564–5449)	9687 (3233–29 030)	13 338 (3079–57 778)	11 883 (2326–60 717)
Weight for age *Z*-score (median (IQR))	−2.7 (−3.4/−1.6)	−2.6 (−3.3/−2.1)	−2.2 (−4.1/−1.1)	−2.0 (−3.1/−0.4)

95% CI, 95% confidence interval; IQR, inter-quartile range.
†Negative: $n = 1244$; positive: $n = 59$.

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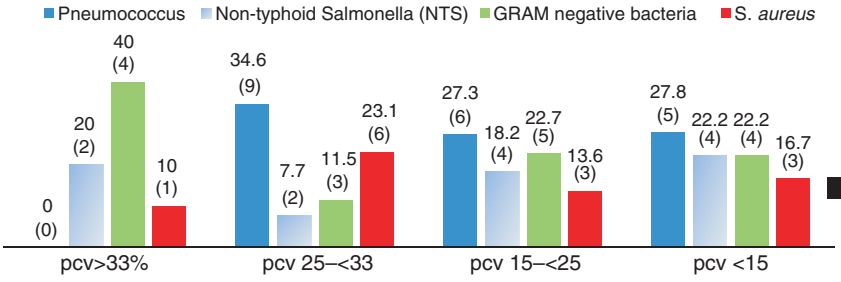

Figure 5 Aetiology (% (*n*)) of bacteraemia cases in severe malaria patients according to haematocrit on admission. *PCV, packed cell volume.

Anaemia and bacteraemia in severe malaria patients

The prevalence of anaemia (PCV < 33%) among severe malaria patients was high (83.0%). The frequency of bacteraemia seemed to be independent of the presence of anaemia and this remained true for the different degrees of anaemia severity, being 6.3% (26/416) among children with mild anaemia, 4.6% (22/474) among those with moderate anaemia and 7.0% (18/256) among severe anaemia cases ($p = 0.4$). Figure 5 illustrates the aetiology of bacteraemia cases among severe malaria patients according to admission haematocrit. *S. pneumoniae* was the most frequent bacterial isolate among all malarial anaemia groups (mild, moderate and severe).

Case fatality rates

Severe malaria accounted for 12% (81/669) of all in-hospital deaths in children <5 years of age. Thirteen children with bacteraemia and severe malaria died. CFR for severe malaria was overall 4.9% (81/1648), rising significantly to 22.0% (13/59) when associated with concomitant bacteraemia.

The impact of having a concomitant bacteraemia was important in some but not all severe malaria syndromic presentations. Among patients with severe malaria and severe anaemia which also had bacteraemia, CFR rose when compared to those without bacteraemia [31.3% (5/16) *vs.* 5.2% (11/211), $P = 0.002$]. Similarly, severe malaria patients with respiratory distress and superimposed bacteraemia had a much higher CFR than those without bacteraemia [32.1% (9/28) *vs.* 5.2% (26/498), $P < 0.0001$]. This increased CFR related to the concomitant bacteraemia could not be confirmed in those severe malaria cases with deep coma, although numbers were very small.

CFRs of the different bacterial isolates among severe malaria patients ranged from 12.5% for *Streptococci* to 33.3% for *H. influenzae*. Among severe malaria bacteraemic patients, CFRs did not significantly vary according to antibiotic use (25.0% for children who received antibiotics *vs.* 17.4% among those who did not; $P = 0.49$).

Table 3 Risk factors independently associated with mortality in children <5 years ($n = 1165$) with severe malaria according to a multivariate logistic regression model.

Risk factor	Adjusted OR	95% CI	*P* (LR χ^2-test 1 d.f.)
Positive blood culture†	6.2	2.8–13.7	<0.0001
Unconsciousness	2.8	1.3–6.1	0.02
Oral candidiasis	7.6	1.4–42.8	0.04
Weight for age *Z*-score (per unit increase)	0.74	0.62–0.88	0.0005
Nasal flaring	3.3	1.8–5.9	0.0001
Hypoglycaemia	3.0	1.2–7.7	0.04
Having been previously visited‡	2.2	1.2–4.0	0.01

LR, likelihood ratio.
†Only includes children with a valid blood culture result (positive versus negative blood culture).
‡Only includes peripheral health posts traditional healers and informal health sector.

Risk factors for death

Independent risk factors for death among severe malaria patients are shown in Table 3. Importantly, a positive blood culture independently increased the odds of death by 6.2 (95% CI 2.8–13.7).

