The Cardiovascular Toxicity of Antimalarial Drugs

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A thesis submitted for the degree of
Doctor of Philosophy

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“It is base to receive instructions from others’ comments without examination of the objects themselves, especially as the book of nature lies so open and is so easy of consultation”

– William Harvey

สามเหลี่ยมเขยื้อนภูเขา

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Abstract

Malaria is an ancient mosquito-borne parasitic disease from which over a thousand – mostly children in sub-Saharan Africa – still die of needlessly every day. For half a millennium, quinine and quinine-like antimalarial drugs have been the mainstay of malaria treatment and prevention. In the 18th century, the chance observation of their ability to quell palpitations led to their becoming the first anti-arrhythmic agents. Some of these anti-arrhythmic antimalarials later came to define the adverse drug reaction of repolarisation-related cardiotoxicity as sudden deaths, ventricular tachyarrhythmias, and electrocardiographic QT interval prolongation were in turn causally associated with their use. With increasing population-level use of antimalarials for malaria elimination, there has been renewed global interest in defining the cardiovascular toxicity of key members of this drug class to guide antimalarial choice and dosage for development and deployment.

In this thesis, I investigate the repolarisation-related cardiotoxicity of the quinoline and structurally-related oral antimalarials mostly widely used in malaria treatment, prevention, and drug development. In Chapter 3, I find that the risk of sudden unexplained death after dihydroartemisinin-piperaquine, a leading candidate for mass drug administration and intermittent preventive therapy for malaria, is no higher than baseline. In Chapter 4, I report how torsade de pointes and other clinically significant arrhythmias have not been documented after front-line antimalarials at standard malaria doses despite extensive use. In Chapter 5, I identify independent effects of malaria severity and fever on the QT interval accounting for the greater post-drug prolongation in malaria patients compared to healthy individuals. In Chapter 6, I present the QT interval prolongation and heart rate reduction from artesunate-amodiaquine compared with other front-line antimalarials and propose bradycardia may underlie amodiaquine-associated asthenia.

I conclude that chloroquine, piperaquine, amodiaquine, and lumefantrine remain safe at World Health Organization-recommended doses and combinations for the treatment, prevention, and global eradication of malaria.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ACT	Artemisinin-based combination therapy
AL	Artemether-lumefantrine
ANOVA	Analysis of variance
AQ	Amodiaquine
ASAQ	Artesunate-amodiaquine
bpm	Beats per minute
C_{max}	Maximum or peak drug concentration
CDC	Centers for Disease Control and Prevention
CI	Confidence (frequentist) or credible (Bayesian) interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CIOMS	Council for International Organizations of Medical Sciences
CiPA	Comprehensive *in vitro* Proarrhythmia Assay
CQ	Chloroquine
CYP2C8	Cytochrome P450 isozyme 2C8
CYP2D6	Cytochrome P450 isozyme 2D6
CYP3A4	Cytochrome P450 isozyme 3A4
CYP450	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane
DEAQ	Desethylamodiaquine
DHA	Dihydroartemisinin
DNDi	Drugs for Neglected Diseases initiative
DP or DHA-PPQ	Dihydroartemisinin-piperaquine
ECG	Electrocardiogram
EMBASE	Excerpta Medica Database

ERG	Evidence Review Group
FDA	Food and Drug Administration
G6PD	Glucose-6-phosphate dehydrogenase
GMEP	Global Malaria Eradication Programme
GRADE	Grading of Recommendations Assessment, Development and Evaluation Working Group
GSK	GlaxoSmithKline
hERG	Human ether-à-go-go related gene
HRP2	Histidine-rich protein 2
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IPD	Individual patient/participant-level data
IPT	Intermittent preventive treatment
IPTi	Intermittent preventive treatment in infants
IPTp	Intermittent preventive treatment in pregnant women
IQR	Interquartile range
IRS	Indoor residual spraying
IST	Intermittent screening and treatment
LDH	Lactate dehydrogenase
LLIN	Long-lasting insecticide-treated bednet
MDA	Mass drug administration
MEDLINE	Medical Literature Analysis and Retrieval System Online
MSAT	Mass screening and treatment
NONMEM®	Non-linear mixed effects modelling
NR4A2	Nuclear receptor subfamily 4, group A, member 2
OFV	Objective function value
PA or PyAS	Pyronaridine-artesunate

QTc	Corrected QT interval
QTcB	Bazett formula-corrected QT interval
QTcF	Fridericia formula-corrected QT interval
QTcS	Study specific formula-corrected QT interval
PROTECT	Pharmacoepidemiological Research on Outcomes of Therapeutics by a European Consortium
RBC	Red blood cell or erythrocyte
RDT	Rapid diagnostic test
SCD	Sudden cardiac death
SD	Standard deviation
SMC	Seasonal malaria chemoprevention
SP	Sulfadoxine-pyrimethamine
SUD	Sudden unexplained death
TdP	Torsade de pointes
UK	United Kingdom of Great Britain and Northern Ireland
US or USA	United States of America
WANECAM	West African Network for Clinical Trials of Antimalarial Drugs
WBC	White blood cell
WHO	World Health Organization
WRAIR	Walter Reed Army Institute for Research
WWARN	WorldWide Antimalarial Research Network

1 Introduction

1.1 MALARIA

Malaria is the most important parasitic disease of humans. It has changed the course empires and revolutions, inflicted more casualties than conflict, and rewritten our genetic code. No other infectious disease has shaped the history or genome of our species as fundamentally as malaria.

1.1.1 Etymology and History

The Chinese word for malaria (疟) has been found inscribed on oracle bones (甲骨) used for divination dating as far back as between 1401 and 1101BC[1]. Paroxysmal fevers causing enlarged spleens were described in the oldest and most important ancient text in Chinese medicine, *The Yellow Emperor's Inner Canon* (黄帝内经), which was composed between the Warring States period and the Qin dynasty (475-206BC). In ancient Greece, malaria was known simply as 'the fever' (πυρετός)[2]. The Greek physician Hippocrates, the 'Father of Medicine', recorded the first descriptions of the disease's characteristic periodic 'tertian' and 'quartan' fevers in his 400BC treatise *On Airs, Waters, and Places*[3].

Malaria was thought to be caused by poisonous fumes from swamps and marshes for two millennia. The name 'malaria' itself has its origins in the old miasma theory of disease[4], with 'mal aria' being medieval Italian for 'bad air'[5]. The word malaria was introduced into the English language by the Scottish geologist and physician John MacCulloch in 1827, who studied the distribution of 'marsh fever' from a topological perspective[6].

In 1880, while working in the Constantine military hospital in Algeria, the French physician Alphonse Laveran became the first person to see the malaria protozoan in a blood smear from a patient who had just died of the disease. He disliked the name malaria, preferring 'paludisme' from the Latin for swamp 'palus', and it is this name which has lived

on as the French term for the disease[7]. However, Laveran's name for the protozoan had no such longevity: the Italian physicians Ettore Marchiafava and Angelo Celli renamed the genus *Plasmodium* just five years later in 1885, after recognising the different stages of development of the malaria parasite in human blood[8].

The identification of the mosquito vector of malaria did not occur until 1897, when the British physician Ronald Ross observed *Plasmodium* oocysts in the guts of female *Anopheles* mosquitoes in India[9]. The *Anopheles* mosquito genus had been discovered and named for the Ancient Greek word for 'useless' (ἀνωφελής) by the German entomologist Johann Wilhelm Meigen in 1818[10].

1.1.2 Aetiology

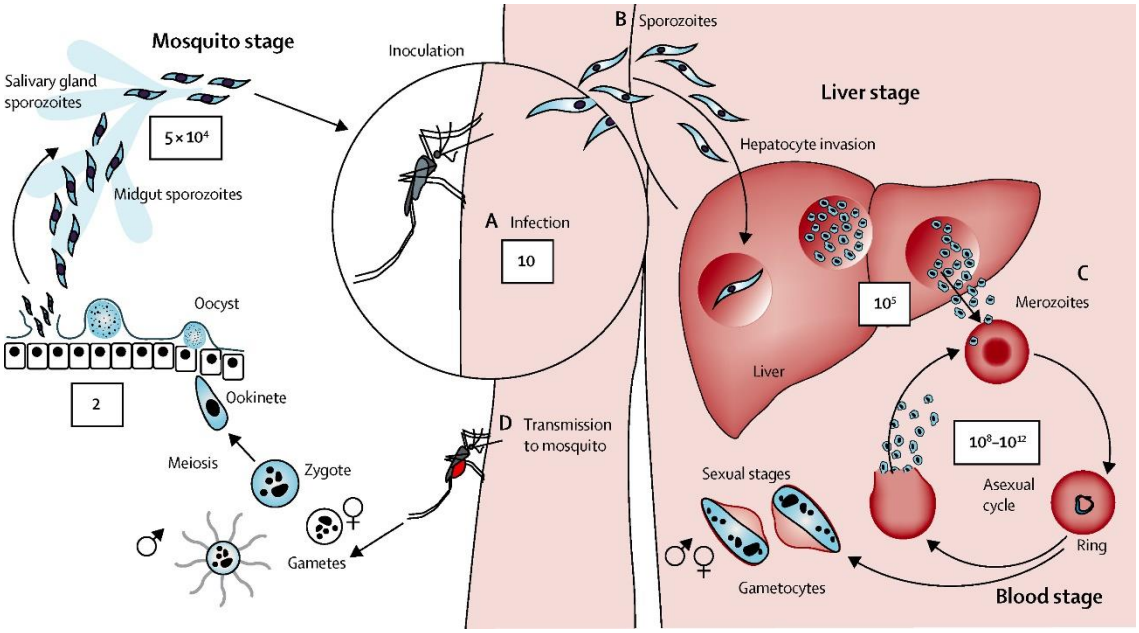
Malaria is caused by the infection of red blood cells by protozoan parasites of the genus *Plasmodium*. Five species are known to cause disease in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*.

Sporozoites of the parasite are inoculated into the human host by the bite of a feeding female *Anopheles* mosquito. In the pre-erythrocytic liver stage, the inoculated motile sporozoites invade and multiply in hepatocytes which mature to become schizonts containing tens of thousands of daughter merozoites. When the hepatic schizont ruptures, it releases these merozoites which invade red blood cells. Thus, the blood stage begins, with serial cycles of trophozoites maturing into erythrocytic schizonts which in turn rupture to release merozoites. These cycles of asexual replication produce the rising parasite numbers which give rise to the signs and symptoms of human disease. A subpopulation of intraerythrocytic parasites switches to sexual development, becoming male and female gametocytes which can transmit malaria to the female *Anopheles* mosquito when they are taken up through a blood meal. In the mosquito midgut, the male gametocyte exflagellates and fuses with the female gamete to form a zygote which

transforms into an ookinete and then an oocyst in the gut wall. Rupture of the oocyst releases sporozoites which migrate to the mosquito salivary glands, thus completing the parasite life cycle[11].

The life cycle of *P. falciparum* in the human and mosquito are shown in Figure 1.1.

Figure 1.1: The Life Cycle of *Plasmodium falciparum* in the Human and Mosquito



Reproduced from a seminal review on malaria[11]. The cycle begins with inoculation of motile sporozoites into the dermis (A; magnified), which then travel to the liver (B); each sporozoite invades a hepatocyte and then multiplies. After about a week, the liver schizonts burst, releasing into the bloodstream thousands of merozoites that invade red blood cells and begin the asexual cycle (C). Illness starts when total asexual parasite numbers in the circulation reach roughly 100 million. Some parasites develop into sexual forms (gametocytes). Gametocytes are taken up by a feeding anopheline mosquito (D) and reproduce sexually, forming an ookinete and then an oocyst in the mosquito gut. The oocyst bursts and liberates sporozoites, which migrate to the salivary glands to await inoculation at the next blood feed. The entire cycle can take roughly 1 month. Estimated numbers of parasites are shown in boxes—a total body parasite burden of 10^{12} corresponds to roughly 2% parasitaemia in an adult.

In *P. vivax* and *P. ovale* infections, a proportion of sporozoites in the human become hypnozoites which lay dormant in the liver and can cause relapses months or years after the initial infection.

1.1.3 Clinical Presentation

The symptoms of malaria develop after the erythrocytic cycle reaches a threshold level of parasites in the blood of roughly 100 parasites/μL (100 million or 10^8 parasites in total – Figure 1.1 section C). Incubation periods vary among species, ranging from 10-14 days for *P. falciparum* and *P. knowlesi*, to 2-3 weeks or even months for *P. vivax* and *P. ovale*, and 18 days or longer for *P. malariae*[12].

The cardinal symptom of malaria is fever, and malaria is often the most common cause of febrile illness in the areas it is endemic. Classical accounts from Hippocrates[3] onwards describe periodic fever spikes corresponding to the length of the erythrocytic cycle of the infecting species: 48 hours or 'tertian' for *P. falciparum*, *vivax*, or *ovale*; and 72 hours or 'quartan' for *P. malariae*. However, these patterns are now observed rarely[12].

Anaemia is a common feature of malaria and is multifactorial in origin[13]. Red cell loss from splenic clearance is typically the leading cause, followed by intravascular haemolysis. Bone marrow suppression and dyserythopoiesis can also contribute.

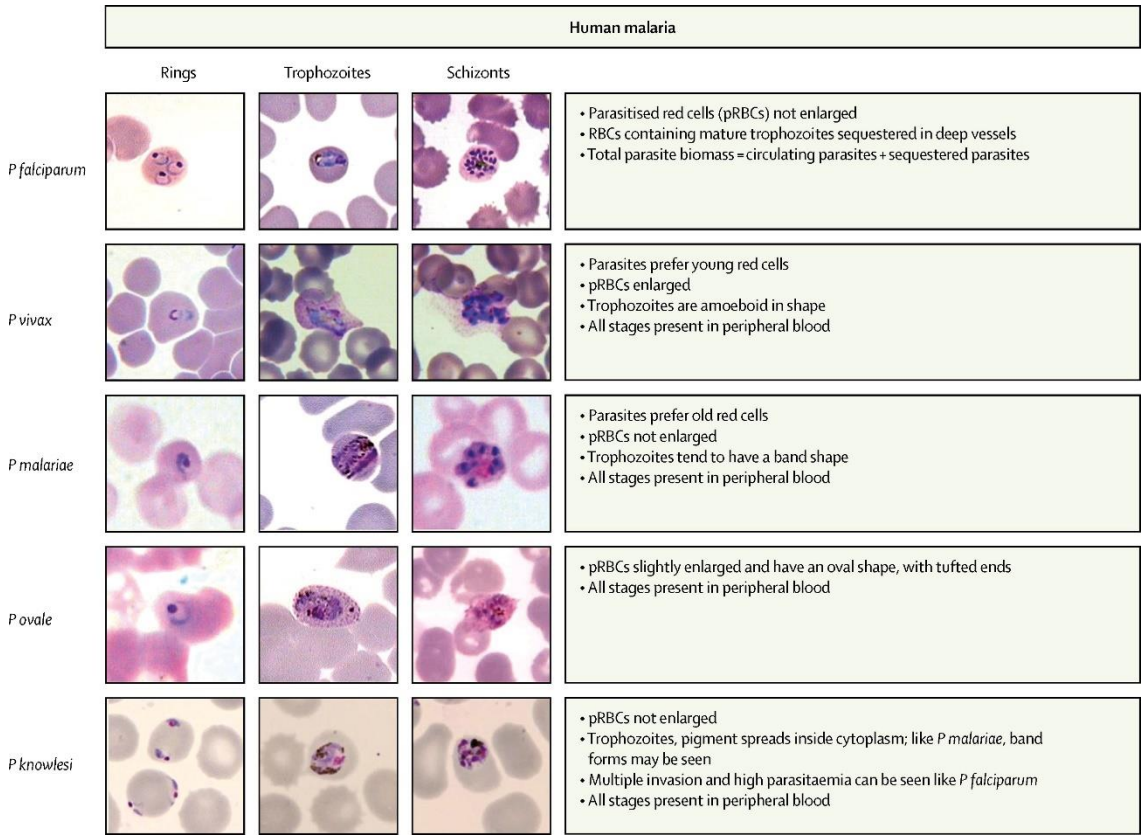
Malaria is divided into two disease presentations which also guide management: uncomplicated and severe. The first symptoms of uncomplicated malaria are non-specific and difficult to distinguish from a systemic viral illness. In addition to fever and chills, these symptoms include: headache, fatigue, lassitude, abdominal pain, diarrhoea, nausea, and vomiting. Clinical diagnosis is therefore unreliable and laboratory diagnosis is needed.

Severe malaria is characterised by organ failure and its presentation varies by age[14]. Prostration, fast deep breathing (of metabolic acidosis), and reduced consciousness (coma) are danger signs used for rapid triage. Other important manifestations are acute lung injury, acute kidney injury, hypoglycaemia, and severe anaemia. Severe malaria has a case fatality rate of 10-20% even with treatment.

1.1.4 Laboratory Diagnosis

Laboratory diagnosis confirming the presence and species of malaria parasites is critical for the initiation of effective species-specific antimalarial drug therapy and to indicate the need for identification of an alternative cause in negative cases. The gold standard for laboratory diagnosis remains light microscopy of stained thick blood films which have greater sensitivity along with thin blood films to quantitate the parasitaemia (Figure 1.2)[12].

Figure 1.2: Blood Films of the Human Malaria Parasites under Light Microscopy



Adapted from a seminal review on malaria[12] and slides skilfully created by Kamolrat Silamut in Thailand. Thin blood films were prepared from specimens taken from patients with clinical malaria, stained with modified Field's stain, and examined by light microscopy under oil immersion at x1000 magnification.

Antibody-based rapid diagnostic tests (RDTs) are another widely-used first-line diagnostic tool for malaria and a range of different devices detecting different antigens is available. However, unlike light microscopy, RDTs do not quantify parasitaemia.

The most commonly available RDT, particularly in Africa, is one based on the highly expressed histidine-rich protein 2 (HRP2) antigen of *P. falciparum*. This test can remain positive for several weeks after acute infection but can be used to diagnose severe malaria in patients who have cleared peripheral parasitaemia. In other parts of the world, RDTs usually also detect the lactate dehydrogenase (LDH) enzyme from all human malaria species, although they are not as sensitive for *P. knowlesi* LDH. HRP2 deletions in the Americas can make HRP2-based tests unreliable[15].

1.1.5 Global Epidemiology

Today, malaria remains endemic in more than 80 countries[16] in tropical and sub-tropical regions (Figure 1.3). Vector and parasite distributions, transmission intensities, human genetics, and drug resistance patterns of malaria are highly location-specific.