Discussion

Severe malaria is the predominant cause of hospital admission among children younger than 5 years of age attending Manhiça hospital (Bassat *et al.* 2008). This study portrays the frequent coexistence of bacteraemia among severe malaria patients, and its detrimental prognostic implications. Despite a substantial degree of overlap between the two diseases, it seems difficult to establish whether one infection predisposes to the other or whether they are both coincidental. Indeed, 5.4% of all admitted patients with severe malaria and valid blood culture results had concurrent bacteraemia, and conversely, 7.3% of

bacteraemic patients had also severe malaria. Bacteraemia substantially increased, as anticipated (Berkley *et al.* 1999; Bronzan *et al.* 2007), the risk of death. Bacteraemia should therefore be considered in future revisions as one of the defining criteria for severe malaria. The remaining independent risk factors for death among malaria cases are concordant with those previously described for the area (Bassat *et al.* 2008), disparities being attributable to differences in sample size. CFRs of patients with both diseases were much higher than CFRs of any of the infections alone. Parasite densities among severe malaria patients with bacteraemia were significantly lower than those among non-bacteraemic, suggesting that some of those low-grade malarial infections could be incidental. This finding contrasts with previous studies (Berkley *et al.* 1999; Bronzan *et al.* 2007) in which parasitaemias in severe malaria patients with or without bacteraemia did not differ significantly, but such studies did not look at all consecutive admissions, and were only based on selected series of severe malaria cases predominantly presenting with cerebral malaria, severe anaemia or respiratory distress. It is important to note, however, that due to the age-related differences in the criteria for taking blood cultures, an important proportion of children older than two years of age may lack a blood culture in admission, as compared to younger children. The fact that only 117 severe malaria patients (15.7%) older than two years of age did not have a blood culture performed reassures us that most severe malaria patients did indeed fulfil severity criteria despite their age for blood to be cultured. The lack of microbiological data on these admitted children may, however, bias our estimates on the magnitude of the coexistence of both diseases in any direction, but should not represent, in any case, a major deviation from reality.

As all blood cultures were performed on admission in this study, both diseases, when coexisting, were simultaneously present on arrival. It is however not possible to ascertain the causal pathway leading to such co-infection, as it is problematic to distinguish whether one patient dies with bacteria or parasites in their blood or as a result of such parasites or bacteria.

A consistent relationship between malaria and NTS bacteraemia has been documented (Graham *et al.* 2000; Walsh *et al.* 2002; Bronzan *et al.* 2007), and several studies have suggested that malaria parasites or malarial anaemia may predispose to NTS bacteraemia (Mabey *et al.* 1987). Other African series (Prada *et al.* 1993; Bahwere *et al.* 2001; Evans *et al.* 2004; Norton *et al.* 2004) confirmed this and also underlined the importance of other GRAM negative bacteria among malaria cases. In contrast to these findings, our data highlight the importance of *S. pneumoniae* as the principal isolate related with severe malaria cases in Manhiça. Difficulties inherent to the isolation of *S. pneumoniae* (such as its slow growth rate and subsequent potential for contamination of the truly infected samples by other faster-growing contaminant bacteria) could partially explain why such infection was rare in previous African studies (Evans *et al.* 2004). The HIV infection, highly prevalent in Manhiça [23.6% positivity among pregnant women attending the antenatal clinic in 2001–2006 (Menendez *et al.* 2008)], has traditionally been considered a risk factor for pneumococcal invasive disease (Greenwood 1999). Unfortunately, no HIV data were available for these patients, and its relevance cannot be confirmed, potentially bringing an important bias to these results. However, the importance of HIV infection may be inferred indirectly by the high prevalence of oral candidiasis among children in the study, an unusual finding in severe malaria patients, but a good marker of immunosuppression. Similarly, the absence of sickle cell disease in Manhiça (Menéndez, personal communication), a genetic trait traditionally associated with an increase in NTS and other GRAM negative bacterial infections, may also contribute to enhance the burden of pneumococcal disease in detriment of NTS.

These data also challenge a clear-cut relation between severe malaria cases with any degree of anaemia and NTS. Indeed, *S. pneumoniae* was again the most frequent isolate in any of the different anaemia groups, and also showed the lowest associated mean admission haematocrit. NTS bacteraemias were associated with higher geometric mean parasitaemias than *S. pneumoniae* bacteraemias, contrary to previous reports (Mabey *et al.* 1987; Brent *et al.* 2006) which linked NTS infections to low-level parasite loads or recently cured malaria episodes. This study did not replicate the previously described relationship between malarial anaemia and NTS bacteraemia, although an association between them may still exist, and might not have been detected due to the relatively small number of severe malaria cases with bacteraemia. The contribution of other factors to the development of anaemia in this setting may partially explain why such an association was not clearly confirmed.

This retrospective analysis of all under-five admissions over four years has given insight on the way malaria and bacteraemia intertwine. Importantly, these data may however underestimate what really occurs in the community (Brent *et al.* 2006), where an important proportion of the burden of these two diseases remains undetected. Individually, these two diseases have an enormous impact on the health of African children, and this study has shown that the coexistence of bacteraemia drastically worsens the clinical prognosis in malaria. The deployment of existing, already registered and effective antibacterial tools, such as

the pneumococcus conjugate vaccine, necessary to decrease the silent but constant burden of bacteraemia, may also have an impact in reducing malaria mortality.

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9. Summary of results and conclusions

Study 1

Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for treating uncomplicated malaria in African children: A randomised open-label, non-inferiority trial.