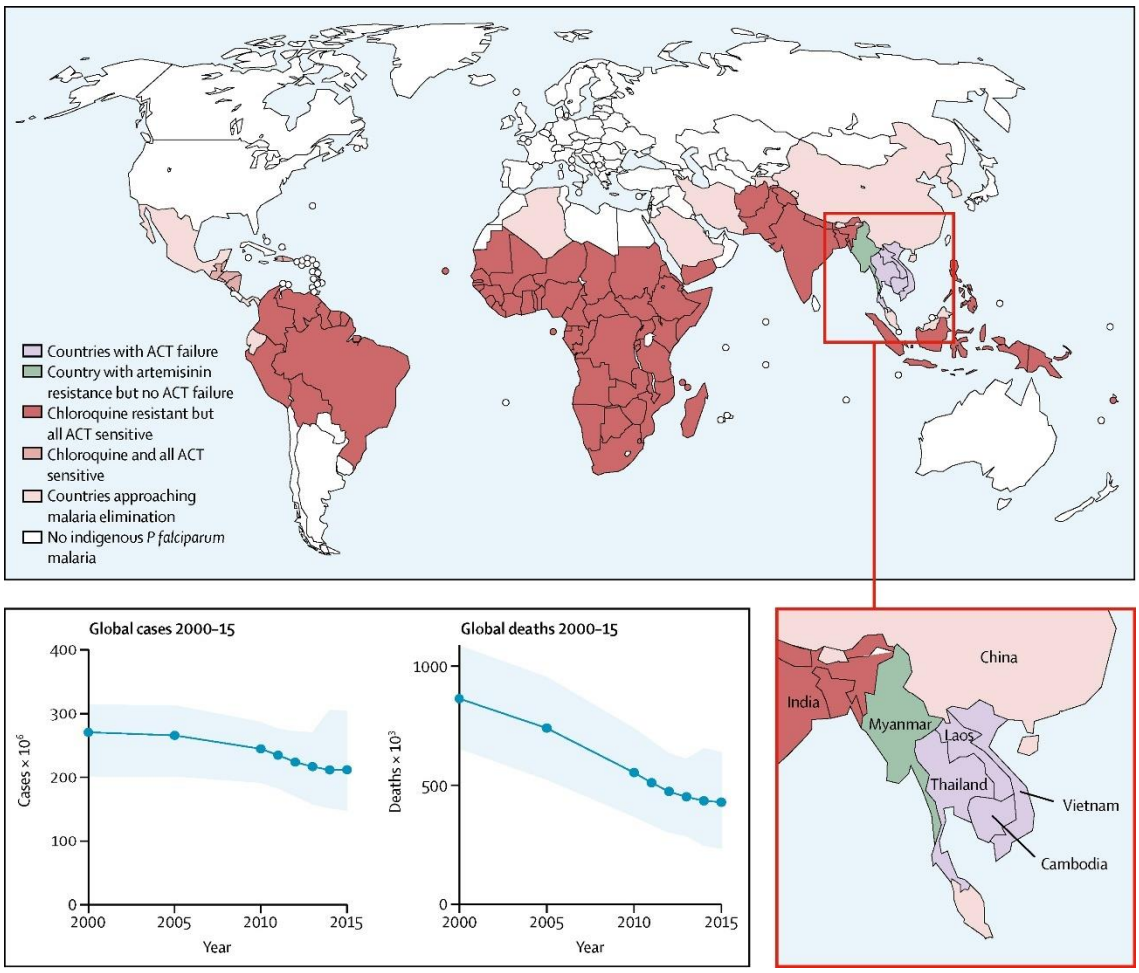
The incidence of malaria is determined by the suitability of the local environment for survival and reproduction of the *Anopheles* mosquito vector. Climate, altitude, vegetation, and level of implementation of control measures are key factors. Malaria therefore thrives in areas of poverty, natural disaster, and war. *A. gambiae* is the most important vector of malaria globally and most commonly found in Africa and the Middle East[17]. *A. dirus* is a major vector in the Asia-Pacific[18], while *A. darlingi* is prominent in the Americas[19].

The most important malaria species globally is *P. falciparum* followed by *P. vivax*, with an estimated 220 million cases of *P. falciparum* and 7.5 million cases of *P. vivax* in 2018[16]. The vast majority of cases and deaths (Figure 1.3), most of which are in children younger than five years, are caused by *P. falciparum* and occur in sub-Saharan Africa where transmission intensity remains high in many areas. Some subregions in Africa, such as the Sahel, have more seasonal transmission concentrated during the rainy season[16]. *P. vivax* has much less of a hold in Africa because of the absence of the Duffy antigen in much of the population. *P. vivax* dominates in the Asia-Pacific and the Americas where it accounts for 50-75% of

cases in these regions[16]. The X-linked recessive glucose-6-phosphate dehydrogenase (G6PD) deficiency, one of the most common inherited enzymopathies present in hundreds of millions of people, protects against *P. vivax* malaria in Asian populations[20,21].

Drug-resistant malaria parasites are a major challenge for malaria treatment and prevention. Resistance has developed in *P. falciparum* to a number of first-line drugs recommended by the WHO, including chloroquine and the anti-folates[22], as well as more recently the artemisinins[23,24] and their partner drugs[24,25]. The geographic distribution of drug-resistant *P. falciparum* malaria is summarised in Figure 1.3. Further information about antimalarial drugs and their use is available in Section 1.2.

Figure 1.3: Global Distribution of Drug-Resistant *P. falciparum* Malaria



Adapted from a seminal review on malaria[12]. The inset graphs show World Health Organization estimates of global annual malaria case numbers and deaths from 2000 to 2015 (with 95% upper and lower uncertainty intervals)[26]. ACT = artemisinin-based combination therapy.

1.1.6 Treatment and Prevention

While there is ongoing and spirited debate about the feasibility of malaria eradication within the next three decades[27,28], it is clear that continued investment into the optimisation and deployment of existing and emerging tools for malaria control and elimination are essential alongside strengthening of health systems.

The three main prongs of malaria treatment and prevention target respectively the *Plasmodium* parasite, *Anopheles* vector, and human host.

1.1.6.1 Antimalarial Drugs

Antimalarial drugs have been the mainstay of the treatment and prevention of malaria for several millennia. A limited number of drug classes is available for use at present although there are several novel compounds at different stages of development[29].

In malaria-endemic regions, antimalarial drugs are used for the treatment of clinical malaria, for preventive therapy in high-risk populations, and in the form of mass drug administration (MDA) for rapid malaria control or for elimination. The objectives of treatment with antimalarial medicines are as follows:

1. Case management

   The clinical objectives of treating malaria are to achieve cure and to prevent progression to severe disease or chronic infection by ensuring as rapid and as complete elimination of the parasite from the blood as possible. The public health objectives of treatment are to reduce onward transmission of malaria and to prevent the emergence and spread of resistance to antimalarial drugs. Uncomplicated malaria is treated with full 3-day oral treatment courses of antimalarial drugs[30]. Severe malaria is treated with parenteral antimalarial drugs for a minimum of 24 hours or until the patient is able to tolerate oral medication[30].

2. Preventive therapy

   Preventive therapies for malaria control are full 3-day oral treatment courses of an antimalarial medicine given to vulnerable populations in order to prevent malarial illness by maintaining therapeutic drug levels in the blood throughout the period of greatest risk. Current WHO-recommended malaria chemopreventive therapies include intermittent preventive therapy of pregnant women (IPTp)[31], intermittent preventive therapy of infants (IPTi)[32], and seasonal malaria chemoprevention (SMC)[33] of children aged 3–59 months.

3. Mass drug administration

   Mass drug administration (MDA) is the coordinated administration of full 3-day oral treatment courses of antimalarial treatment to a population at risk, regardless of the presence of infection. By providing therapeutic concentrations of antimalarial drugs to as much of the population at risk as possible, MDA clears infections from asymptomatic individuals who have sufficient immunity to control infection at a low level without overt illness. This reduces onward transmission and prevents reinfection during periods of post-treatment prophylaxis. The WHO now recommends that MDA of antimalarial medicines be considered for the elimination of malaria in areas approaching interruption of transmission, where there is good access to treatment, effective implementation of vector control and surveillance, and minimal risk of infection being reintroduced[34].

Antimalarial medicines are also used for prophylaxis in travellers visiting malaria-endemic areas for defined periods of time[30].

Further information about the history, pharmacoepidemiology, and clinical pharmacology of antimalarial drugs is available in Section 1.2.

1.1.6.2 Vector Control

Vector control is an essential component of malaria prevention. The main tools in widespread deployment are long-lasting insecticide-treated bednets (LLINs)[35] and indoor residual spraying (IRS)[36] with insecticides.

LLINs are highly effective at reducing malaria-related morbidity and mortality, particularly where transmission intensity is high and vectors bite indoors at night. Pyrethroids are the only class of insecticide used for treatment of LLINs. The extent to which pyrethroid resistance of *Anopheles* mosquito vectors affects LLIN effectiveness is unclear at present[37-39].

IRS is another proven and important vector control measure[40]. Pyrethroids are also the most commonly used insecticide for IRS, along with three other classes: organophosphates, carbamates, and occasionally the organochlorine dichlorodiphenyltrichloroethane (DDT). The non-pyrethroid classes are being used increasingly as part of global insecticide resistance management strategies[41]. However, 73 countries have confirmed resistance to at least one of the four insecticide classes between 2010 and 2018[16]. New insecticides are needed urgently.

1.1.6.3 Vaccines

More than thirty years in the making, the RTS,S/AS01 vaccine based on the *P. falciparum* circumsporozoite protein is the first malaria vaccine to be tested in phase III clinical trials.

Earlier studies of the three-dose regimen demonstrated modest efficacy (29.9% against first and 16.8% against all episodes of clinical malaria) lasting no more than four years which waned with time and malaria exposure[42,43]. A larger study of children (5-17 months; n = 8,922) and infants (6-12 weeks; n = 6,547) with three to four years of follow-up found that the addition of a booster dose to the three-dose regimen improved efficacy against

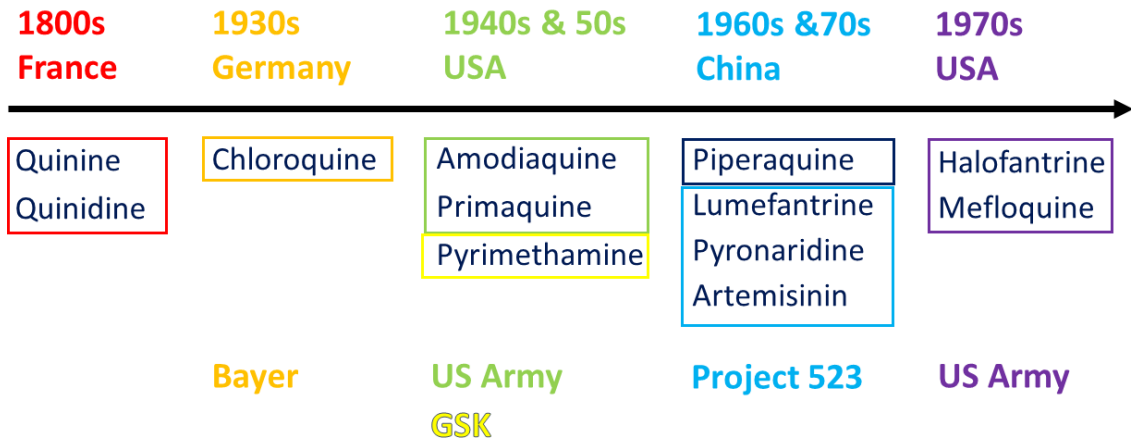
clinical malaria (children: 36.3% vs 28.3%; infants: 25.9% vs 18.9%), and severe malaria (children: 32.2% vs 1.1%; infants: 17.3% vs 10.3%). No significant efficacy against severe malaria was seen in young infants or children who did not receive a booster dose of vaccine. The proportional increase in efficacy against clinical malaria associated with booster vaccination was similar in children and young infants, but efficacy after the booster dose remained lower in those who received their primary vaccination aged 6-12 weeks compared to at the age of 5-17 months. Of note, this trial also identified a meningitis safety signal in the older children[44].

The WHO is supporting a pilot programme of the four-dose regimen of the RTS,S/AS01 vaccine in Ghana, Kenya, and Malawi to generate evidence and experience about its use in the context of routine vaccination[45], but concerns remain about the vaccine's safety and efficacy[46].

1.2 ANTIMALARIAL DRUGS

The safety pharmacology of antimalarial medicines is a vast topic. This section provides an overview of the most widely used antimalarial drugs currently recommended by the WHO for the treatment and prevention of malaria[30]. Figure 1.4 shows when each of these antimalarial drugs was isolated or synthesised.

Figure 1.4: Year and Country of Discovery of Major Antimalarial Medicines



Quinine and quinidine were isolated by independent French chemists. From the 1900s onwards, the World Wars and other major conflicts in the Asia-Pacific massively increased demand for antimalarial medicines. These catalysed large-scale antimalarial discovery and development programmes which were collaborations among the militaries of global powers, academic institutions, and pharmaceutical companies. All of the antimalarials listed which were discovered in the 20th century were part of such programmes, with the exception of pyrimethamine and piperaquine. Pyrimethamine was synthesised by the Nobel laureate Gertrude Elion in Burroughs-Wellcome, now part of GlaxoSmithKline (GSK). Piperaquine was synthesised independently by the Shanghai Pharmaceutical Company Research Institute and the French pharmaceutical company Rhône-Poulenc, later part of Sanofi.

This section is organised by drug class and focuses on the quinoline and structurally related antimalarials, in particular the 4-aminoquinolines most recently co-formulated with an artemisinin in a WHO-recommended artemisinin-based combination therapy (ACT), for which there have been recent concerns about cardiac safety[47].

ACTs are now the gold standard oral treatment for uncomplicated malaria and the first-line antimalarial treatment in more than 80 malaria-endemic countries[16]. They contain a rapidly acting artemisinin derivative combined with a more slowly eliminated partner drug. Most of the partner drugs in use and several of those in development are structurally related quinoline or quinoline-like compounds, some of which prolong the electrocardiographic (ECG) QT interval[48]. These medicines are deployed extensively, with over 400 million treatments now distributed annually, and 2.2 billion courses of ACTs delivered between 2005 and 2015[47].

1.2.1 The Artemisinins

Artemisia herbs or qinghao (青蒿) have been part of the traditional Chinese medicine pharmacopeia for more than two millennia. The first description of their use was recorded in *Shen Nong's Herbal Classic* (神农本草经), the oldest compendium of Chinese herbal therapies dating from the Qin and Han dynasties (221BC-220AD). Their application for relief of periodic fever was first documented in the Eastern Jin dynasty (317-420AD) manual *A Handbook for Prescriptions for Emergencies* (肘后备急方). In 1972, artemisinin or qinghaosu (青蒿素) was isolated from the leaves of *Artemisia annua*, also known as sweet wormwood, as part of Project 523, the secret Chinese military project initiated during the Cultural Revolution for antimalarial discovery in support of the North Vietnamese in the Vietnam War[49]. The Chinese chemist Tu Youyou (屠呦呦) received the 2015 Nobel Prize in Medicine for her role in discovering artemisinin[1].

The artemisinins all have broad stage-specificity against blood-stage parasites, from the ring stages through to early schizonts[30]. Artemisinin is a sesquiterpene lactone endoperoxide. Dihydroartemisinin (DHA), the active metabolite of artesunate and

artemether, is obtained by reduction of artemisinin. Artesunate is a hemisuccinate derivative of DHA, while artemether is the less active methyl ether derivative[30].

Of these artemisinins, artesunate is the most water-soluble and is used for intravenous administration, but can also be administered orally, rectally, and intramuscularly. Parenteral artesunate is the first-line treatment for severe malaria recommended by the WHO. Where parenteral artesunate is not available, intramuscular artemether is recommended in preference to quinine[30]. Artemether is also co-formulated with lumefantrine as an ACT (see 1.2.2.1).

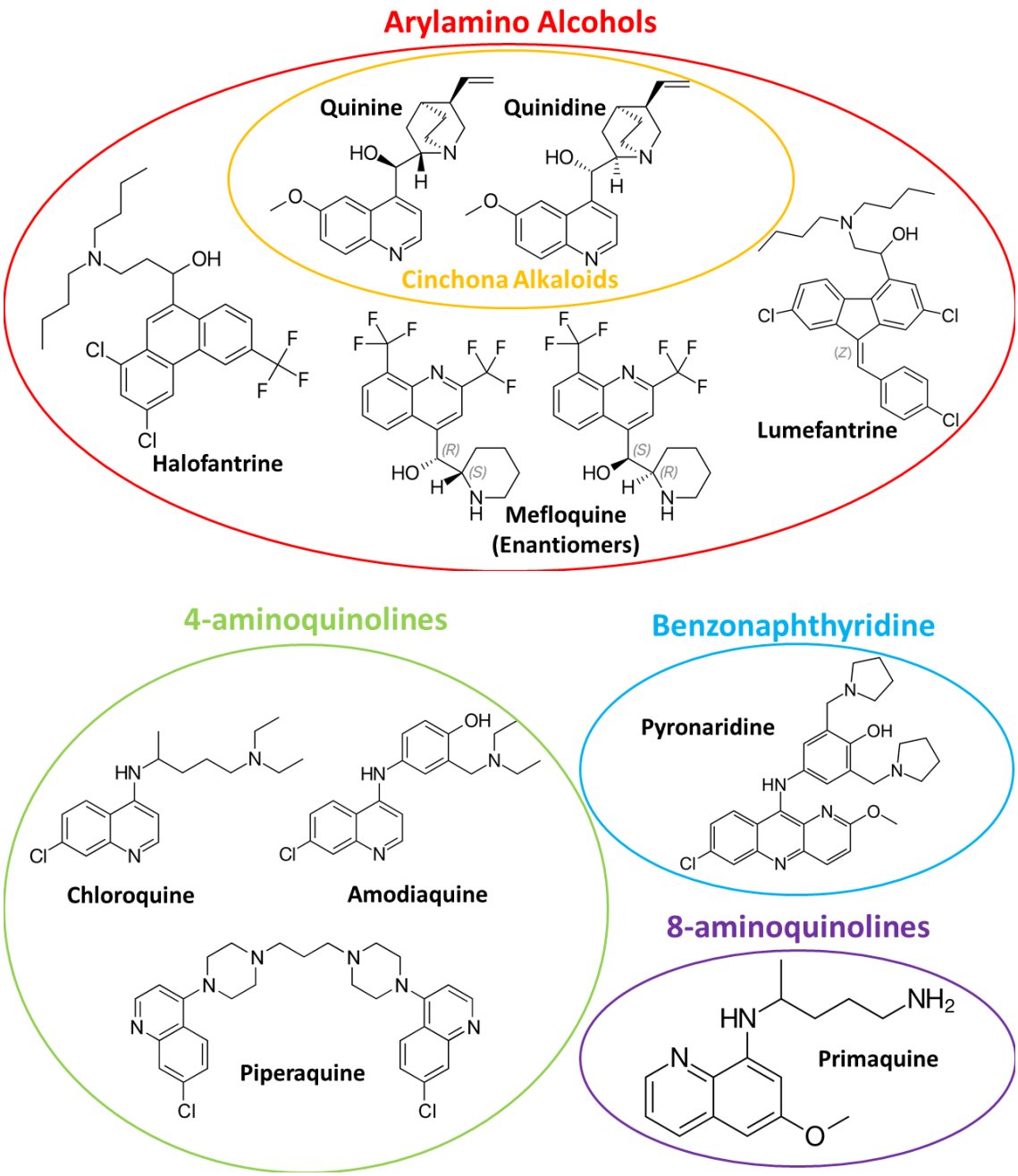
Post-artesunate delayed haemolysis has been reported in non-immune returning travellers 1-3 weeks after treatment with intravenous artesunate for severe malaria at a rate of approximately 20%[50] but is much less common in African children living in a high transmission setting who have high parasite densities[51]. These haemolytic episodes are triggered by the delayed splenic clearance of pitted red blood cells, the erythrocytes from which malaria parasites are expelled after being killed by artesunate[52], and are a predictable event related to the life-saving effects of antimalarial treatment.