Results

Recruitment and compliance

- 1553 children aged 5 years or younger were recruited and randomly (2:1) assigned to receive either DHA-PQP (n=1039) or AL (n=514), in 5 different African countries (Mozambique, Uganda, Burkina Faso, Zambia and Kenya).
- 88.5% of patients in the DHA-PQP arm and 76.1% in the AL arm completed the day 28 assessment.
- 99.7% (1548/1553) of patients qualified for the primary analysis population (intention to treat/safety), and 91.0% (1413/1553) for the co-primary per protocol population.
- Randomisation generated comparable between countries and overall in terms of baseline characteristics.

Safety and tolerability

- Two deaths (one per group) occurred during the study. In Uganda, a 3 year old girl died 24h after commencing treatment with DHA-PQP, and sepsis or severe malaria was considered the most likely cause. In Mozambique, an 18 month old girl died 7 hours after her first dose of AL. Severe malaria was considered the most likely cause of death, although other aetiologies could not be ruled out.
- Both drugs were well tolerated with the majority of adverse events of mild or moderate severity, and consistent with symptoms attributable to malaria.

- There were no significant differences in the occurrence of events, including serious adverse events.
- Gastrointestinal tolerability of both drugs was similar, with the majority of events being mild.
- Cutaneous adverse events were infrequent, and mainly involved minor dermatitis or rash.
- The proportion of patients with serious adverse events was low (<2% in both groups), most being infectious.
- No significant electrocardiographic abnormalities were seen in any of the treatment groups. Significant increases (≥ 60 ms) in the QTc interval between day 0 and day 2 were observed in <3% of patients, but none of those had any associated clinical symptomatology.

Efficacy

- Primary efficacy endpoint estimates (PP population, PCR-corrected day 28 cure rate) were similarly high in both treatment arms: 95.7% in the DHA-PQP group and 95.7% in the control group.
- These estimates were slightly lower but still similar for the co-primary ITT population: 90.4% in the DHA-PQP group and 90.0% in the control group.
- The lower bound of the one-sided 97.5% CI calculated around the difference between the day-28 cure rate point estimates in both groups was -2.24% (PP), and thus within the pre-specified -5% non-inferiority limit. In the ITT population this was also confirmed (-2.80%).
- The day 42 PCR-corrected cure rates were lower than those at day 28 but similar for the two treatments, irrespective of the study population analysed.
- Infants (age 6-11 months) treated with DHA-PQP (n=129) responded as well to treatment as older children, with day 28 PCR-corrected cure rates being above 90% in both treatment groups. Non-inferiority of DHA-PQP in this age group could however not be confirmed as the study was not powered for this purpose.

- PCR uncorrected cure rates were significantly higher in the DHA-PQP group, both at day 28 and day 42, irrespective of the study population analysed.
- In terms of country specific day 28 PCR corrected cure rates, heterogeneity tests suggested that there were statistically significant differences among countries. Those differences were in size of the treatment effect rather than in its direction (with the exception of Kenya).
- Sensitivity analyses using the WHO standard definitions (survival analysis) confirmed that treatment failures at day 28 were similarly low in both groups.
- PCR uncorrected treatment failure rates at day 28 or day 42 (survival analysis) were both significantly lower in the DHA-PQP group than in the AL. This was mainly due to more late failures in the AL group, shown subsequently to be new infections, rather than recrudescences.
- In the ITT population, new infections until day 42 occurred significantly less in the DHA-PQP group (12.14%) than in the AL group (21.96%). This was also confirmed for the PP population.
- Parasite clearance time was rapid in both treatment groups. Median parasite-clearance time (2 days) did not differ significantly between groups.
- More than 97% of patients were afebrile in both treatment groups by day 2. Fever clearance time showed no differences by group.
- Haematological recovery was adequate and comparable between treatment groups. However, the change in haemoglobin from baseline to last available data was significantly higher in the DHA-PQP group (17.10g/L) than in the AL group (14.91%, p=0.036).

<u>Effects on gametocytes</u>

- Gametocyte prevalence at recruitment (~12%) was similar at both study arms.
- Gametocyte carriage (measured as rate of person-gametocyte-weeks) was significantly higher in the DHA-PQP group (ITT: 43.97/1000) than in

the AL group (21.43/1000; p=0.005). This was also true for the PP population.