The artemisinins are generally accepted not to have significant effects on the QT interval[53].

1.2.2 The Quinoline and Structurally Related Antimalarials

The chemical structures of the quinoline and quinoline-like antimalarials discussed in this thesis are shown grouped by structural similarity in Figure 1.5.

Figure 1.5: Chemical Structures of the Quinoline and Structurally Related Antimalarials



1.2.2.1 Arylamino Alcohols

The arylamino alcohol antimalarials include the cinchona alkaloids quinine and quinidine as well as mefloquine, halofantrine, and lumefantrine. Of these, quinine, mefloquine, and lumefantrine are listed in WHO and national guidelines for the treatment of malaria[16,30].

Quinine and Quinidine

In 1630, the Jesuit Barnabé de Cobo returned to Spain from Peru and brought with him the bark of the cinchona tree native to South America. 'The Jesuit's powder' as the cinchona bark became known, was promoted as a treatment for fever and malaria. Quinine was not isolated from the cinchona bark until about 200 years later by the French chemists Joseph Pelletier and Jean Bienaimé Caventou[54].

Quinine was being used by the United States (US) military by 1830. The US medical officer Benjamin Harney demonstrated the efficacy of large doses to treat periodic fevers[55] during the Second Seminole War in Florida (1838-1842), During the American Civil War (1861-1865) more than 25,000kg of quinine and other cinchona products were deployed[56].

Efforts to synthesise quinine were begun in 1856 by the British chemist William Henry Perkins, but this goal was not accomplished until 1944 and has never been achieved on a commercially viable scale. After four years of searching, the British trader Charles Ledger and his Amerindian associate Manuel Mamani found a variety of cinchona with a high quinine content and sold the seeds of this tree to the Dutch government in 1865 after the director of London's Kew Gardens rejected the offer. These seeds formed the basis of what would become the lucrative Dutch monopoly on quinine in the early 20th century: Dutch plantations in Java were soon producing more than 90% of the world's supply of quinine[54]. Quinine remained the only treatment for malaria until the introduction of chloroquine.

Quinine kills the large ring and trophozoite asexual parasites. It is gametocytocidal against *P. vivax*, *P. ovale*, and *P. malariae*, but not *P. falciparum*. Quinine undergoes extensive hepatic biotransformation, primarily by the CYP3A4 enzyme, into several metabolites, and is both a substrate and inhibitor of CYP2D6[30].

Quinine is now recommended by the WHO for the treatment of severe malaria when parenteral artesunate and artemether are not available[30]. Adverse effects have been known to affect adherence to the drug. During World War I, the British physician Ronald Ross, discoverer of mosquito transmission of malaria, famously created what he termed 'quinine concentration camps' to enforce intensive treatment for tens of thousands of soldiers returning from Greece with chronic malaria to get them fit for the Western front[57]. The common adverse effects after quinine came to be known as 'cinchonism'. Mild presentations of cinchonism include tinnitus, hearing loss, dizziness, nausea, dysphoria, and occasionally disturbed vision. More severe presentations include marked auditory loss, vertigo, vomiting, diarrhoea, and visual loss[30]. Other neuropsychiatric effects like delirium have also been reported[58]. Hyperinsulinaemic hypoglycaemia can occur after treatment for severe malaria, and is more common at the extremes of age and in pregnant women[59].

Quinine is known to prolong the ECG QT interval[48,60], but cardiotoxicity is much less frequent than with quinidine[48]. Hypotension after rapid administration, such as in an intravenous bolus, can occur. Intravenous administration by continuous infusion is therefore recommended[61] (see 1.3.3.1).

Quinine has a narrow therapeutic index and can accumulate in patients with severe malaria as well as those with liver and kidney disease[30]. Overdosage can cause oculotoxicity, retinal toxicity, and cardiotoxicity, and can be fatal[62].

Quinidine is another of the cinchona alkaloids and the dextrorotatory stereoisomer of quinine. The chance discovery of its effects on heart rhythm led to it becoming the first anti-arrhythmic and the prototype drug for repolarisation-related cardiotoxicity (see 1.3.1).

Mefloquine and Halofantrine

Mefloquine[63] and halofantrine[64] were discovered in the 1970s by the US Walter Reed Army Institute of Research (WRAIR) antimalarial drug discovery programme which was established after the US sustained significant military losses from malaria during the Vietnam War[65]. Both drugs swiftly received US Food and Drug Administration (FDA) approval in 1989 and 1992 respectively[56], and mefloquine became an important military drug for malaria chemoprophylaxis. However, major safety concerns soon emerged.

Mefloquine is recommended by the WHO for weekly prophylaxis of all species of malaria, and as an ACT with artesunate[66] for the treatment of uncomplicated malaria[47]. It is metabolised in the liver by CYP3A4, largely to an inactive metabolite. Neuropsychiatric adverse effects began to be reported in the early 1990s, which described changes in mood, thought, cognition, sleep, and behaviour[58]. The estimated incidence of seizures, encephalopathy, or psychotic reactions after mefloquine ranges from 1 in 10,000 healthy people on chemoprophylaxis, 1 in 1,000 uncomplicated malaria patients in Asia, 1 in 200 malaria patients in Africa, to 1 in 20 patients recovering from cerebral malaria[47]. Mefloquine is therefore not recommended for follow-up treatment after cerebral malaria, and should also be avoided as prophylaxis for travellers in whom fine motor coordination or sudden onset confusion may be dangerous, such as pilots and drivers[47]. While these neuropsychiatric adverse effects were previously considered generally self-limiting and reversible on drug discontinuation, it is now recognised they can last for years after use[67]. In 2013, the US FDA published a patient safety alert and added a black box warning to the drug label about the long-term neuropsychiatric effects of mefloquine[68].

Halofantrine causes dangerous arrhythmias and fatal cardiotoxicity with the index case of sudden death reported in 1993[69] (see 1.3.2.4). It has never been recommended for the treatment of malaria by the WHO[47].

Lumefantrine

Lumefantrine, also known as benflumetol, was synthesised by Project 523 of China in the 1960s. Chinese researchers at the Beijing Academy of Military Medical Sciences started investigating combining artemether with lumefantrine in the 1980s, and from 1994 development was undertaken in collaboration with Novartis, the Swiss pharmaceutical company[70] (see also 1.3.2.4).

Like artemether, lumefantrine is highly lipophilic and more readily absorbed when co-administered with fatty foods or milk[71]. Lumefantrine exhibits high interindividual variability in bioavailability and time to maximum concentration, primarily due to fat-dependent absorption. Both artemether and lumefantrine are extensively metabolised in the liver, primarily by the CYP3A4 enzyme[72]. Reassuringly, healthy volunteer studies did not find clinically significant interactions between artemether or lumefantrine with CYP3A4 inhibitors like ketoconazole[73] or substrates like mefloquine[74,75]. The active metabolite of lumefantrine is desbutyl-lumefantrine[76].

Lumefantrine is available as a fixed-dose combination ACT with artemether and has never been available as monotherapy. Artemether-lumefantrine (AL) was the first fixed-dose ACT to be pre-qualified by the WHO in 2004. AL in a six-dose course administered twice daily over three days is recommended by the WHO for the treatment but not prevention of malaria[30]. AL is generally well-tolerated with a wide therapeutic index[30], and is the first-line treatment for uncomplicated malaria in more than 60 countries around the world[16]. A new solid dispersion formulation of lumefantrine is being developed by Novartis for use as a partner drug in emerging single dose treatments for malaria[77].

1.2.2.2 4-aminoquinolines

Chloroquine

Chloroquine is one of the most extensively-used drugs in humans. With hundreds of metric tonnes dispensed annually since the 1950s and a terminal elimination half-life of over a month, it could well be the drug to which humans have been most exposed[48].

By World War I, the global supply of quinine was controlled by the Dutch who had tens of thousands of cinchona trees in their Javan plantations. This catalysed an intensive search for synthetic antimalarials by German scientists. Chloroquine was discovered in 1934 by Han Andersag of Bayer Laboratories, but was initially considered too toxic for further development[78]. In 1941, chloroquine was licensed by Bayer to Winthrop, the US pharmaceutical company. Further development of chloroquine was undertaken in World War II as part of the large-scale US Army World War II antimalarial research programme which intensified after the Japanese seized Java in 1942[79]. After clinical studies demonstrating its safety and efficacy, chloroquine received US FDA approval in 1949 for the treatment and prevention of malaria[56].