Conclusions

- DHA-PQP was as efficacious as AL in treating African children aged 6-59 months with uncomplicated malaria.
- Importantly, efficacy seemed maintained in infants (6-11 months of age), although the study was not powered to show non-inferiority in this age group.
- These efficacy data in infants, coupled with PK studies performed in a subgroup of this study population, suggest that infants may not require an increase in the dose of DHA-PQP.
- It has been argued that the structural similarity between chloroquine and piperaquine could compromise piperaquine's efficacy in areas of high chloroquine resistance. This study challenges this, as DHA-PQP was found equally efficacious in the 5 African countries despite their high underlying chloroquine resistance.
- DHA-PQP was better than AL improving the patient's haematological recovery.
- DHA-PQP showed a better post-treatment prophylactic effect, and prevented more new infections than AL, possibly as a result of piperaquine's longer elimination half-life (~20 days) compared to lumefantrine (4-10 days).
- While this is clearly an advantage for the individual, at the population level it may increase the risk of selecting resistant parasites among the new infection.
- Large-scale deployment of DHA-PQP should therefore be coupled with the careful monitoring of resistance.
- The lower dose of artemisinin derivative used in DHA-PQP may explain why this drug seems to have a weaker gametocydal effect than other ACTs. This may have negative public health effects in terms of increasing the transmission potential, but should be balanced-out with its

better post-treatment prophylactic effect, particularly useful in areas of high transmission.

- The two treatments had similar safety profiles.
- DHA-PQP was well tolerated, and had few adverse events of clinical relevance.
- At therapeutic doses, DHA-PQP and AL do not have any clinical significant cardiotoxicity. There is no evidence either to support a risk for tipe I hypersensitivity reactions.
- The lack of major safety problems in the more than 1000 children treated with DHA-PQP is reassuring but should not slow down the development of pharmacovigilance systems where those new drugs may be deployed.
- DHA-PQP is a safe, efficacious, tolerable and affordable new antimalarial treatment option in Africa.

Study 2

Efficacy and safety of artemether-lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial.

Results

Recruitment and compliance

- 899 children aged 12 years or younger were randomly assigned to either dispersible (n=447) or crushed tablets (n=452), in 8 sites in 5 different African countries.
- More than 85% of patients in each treatment group completed the study.
- 4% of patients withdrew during the 3-day treatment period.
- 90% (812/899) of patients qualified for the primary analysis population (modified intention to treat).
- Randomisation produced two similar groups in terms of baseline characteristics.

Safety and tolerability

- Three patients died during the study, but none of the deaths was deemed related to the study drug. One was a complication of a new *P. falciparum* infection, the other was secondary to a haemorrhage caused by a scarification performed by a witch doctor, and the third one was related to a unspecified infection (non-malaria) accompanied by severe dehydration.
- Tolerability was adequate in both treatment groups with no difference in pattern and overall frequency of adverse events.
- The proportion of patients with serious adverse events was low (1-2% in both groups), most being infectious.
- Few patients developed danger signs of malaria or severe malaria (three patients on dispersible tablets and four on crushed tablets).
- No new or unexpected adverse event was seen.

- Most commonly reported adverse events were symptoms of malaria.
- The most frequent drug-related adverse event was vomiting (7.4% children receiving the dispersible formulation vs. 9.3% receiving the crushed ones).
- No clinically relevant neurotoxic (including events related to the auditory system) effects were seen during the study.
- The proportion of children with abnormal haematological or biochemical values was similar in each treatment arm at day 28.
- No clinically relevant findings or differences between groups were found in the electrocardiographic assessment. The study showed a slight increase in QTc interval, similar for both treatment arms, but not associated with clinical symptoms.

Pharmacokinetics

- Population pharmacokinetics was used to estimate the different PK parametres.
- The maximum concentration of artemether plus dihydroartemisinin (artemether's metabolite) did not suffer substantially across bodyweight groups.
- No difference in lumefantrine pharmacokinetics between treatment groups was apparent.

Efficacy

- Primary efficacy endpoint estimates (PCR-corrected day 28 cure rate) were similarly high in both treatment arms: 97.8% (95%CI 96.3-99.2%) in the dispersible tablet group and 98.5% (97.4-99.7%) in the crushed tablet group.
- Cure rates were also high (above 95%) and comparable between study arms for the other study analysis populations (ITT, PP).
- The lower bound of the one-sided 97.5% CI calculated around the difference between the day-28 cure rate point estimates in both groups was -2.7%, and thus within the pre-especified -5%% non-inferiority limit.

- Cure rates were generally similar across bodyweight groups for both treatments.
- PCR corrected cure rates at days 14 and 42 were also high and similar for the two formulations, irrespective of the study population analysed.
- Median time to fever clearance (7.9h *vs.* 7.8h) and median parasite-clearance time (34.3h *vs.* 34.9h) did not differ significantly between groups.
- Gametocyte clearance could not be assessed because too few patients had gametocytes at baseline (<5%), and only less than 1% of the patients in both groups had detectable gametocytes after day 8.
- Early (2 cases) and late clinical failures (5 patients) were rare throughout the study.
- The proportion of patients with late parasitological failure was similar between treatments (11.4% vs. 13.4%).
- The proportion of patients with ACPR (day 28 to day 42) was also similar between the two groups.