The Global Malaria Eradication Programme (GMEP) was launched by the WHO in 1955 using mass treatments with chloroquine and spraying with the insecticide DDT to eliminate malaria outside of Africa. While malaria was successfully eliminated from several parts of the world, the programme foundered with the emergence of chloroquine and DDT resistance. The GMEP was abandoned by 1969[80].

Chloroquine is currently recommended by the WHO for the treatment of *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* malaria[30]. It is metabolised in the liver by CYP2C8 and CYP3A4, mostly to monodesthylchloroquine, which has similar antimalarial activity[81].

Chloroquine is generally well-tolerated at recommended treatment doses for malaria, but is dangerous in overdose, and death from cardiac arrest can occur within a few hours. In chronic use at higher doses for rheumatological disorders, chloroquine can be associated with cardiomyopathy[82], conduction disorders[83], and ECG QT interval prolongation[83]. It was also associated with lethal hypotension with rapid parenteral administration for treatment of severe malaria which is no longer recommended[84] (see 1.3.3.1).

Pruritus after chloroquine is very common, especially in darker-skinned individuals[85]. More rarely, neuropsychiatric disorders like seizures and psychosis have also been reported[58]. Retinopathy can occur in chronic use and screening is recommended every five years for individuals without additional ophthalmological risk factors[86].

Amodiaquine

Amodiaquine was first synthesised by the US World War II antimalarial research programme[79,87] which also developed chloroquine. It was later sold under the trade name of Camoquin® by the American pharmaceutical company Parke-Davis[87]. Following multiple reports of its effectiveness against *P. falciparum* malaria, amodiaquine became widely used for treatment of uncomplicated malaria in sub-Saharan Africa at a total dose of 30-35mg/kg over three days from the 1950s and was subsequently adopted for malaria prophylaxis in travellers in the 1980s at a weekly dose of 400mg. However, reports soon emerged of fatal drug-related hepatotoxicity and neutropaenia in travellers taking amodiaquine prophylaxis[88-90]. Amodiaquine was withdrawn by the WHO from the list of recommended antimalarials in 1990[91] before being reinstated in 1996 in light of the findings of a Cochrane review[92] which supported the continued use of amodiaquine for the treatment – but not prophylaxis – of uncomplicated malaria alongside continued surveillance for both effectiveness and toxicity.

In 2008, artesunate-amodiaquine (ASAQ), the fixed-dose formulation of amodiaquine and artesunate, became the first major success of a public-private partnership, the Drugs for Neglected Diseases initiative (DNDi), in development and WHO pre-qualification by of an ACT[93]. Several years later, the WHO recommended addition of amodiaquine (AQ) to sulfadoxine-pyrimethamine (SP) for seasonal malaria chemoprevention (SMC) in young children (aged 3-59 months) living in areas of seasonal high-intensity malaria transmission in the Sahel subregion of Africa[33]. Following the publication of case series of extrapyramidal reactions after artesunate and amodiaquine[94,95], this group of adverse drug reactions was added to the Summary of Product Characteristics for Artesunate Amodiaquine Winthrop Tablets in 2014[96]. Today, ASAQ is the first-line oral antimalarial in more than 20 countries in sub-Saharan Africa[16] where malaria is endemic and SP + AQ remains the WHO-recommended drug combination for SMC in African children.

The mechanisms of toxicity underlying these rare adverse drug reactions from amodiaquine continue to be investigated. Amodiaquine has been shown to have a direct[97] and dose-dependent[98] toxic effect on the bone marrow cells of patients who developed agranulocytosis after the drug when bone marrow aspirates from these patients were cultured *in vitro*. Agranulocytosis may also occur secondary to bioactivation of amodiaquine to a cytotoxic reactive quinoneimine metabolite[99]. Anti-drug IgG antibodies have been detected in patients receiving amodiaquine doses in prophylactic but not treatment regimens[100], supporting an antibody immune-mediated mechanism for hepatoxicity and neutropaenia. A new murine model of amodiaquine-induced liver injury suggests cell-mediated immune responses modulated by ciclosporin may also play a part[101]. In addition, amodiaquine and chloroquine have been found to be agonists of the nuclear orphan receptor Nurr1. Nurr 1, also known as nuclear receptor subfamily 4, group A, member 2 (NR4A2), is essential for the development and maintenance of midbrain dopaminergic neurons in adult brains. Amodiaquine triggers abnormal involuntary

movements in L-DOPA treated rats[102]. Nurr1 activation could provide a molecular basis for the extrapyramidal reactions observed in ASAQ-treated young Africans.

These investigations have yet to present a clear approach for minimising the risk of occurrence of adverse drug reactions in the clinical setting beyond pragmatic changes in dosing regimen. In cases reported to the United Kingdom Committee of Safety of Medicines between 1984 and 1986, hepatic injury and agranulocytosis occurred after weekly malaria prophylaxis at a mean total dose of 2.8g (range: 0.8-4.8) over a mean period of 6.9 weeks (range: 1-12)[90]. Preventive use of amodiaquine is now in smaller total doses at a lower frequency than in prophylaxis. From 2012, the WHO-recommended use of amodiaquine for malaria prevention is in SMC of African children and comprises a single dose of SP with amodiaquine at 229.5mg (age 3-12 months) or 459mg (age 12-59 months) divided over three days each month of the rainy season for up to four months a year[33]. There have been no major safety concerns with SMC with SP + AQ to date[103] with millions of children treated every year. Evidence from large studies of hundreds of thousands of children[104] suggests hepatotoxicity and extrapyramidal reactions after SMC are very rare[105].

In the treatment of uncomplicated malaria with ASAQ, the amount of amodiaquine in the fixed-dose combination was reduced to 540mg from 612mg in the non-fixed dose combination in consideration of weight-for-age reference data for designing age-based dosing regimens[106]. The Ghanaian Ministry of Health has recommended twice daily dosing of ASAQ alongside the WHO-recommended once daily regimen since 2006. However, polymorphisms in CYP2C8, the main enzyme for amodiaquine metabolism by the liver into desethylamodiaquine (DEAQ)[107], also contribute to interindividual variability in amodiaquine clearance[108]. There is no evidence to date that either of these changes has resulted in a reduction in the incidence of adverse events after treatment of uncomplicated malaria with ASAQ[94,109,110].

Data on the cardiovascular effects of amodiaquine are scarce[47] and warrant further investigation, particularly in view of its structural similarity to chloroquine.

Piperaquine

Piperaquine, a bisquinoline compound of the 4-aminoquinoline group, was synthesised independently by the French pharmaceutical company Rhône-Poulenc and the Shanghai Pharmaceutical Industry Research Institute in China in the 1960s[111]. Before being combined with DHA in the current ACT formulation, some 140 million piperaquine treatments were consumed in China for individual and mass treatments between 1978 and 1992 until the emergence of piperaquine resistance prompted a change in national treatment policy[112].

Like chloroquine, piperaquine provides extended post-treatment prophylaxis resulting from its 20-30 day long terminal elimination half-life[113]. A large amount of co-administered fat has been found to increase piperaquine concentrations significantly[114,115] while a small amount of fat in normal meals does not[115-117].

Dihydroartemisinin-piperaquine (DHA-PPQ) is now available as a co-formulated ACT[118]. Unlike AL which has twice a day dosing, DHA-PPQ is administered once daily over a 3-day treatment course. DHA-PPQ is well-tolerated and adverse effects are rare. These properties along with its long post-treatment prophylaxis make it an attractive choice for preventive therapies and mass drug administration.

DHA-PPQ has been a first-line ACT for the treatment of uncomplicated malaria in East and Southeast Asia for over a decade. However, its effectiveness in case management has been diminished by the emergence of artemisinin and piperaquine resistance which have now spread across the region[23-25].

The major safety consideration for DHA-PPQ has been repolarisation-related cardiotoxicity. DHA-PPQ was found to cause prolongation of the ECG QT interval in a phase I study during drug development particularly after a high fat and high calorie meal[119] (see 1.3.2.4). Concerns about the effects of DHA-PPQ on cardiac electrophysiology have motivated a global review of the cardiotoxicity of antimalarials[47].

1.2.2.3 Benzonaphthyridine Derivatives

Pyronaridine is a benzonaphthyridine derivative which was first synthesised in 1970 by the Institute of Chinese Parasitic Disease of the Chinese Academy of Preventive Medicine as part of Project 523. It has been used in China as monotherapy for the treatment of malaria for more than three decades[120]. Pyronaridine is now co-formulated with artesunate as an ACT, which was pre-qualified by the WHO as Pyramax® by Shin Poong in 2012.

The main safety concern for pyronaridine-artesunate (PA) has been an increased risk of drug-induced liver injury as defined by elevation of liver transaminase enzymes to more than five times the upper limit of normal[121]. A large safety study in African children has demonstrated that retreatment with PA did not increase the risk of raised hepatic transaminases or other adverse events[122]. PA is indicated for the treatment of both *P. falciparum* and *P. vivax* malaria[123], and was added to the WHO Essential Medicines List and Essential Medicines List for Children in 2017.

1.2.2.4 8-aminoquinolines

Like amodiaquine, primaquine was synthesised as part of the US Army World War II antimalarial research programme led by the Office of Scientific Research and Development[79,124]. The US Army undertook large-scale safety and efficacy studies in the 1950s when relapsing *P. vivax* malaria emerged as a problem in troops returning from the Korean War[56].

Primaquine is the only generally available antimalarial which kills the dormant liver stages of *P. vivax* and *P. ovale* malaria, and therefore prevents their relapse, i.e. radical cure. It is also a potent gametocytocide for reduction of onward transmission of *P. falciparum* malaria. Primaquine is used for these indications in conjunction with a blood schizonticide, typically an ACT or chloroquine. Genetic polymorphisms which result in lower levels of CYP2D6 enzyme activity reduce bioactivation of primaquine and may result in treatment failure[47].

The most important adverse event associated with primaquine is haemolysis in individuals with G6PD deficiency which can require blood transfusion. Risk of haemolysis is substantially lower after single low-dose primaquine (0.25mg/kg) for interruption of *P. falciparum* malaria transmission than for radical cure[125]. Policies and practices of G6PD testing vary widely in malaria-endemic countries[126].

1.2.3 The Antifolates

Sulfadoxine-pyrimethamine (SP) is a combination therapy of two antimalarial drugs which inhibit folic acid synthesis in protozoa. Sulfadoxine is a sulfonamide which inhibits dihydropteroate synthetase, while pyrimethamine is an inhibitor of dihydrofolate reductase. The combination of two drugs interfering with folic acid synthesis at different points in the pathway results in a higher level of anti-folate activity than monotherapy.

SP has been in use since the 1950s and is generally well-tolerated at recommended treatment doses. Consistent with other sulfanomides, serious adverse effects include potentially fatal skin reactions such as erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis[47].

SP has an important role in the prevention of malaria in vulnerable populations. It is the first-line drug recommended by the WHO for intermittent preventive therapy in pregnant women (IPTp) and infants (IPTi), although resistance is reducing its effectiveness and fuelling the search for therapeutic alternatives[22]. It is administered with amodiaquine (SP + AQ) for monthly seasonal malaria chemoprevention of children aged 3-59 months in areas of seasonal high-intensity malaria transmission during the rainy season in the Sahel subregion of Africa[33].

SP is also available as an ACT with artesunate. This ACT is a preferred treatment for uncomplicated malaria in Southwest Asia[16].

1.3 ANTIMALARIALS AND THE CARDIOVASCULAR SYSTEM

1.3.1 The First Anti-Arrhythmic Agents

The chance discovery of the ability of antimalarials to terminate irregular heart rhythms led to their becoming the first anti-arrhythmic agents. "Long and rebellious palpitations have ceded to this febrifuge", wrote the French royal physician Jean-Baptiste de Sénac in his seminal 1749 treatise on the anatomy, function, and diseases of the heart (*Traité de la structure du coeur, de son action, et de ses maladies*). The febrifuge in question was the cinchona bark, from which the alkaloids quinine and quinidine were later isolated in turn by the French chemists Pierre-Joseph Pelletier and Joseph Bianaimé Caventou in 1820, then Étienne Henry and Auguste Delondre in 1833[54].

Quinidine, the dextrorotatory stereoisomer of quinine, is the prototype for drugs causing repolarisation-related cardiotoxicity manifest as torsade de pointes (TdP), a potentially lethal polymorphic ventricular tachycardia associated with electrocardiographic QT interval prolongation. Sudden deaths and abrupt syncope after quinidine for the treatment of infectious diseases and arrhythmias have been reported since the 19th century[127]. The first case series of quinidine use (n = 460) identified a rate of symptomatic cardiotoxicity of about 1%[127]. In the 1920s, the Dutch cardiologist Karel Frederik Wenkebach started using quinidine for chemical cardioversion of atrial fibrillation[128]. In the 1960s, quinidine became the first drug to be associated with ventricular fibrillation, symptomatic TdP (syncope), and marked QT interval prolongation[129] such that iatrogenic QT interval prolongation came to be known as the 'quinidine effect'. Quinidine continued to be used as an anti-arrhythmic agent and antimalarial over the next century, but has been discontinued by Eli Lilly as of 2019[130].

Around the time of the formal description of TdP by François Dessertenne in 1966, it soon became apparent that even drugs prescribed for non-cardiovascular indications could cause TdP and sudden death, although at rates several of magnitudes lower than anti-arrhythmics. The first of these non-cardiovascular drugs identified was the phenothiazine anti-psychotic thioridazine[131,132]. Dozens more followed across many therapeutic categories, but drug-induced repolarisation-related cardiotoxicity remained an electrocardiographic curiosity for another two decades throughout the 1970s and 1980s.

1.3.2 Drug-induced Repolarisation-Related Cardiotoxicity

1.3.2.1 Regulatory Landscape

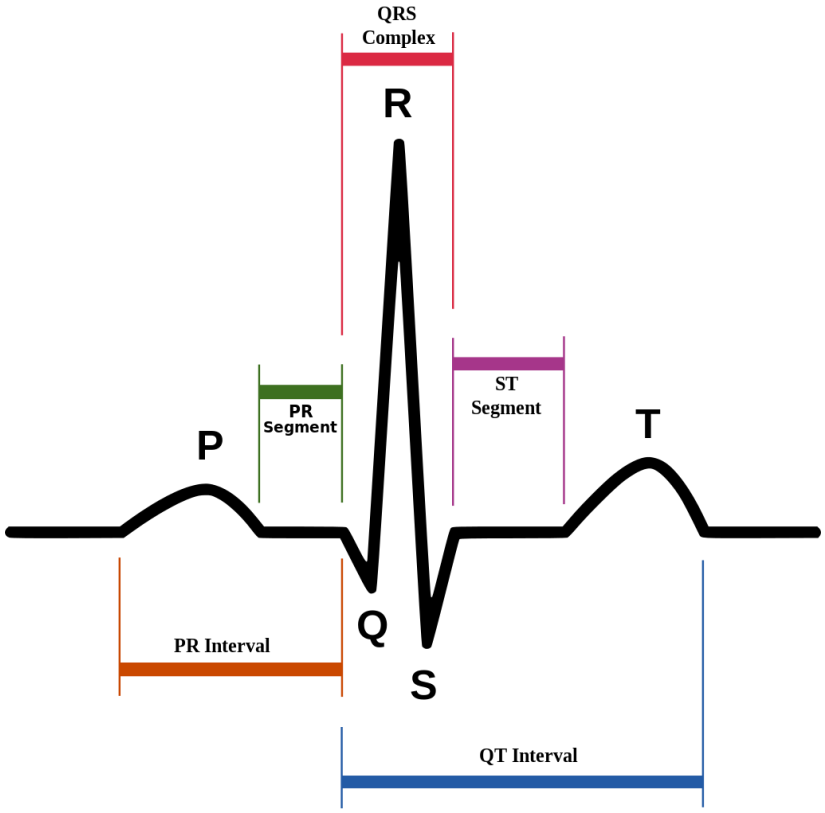
Drug-induced repolarisation-related cardiotoxicity was first thrust into the regulatory spotlight in the early 1990s following a high-profile case of symptomatic TdP in a patient who had taken the popular antihistamine terfenadine at the recommended prescribed dose[133]. Terfenadine was never granted over-the-counter status, and was subsequently withdrawn from market in the late 1990s with the development of its non-QT prolonging metabolite fexofenadine as an antihistamine.

The assessment of QT interval prolongation has since become an important part of the regulatory evaluation of new medicines as well as one of the most common reasons for drug withdrawal and relabelling[134]. Both the European Medicines Agency and the US FDA have adopted the International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) pre-clinical S7B[135] and clinical E14[136] ('Thorough QT') guidelines for evaluating QT interval prolongation and the proarrhythmic potential of new medicines.

1.3.2.2 Cardiac Electrophysiology

The electrocardiographic QT interval represents the ventricular action potential, i.e., the interval between ventricular depolarisation and repolarisation, as determined by the dynamic and fine balance of electrical currents mediated by ion channels on ventricular cardiomyocytes. These action potentials vary from cell layer to cell layer. The QT interval reflects the summation of these potentials (Figure 1.6).