Conclusions

- The dispersible, cherry-flavoured, formulation of AL was not inferior in efficacy to crushed tablets in African children with uncomplicated *P. falciparum* malaria.
- PCR-corrected day-28 cure rates were high for both formulations in children. Cure rates were also similar and high (>90%) for the secondary efficacy endpoints.
- Both formulations had a similar safety profile.
- No differences were seen in the response to treatment across the various bodyweight groups.
- No differences were seen between the two formulations in terms of clearance of asexual parasites and fever, which was rapid for both treatments.
- Analyses performed in the non-primary study populations were in agreement with the primary analysis and confirmed the robustness of the data.

- Both formulations seemed to have a similar prophylactic effect.
- The dispersible formulation may contribute to a better therapeutic compliance and consequently become a useful alternative to the conventional crushed tablets for the treatment of uncomplicated malaria in children.
- Assuming an equal efficacy, this formulation may, by overcoming barriers to access, guarantee a better effectiveness.

Study 3

Malaria in rural Mozambique. Part II: children admitted to hospital.

Results

Clinical presentation of children with malaria admitted to the hospital

- 49.1% (4080/8311) of all children <15 years of age admitted during a 2 year- period had a diagnosis of clinical malaria.
- More than a quarter (27%) of all admitted malaria cases fulfilled the definition of severe malaria (13.2% of all admitted patients).
- Males accounted for a significantly higher proportion of malaria and severe malaria admissions.
- 58.1% of all malaria cases occurred in children younger than 2 years of age.
- Infants carried the brunt of severe malaria accounting for almost 30% of the cases, but malaria was extremely rare in the neonatal period.
- One third of all malaria cases requiring admission did not have fever on arrival.
- Mean PCVs among malaria patients significantly increased with increasing age.
- Severe malaria cases had a significantly lower mean PCV than uncomplicated cases (23.4% *vs.* 25.2%, p<0.0001)
- Severe malaria cases had a significantly lower geometric mean parasitaemia than uncomplicated ones (14756 parasites/μL *vs.* 20616 parasites/μL, p<0.0001).
- Malaria cases, severe malaria cases, severe malarial anaemia episodes and malaria deaths were significantly more frequent during the rainy season (November-April).

Case fatality rates of children with malaria

- There were 62 deaths attributable to malaria, representing 18.7% of all paediatric in-hospital deaths.

- The majority of malaria deaths were clustered in the first two years of life, but up to 10% of the deaths occurred in children older than 5 years of age.
- CFR for malaria was significantly lower than for other diagnoses (1.6% *vs.* 7.3%, p<0.0001). CFR for severe malaria rose to 4.4%.
- More than 50% of all malaria deaths occurred within the first 48 hours of admission

Characterization of severe malaria cases

- Prostration (55.0% of total severe malaria cases), respiratory distress (41.1%) and severe anaemia (17.3%) were the three most frequent criteria to define severe malaria.
- Deep coma, which was infrequent (2.2%) had the highest associated CFR (18.2%), followed by hypoglycaemia (CFR 16.2%) and respiratory distress (CFR 6.2%).
- Geometric mean parasitaemias were significantly higher for the cerebral malaria syndrome (26242 parasites/µL) than for the respiratory distress (11426) or severe anaemia (11924) ones.
- Age distribution of these three classical severe malaria syndromic presentations suggested that patients had severe anaemia or respiratory distress at younger ages than cerebral malaria.
- 78.4% of children with severe malaria presented on admission with a single severe malaria criterion, 16.6% with two, and only 5.1% with three or more. CFRs rose with increasing number of criteria on admission (p<0.0001).

Risk factors for death in children admitted with malaria

- In children aged one to seven months, severe anaemia (OR 9.5, 95%CI 2.0-45.5) and Inability to look for mother's breast (OR 4.6, 95%CI 1.1-19.1) were independently associated with an increased risk of death.
- In children 8-59 months, independent risk factors for death were:
    - Hypoglycaemia (OR 8.2, 95%CI 2.2-31.0)
    - Indrawing (OR 5.8, 95%CI 2.8-12.1)

- Malnutrition, as measured by unit increase of the WAZ score (OR 0.66, 95%CI 0.53-0.82)
- Inability to sit (OR 4.5, 95%CI 2.1-9.3)
- History of vomiting (OR 2.4, 95%CI 1.1-5.0)

Minimum community-based incidence rates (MCBIRs)

- Malaria incidence in the neonatal period was negligible
- Children from 6 months to 2 years of age had the higher MCBIRs for malaria and severe malaria.
- MCBIRs for clinical malaria during the whole study period were 90/1000 CYAR in infants, 86 in children aged 1 to <5 years, and 6 in children >5 years.
- MCBIRs for severe malaria during the whole study period were 27/1000 CYAR in infants, 23 in children aged 1 to <5 years, and 2 in children >5 years.
- Overall, MCBIRs for malaria and severe malaria were twice as high during the first year of study than during the second one. The age pattern also changed between the first and second years of the study.

Conclusions

- The burden of malaria morbidity and mortality is borne mainly by infants and young children, and this may be relatively independent of the intensity of transmission.
- Malaria control interventions targeted at early infancy (such as IPTi, for instance) may therefore have a significant impact in reducing the burden of malaria in this area.
- The substantial malaria burden among older children seen at the outpatient department, and the potential underestimation of the disease among the older admitted children suggests that malaria control programmes cannot disregard this specific age group.
- More than half of the malaria deaths occurred within the first 48 hours of admission, leaving little time for antimalarial treatment to have an effect.

Early recognition of severe malaria cases at home or at the community level, and prompt pre-referral treatment with effective and easy to administer antimalarials, may improve their survival likelihood.

- Hypoglycaemia and respiratory distress were found to be independent risk factors for death and presented high associated CFRs.
- Malnutrition was also found to be an independent risk factor for death, but this study could not assess the prevalence of HIV co-infection, which presumably plays a significant role in the outcome of malaria illness or in the vulnerability to develop a more severe presentation of the disease.
- Neither deep coma nor impaired consciousness were associated in this study with a higher risk of dying, contrary to what most studies have shown. This could be related to the low prevalence of these conditions, despite them associating high CFRs.
- Prostration was highly prevalent and a clear risk factor for death in both age groups. Identifying prostrated children on arrival may be challenging but is likely to have a great impact on their survival.
- Anaemia, from mild to life threatening, is highly prevalent in this setting and a clear risk factor for death among infants. Interventions focussed on this age group and targeted at preventing anaemia could be highly beneficial.
- The level of parasitaemia on admission is not directly related to a higher risk of death, and more than a third of malaria patients did not show fever on arrival. Thus, relying on the presence of fever or on the parasite count for identifying severe cases on admission may be dangerous.
- MCBIRs for malaria derived from this study are high and concordant with previous data from the area. The yearly variations found in this study could illustrate the high interanual variability that can occur within the same malaria endemic area or more likely reflect a true decline in the malaria burden.
- Hospital-based results underestimate the real burden of disease and are greatly influenced by health-seeking behaviour.

Study 4

Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican Hospital

Results

Severe malaria and bacteraemia in children admitted to the hospital

- Almost a half (7043/14337, 49.1%) of the admitted patients during the four year-long study period had malaria, and a quarter of these (n=1780, 25.3%) fulfilled the severe malaria definition.
- Among severe malaria patients with a valid blood culture performed, 12.8% (207/1616) grew contaminant microorganisms, and 0.3% (5/1616) grew *S. viridians*.
- Among severe malaria patients with a valid blood culture result (excluding contaminants and S. viridians), 5.4% (76/1404) had bacteraemia.
- Among severe malaria patients with bacteraemia, *S. pneumoniae* was the most frequent isolate (26%, n=20), followed by Gram negative bacteria (21.1%, n=16), *S. Aureus* (17.1%, n=13) and NTS (15.8%, n=12).
- *S. pneumoniae* was also the most frequent isolate among all other non-malaria admissions.
- *S. pneumoniae* was also consistently the most important isolate in each of the different severe malaria syndromes, except for patients with convulsions or prostration.
- Prevalence of bacteraemia among the different severe malaria syndromes was variable and ranged from 1.7% among those cases with convulsions to 12.5% in patients with Hypoglycaemia.
- Bacteraemic children with severe malaria had a higher CFR, were more severely malnourished and had lower mean parasitaemias than severe malaria patients with a negative blood culture.
- No differences were found regarding the prevalence of NTS bacteraemia among children with severe malaria and non-malaria patients.

- Respiratory bacteria (*S. pneumoniae* and Hib) were isolated more frequently from patients with severe malaria and respiratory distress (43.2%) than from patients without it (20.5%, p=0.03)
- Among severe malaria patients, the presence of hypoglycaemia, hepatomegaly and oral candidiasis was significantly more frequent in patients with bacteraemia than in those without it. Contrarily, the presence of convulsions was less frequent.

Anaemia and bacteraemia in severe malaria patients

- We found a high prevalence of anaemia (of any degree, i.e. PCV<33%) among severe malaria patients (83.0%).
- The frequency of bacteraemia seemed to be independent of the presence of anaemia, regardless of the severity of anaemia.
- *S. pneumoniae* (and not NTS) was the most frequent bacterial isolate among all different malarial anaemia groups (Mild, moderate and severe).

Case fatality rates

- Severe malaria accounted for 12% (81/669) of all in-hospital deaths in children <5 years of age.
- 13 children with bacteraemia and severe malaria died.
- CFR for severe malaria was overall 4.9% (81/1648), rising significantly to 22.0% (13/59) when associated with concomitant bacteraemia.
- Severe malaria patients with severe anaemia or respiratory distress significantly increased their likelihood of dying whenever bacteraemia was concomitantly present. Counter intuitively, this could not be confirmed for cerebral malaria patients, although numbers were very small.
- CFRs of the different bacterial isolates among severe malaria patients ranged from 12.5% for *Streptococci* to 33.3% for Hib.
- CFRs among severe malaria patients with bacteraemia did not significantly vary according to antibiotic use.

Risk factors for death

- Risk factors independently associated with mortality in children <5 years of age with severe malaria were similar to those published in a previous revision (see article 3).
- Importantly, a positive blood culture independently increased the odds of death by 6.2 (95% CI 2.8-13.7%).

Conclusions

- The coexistence of bacteraemia and severe malaria is frequent and has detrimental prognostic implications.
- Concomitant bacteraemia substantially increased the risk of death among severe malaria patients.
- Bacteraemia should be included in future guidelines as one of the defining criteria for severe malaria.
- Determining whether one disease predisposes to the other or both are coincidental is challenging, despite their substantial overlap. However, as all blood cultures were performed on admission in this study, both diseases when coexisting, were simultaneously present on arrival.
- Parasite densities among severe malaria patients with bacteraemia were significantly lower than those among non-bacteraemic, suggesting that some of those low-grade malarial infections could be incidental.
- These data highlight the importance of *S. pneumoniae* as the principal isolate related with severe malaria cases in Manhiça. This contrasts with previous data from other sites suggesting that severe malaria was more frequently associated to NTS or other Gram negative bacteraemia.
- The high prevalence of HIV infection in Manhiça and the low prevalence of hemoglobinopathies in the area may partially explain the high burden of pneumococcal disease in detriment of NTS.
- These data also challenge a clear-cut relation between severe malaria cases with any degree of anaemia and NTS, but do instead underline the relationship between malarial anaemia and *S. pneumoniae*.

- These hospital-driven data probably underestimate what really occurs in the community, where an important proportion of the burden of these two diseases remains undetected.
- Measures envisaged to decrease the burden of invasive bacterial disease, such as the pneumococcus conjugate vaccine, may also have an impact in reducing malaria mortality.

10. General conclusions

Study 1

1. DHA-PQP has a similar efficacy and safety profile as AL and is therefore a good alternative for the treatment of uncomplicated *P. falciparum* malaria in African children.
2. The potential detrimental effects on transmission related to the increase on gametocytaemia caused by DHA-PQP need to be balanced out with its better post-treatment prophylactic effect, when compared to AL.

Study 2

3. The dispersible formulation of AL has a similar high efficacy and good safety profile than the traditional crushed AL tablets.
4. This formulation will presumably enhance compliance among the younger children, as it has been designed to be more easily swallowed and has a paediatric-friendly cherry taste.
5. Assuming an equal efficacy to conventional crushed tablets, the deployment of this formulation may be advantageous in terms of public health, as an increased compliance may therefore enhance its effectiveness.

Study 3

6. In settings such as Manhiça, where the burden of clinical malaria cases and malaria deaths falls on children less than 2 years of age, malaria control strategies need to target this age group, with special emphasis on infants.
7. Better recognition of signs and symptoms associated with a bad prognosis among severe malaria patients, and prompt treatment at the health facilities or even at the community level, may improve the survival likelihood of those children.
8. Minimum community-based incidence rates of severe malaria in Manhiça are high and show significant year to year variability.

9. The coexistence of bacteraemia and severe malaria is frequent and has detrimental prognostic implications.
10. In settings with high HIV prevalence, *S. pneumoniae* can replace NTS and other gram negative bacteria as the most frequent cause of bacteraemia in patients with severe malaria.
11. Development and use of improved prevention tools as well as clinical management of bacteraemia may result in reduced malaria mortality.

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12. Acknowledgements

The origins of this work can be traced back to the spring of the year 1998, when, as a (rather annoyed and possibly annoying) medical student I developed an urge to spend my summer holidays working in a developing country. At that stage, Pedro Alonso, who would later become my friend, boss and mentor, gave me an opportunity that I could not decline: to spend a couple of months in CISM, a newly created research centre in Manhiça, a rural Mozambican area. While CISM was in 1998 at a very embryonic stage of its development, with no more than 25 people working there, it had already a very distinctive *Alonsian* look, and aspired to become a leading research centre in Africa with all the enthusiasm and none of the defects that most of the already existing research centres in Africa still showed. Together with my dear friend Begoña Benito, I spent there two of the best months of my medical training, during which, not only I immersed myself in the clinical work at the wards, but also had my first contact with research, collecting nasopharyngeal aspirates from healthy volunteers at the community. This first African experience was also an eye-opening one, as I clearly understood three crucial things: First, as very little exams could be performed in the hospital one needed to rely in one's clinical skills, and therefore becoming a good clinician needed to be a priority. Second, as most of the patients brought to the hospital were children, one needed to understand and be able to treat this specific population. Finally, clinicians had an important responsibility to continue doing their clinical work, but if they wanted to have a wider impact, they needed to provide solutions applicable to the wider community, and this could very effectively be performed by combining clinical work with research. When I returned to Barcelona, and soon after starting my last year of medical school, I was again given the opportunity to travel to Africa to stay for a month at Saint Francis Hospital, in Ifakara, Tanzania. David and Joanna Schellenberg received me in Ifakara with great fondness, and I confirmed there that my professional future could only contemplate going back to Africa to work as a clinician and a researcher.

Once I finished medical school, in June 1999, and against my impatient desires to leave Barcelona and establish myself as a clinician in Africa, I studied for the Spanish national specialization exams (MIR) and managed to get a place in

Barcelona's Hospital de la Vall D'Hebron, where I would spend the following 4 years becoming a paediatrician. Four people were of paramount importance during my 4 years spent in this hospital: Two fellow residents -Dominique Vizmanos and Pere Soler- , both great paediatricians and now among my best friends, made those 4 years of hard work easier and happier. Dr. Jordi Almar, a fantastic neonatologist was also responsible for many joyful breakfasts and my first ever published scientific paper. Finally, Su Boronat, an extraordinary paediatric neurologist and a truly caring person helped me become a better doctor, and also a better human being.

Despite those very joyful years, Africa remained vividly in my head, and when during my third year of specialization Pedro Alonso called me asking whether I could be interested in participating as a paediatrician in a malaria candidate vaccine study in Manhiça, I was the happiest person on earth. I managed to convince the hospital manager that this was a great offer not only for me but also for the hospital, and he maliciously agreed to let me go to Mozambique for 4 months if I could anticipate all the nightshifts of those four months in advance. Stubborn as I've always been, I managed to do an extra 27 nightshifts in the 4 months preceding my departure, and I became the first ever paediatrics resident in the hospital that was legally able to move to Africa as part of the training. The effort was worth, and during the four months spent in Manhiça I had the chance of working with the best possible team in a revolutionary study that would change many of the dogmas established around African research centres and malaria vaccine trials. From those great days I have very fond memories, but also one sad one: not being present to bid farewell to my dear grandmother "Mami", who died in Barcelona on the 22nd of August 2003.

Once back in Barcelona, I finished my paediatrics training not without, once again, fooling the manager of the hospital to allow me to spend further 5 months studying a Masters course in Tropical Medicine and International Health while working full-time at the hospital. Again, this was not an easy venture, but I managed to finish both my paediatrics residency and the MsC course in one piece. During that course I first met a fellow paediatrician, Montse Renom, together with whom I would closely be working in different projects a few years later.

Once more adequately trained, I was offered the chance to move to Mozambique, to work at the *Centro de Investigação em Saúde da Manhiça* (CISM). I worked and lived there for three full years, during which I had the chance of practicing as a paediatrician, and starting my own research. Severe malaria became my first point of interest, but as it usually occurs in Manhiça, I ended up expanding the scope of my interest to many other subjects, most of them however malaria-related. Many people contributed both to my research and happiness while I lived in Manhiça. Mozambicans have always been kind at heart and welcoming to me. Three different Mozambicans were CISM's coordinators during my years there: Eusebio Macete, Betuel Sigaúque and Ariel Nhacolo. Although very different in their character, the three shared a very professional attitude towards the job, and I believe I always established with each of them a very affectionate and respectful relation. With Betuel I also had the chance of sharing responsibilities in the clinical wards and of working in different projects, mostly related to respiratory infections, and to date we still maintain a robust friendship for which I'm thankful. The administration department, led by Gonzalo Vicente on my arrival, was always of great help to me, not only on the personal level but also on the professional one. The three other successive administrators (Joan Vives, Sergi Noguera and Jacinto Chilengue) all became good friends and colleagues. Manhiça would not be the same if it had not had dedicated people like them working day and night to solve the possible and the impossible. I'm very grateful too to other people working there such as Abel Detepo (master of ceremonies), Jaume Tarragó (one of my first good friends in Manhiça), Rosaria, Constancia, Sheila or Carmina. However, my everyday work in Manhiça always had a heavy clinical component, and in that sense I want to express my gratitude to all clinicians with whom I worked there. With some of them (Pedro Aide, Jahit Sacarlal and Eusebio Macete), I had already closely worked during the malaria vaccine trial. Pedro Aide and I arrived to Manhiça at the same moment, and I believe we grew together as researchers. I admire Jahit's work capability, and his involvement in any study guarantees a Ganesh-like sense of protection. Other doctors such as Betuel Sigaúque, Azucena Bardají, Sónia Machevo, Tacilta Nhampossa, Emili Letang, Catarina David or Montse Renom shared many long days and nights at the hospital, and I learned immensely from all of them on

how to work in an African rural hospital. Azucena was also my best neighbour, with a tortilla de patatas always ready to improve my dinners. Emili always opened his house for late night discussions, and was a faithful jogging companion. I am also grateful to other clinicians, and in particular to Madalena Ripinga, and the medical agent Berta whom I spent lots of time in my rotations through the ICD, and I must not forget the 24-hour availability of enfermeros Manuel, Sergio and Mónica to help me with giving adequate attendance to the most severe kids. Finally, I'm glad to have met the many residents, medical students and estagiarios that for longer or shorter periods passed through Manhiça.

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Quique Bassat, Spring 2009


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