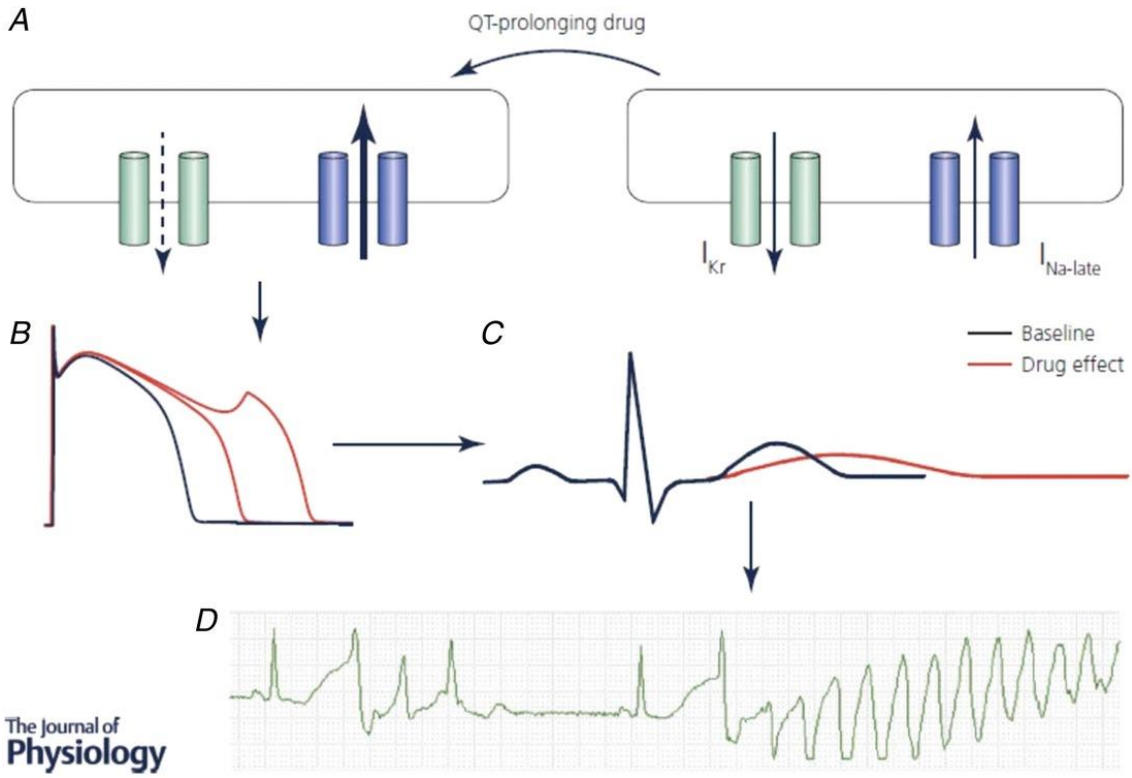
Figure 1.6: The Surface Electrocardiogram in Normal Sinus Rhythm



By far the most common mechanism by which drugs cause QT interval prolongation is by blocking the human ether-à-go-go related gene (hERG) potassium channel, the voltage-gated ion channel that mediates the rapid component of the delayed rectifier potassium current, I_{Kr}. Blockade of the hERG channel lengthens ventricular repolarisation and the duration of the ventricular action potential. This is reflected on the surface ECG as a

prolonged QT interval; it may also result in the reactivation of inward, mainly calcium, depolarizing currents, thereby generating early afterdepolarizations. Under the right spatial and temporal heterogeneity of refractoriness in ventricular cardiomyocytes, early afterdepolarizations can trigger TdP. In the majority of cases, TdP is self-terminating, but if sustained, it can degenerate into ventricular fibrillation and cause sudden cardiac death[137] (Figure 1.7).

Figure 1.7: Chain of Molecular and Cellular Events Causing Drug-induced Torsade de Pointes



Adapted from a major review article[138]. The first event (A) is drug block of the human ether-à-go-go related gene (hERG) potassium channel, leading to decrease in the delayed rectifier potassium current, I_{Kr}; recent evidence has also suggested an increase in the late sodium current, $I_{Na\text{-}late}$ may also contribute. This in turn results in prolongation of action potentials and generation of triggered activity from early afterdepolarisations (B), and QT interval prolongation (C), which can result in the polymorphic ventricular tachycardia torsade de pointes (D)[138].

The relationship between QT interval prolongation and TdP is not straightforward. Drugs that cause QT interval prolongation lead to life-threatening tachyarrhythmias in only a small proportion of patients. Sudden cardiac death can also occur in individuals whose QT intervals are within the normal range. Prolongation of the QT interval is therefore a sensitive but not specific marker of an increased risk of TdP. Although imperfect, QT interval prolongation remains at present the best available and most commonly used surrogate indicator for TdP risk[139].

Experience with both QT interval-prolonging medicines and congenital long QT syndrome suggests[47]:

- A QT/QTc[i] interval >500 milliseconds[ii] is associated with a higher risk of TdP and sudden cardiac death;
- Among drugs with QT interval-prolonging potential, antiarrhythmics have been associated with TdP in 1–5% of exposed subjects, while non-cardiovascular drugs have been associated with TdP at much lower frequencies, e.g., one in 100 000 exposures for the quinolone antibiotic moxifloxacin[138];
- TdP degenerates into ventricular fibrillation in approximately 10% of cases;

[i]The measured QT interval is routinely adjusted for heart rate using one of a range of correction formulae to account for the inverse relationship between the QT interval and heart rate. QTc refers to this corrected QT value.

[ii]There is a no consensus concerning the choice of upper limit values for absolute QT/QTc interval and changes from baseline. While lower limits increase the false-positive rate, high limits increase the risk of failing to detect a signal for concern. In clinical trials, a QT/QTc prolongation >500 milliseconds during therapy has been a threshold of particular concern. International guidelines recommend conducting multiple analyses using different limits as a reasonable way to approach this uncertainty, including:

- Absolute QTc interval prolongation:
  - QTc interval >450 milliseconds
  - QTc interval >480 milliseconds
  - QTc interval >500 milliseconds
- Change from baseline in QTc interval:
  - QTc interval increases from baseline >30 milliseconds
  - QTc interval increases from baseline >60 milliseconds

- The incidence of drug-induced TdP and life-threatening ventricular arrhythmias has been reported as 3.2–13 per million person years in active surveillance studies conducted in Europe[140-142]; very little evidence has emerged from tropical areas.

1.3.2.3 Torsade de Pointes Risk Factors

Apart from hERG channel blockade, several risk factors decrease repolarisation reserve[143] and facilitate the development of arrhythmias in individual patients. These include:

- Pharmacokinetic-pharmacodynamic effects, e.g., CYP450 inhibition from drug interactions leading to higher drug levels;
- Female sex, which is associated with a risk that is approximately two-fold greater after puberty[144];
- Structural heart disease, e.g., ischaemic cardiomyopathy, left ventricular hypertrophy;
- Genetic defects of cardiac ion channels, including subtle genetic polymorphisms[145];
- Electrolyte disturbances, e.g., hypokalaemia, hypomagnesaemia, hypocalcaemia;
- Bradycardia, e.g., from increased vagal tone;
- Hepatic impairment, e.g., from alcoholic cirrhosis;
- Concomitant use of medicines that prolong the QT/QTc interval.

Terfenadine at therapeutic doses in healthy individuals produces minimal QT interval prolongation. The index patient who had developed symptomatic TdP after terfenadine, a 39-year-old woman, had taken the drug at the recommended prescribed dose but was also taking ketoconazole, a CYP450 inhibitor. This drug-drug interaction would have significantly reduced terfenadine's extensive pre-systemic metabolism, causing plasma concentrations of this QT-prolonging parent drug to rise dramatically, thus resulting in marked QT interval prolongation (>60 milliseconds) and TdP[146].

1.3.2.4 Antimalarial Drug Development

The first report of drug-associated sudden death after an oral antimalarial emerged from the Thai-Burmese border also in the early 1990s. A 37-year-old post-partum Karen woman with a history of 'fainting easily' had collapsed and died after her ninth and final dose of halofantrine for recrudescent uncomplicated *P. falciparum* malaria after having previously been treated with mefloquine[147]. Halofantrine, a lipophilic phenanthrene methanol of the arylamino alcohol group of antimalarials, soon came to be associated with extreme QT interval prolongation[147,148], TdP[148] and more than 30 reports of sudden cardiac death[69]. The WHO has never recommended halofantrine for the treatment of malaria.

Artemether-lumefantrine (AL), the first co-formulated ACT with a similarly named arylamino alcohol as the partner drug, was being developed at around the same time. Concerns that lumefantrine could have the same cardiotoxic effects as halofantrine motivated the first cardiac safety evaluations of new ACTs in field trials. These studies compared AL with the other arylamino alcohols in use at the time, mefloquine[149] (co-administered with artesunate) and halofantrine[150], as well as the 4-aminoquinoline chloroquine[151], and did not find significant QT interval prolongation or cardiotoxicity. In addition, healthy volunteer studies did not find significant interactions between AL and mefloquine[74] or ketoconazole[73]. AL was pre-qualified by the WHO as Coartem® by Novartis in 2004[29] and has been on the WHO Essential Medicines List from 2007.

More recently, dose-dependent QT interval prolongation was observed in healthy volunteers in a phase 1 'Thorough QT'[136] assessment[119] of the ACT dihydroartemisinin-piperaquine (DHA-PPQ) in fed and fasted conditions – piperaquine is a bisquinoline of the 4-aminoquinoline group. These findings, along with increasing use of DHA-PPQ in population-based mass treatment programmes of healthy individuals in malaria-endemic areas have renewed interest in the repolarisation-related cardiotoxicity of the quinoline

and structurally related antimalarials[47]. DHA-PPQ was approved by the European Medicines Agency in 2011[152] and pre-qualified by the WHO as Eurartesim® by Sigma Tau in 2015[29]. It has been recommended by the WHO Guidelines for the Treatment of Malaria for case management of uncomplicated malaria from 2015[30].

1.3.3 Other Serious Cardiovascular Adverse Effects

1.3.3.1 Hypotension

The hypotensive potential of the cinchona alkaloids, quinine and quinidine, following intravenous injection was recognised when this method of administration was introduced over a century ago. Until the 1940s, parenteral quinine was given by intravenous or intramuscular injection. Transiently high plasma concentrations during the distribution phase caused hypotension. The slow intravenous infusion method was introduced by John Strahan[61] in prisoner of war camps during World War II: it allowed adequate distribution, was found to be considerably safer, and has been the recommended method of administration of quinine ever since. In contrast, quinidine is predictably hypotensive even with rate-controlled infusions[153] due to competitive alpha-adrenergic blockade[154,155].

Chloroquine hypotension from peripheral vasodilation and negative inotropy was the probable cause of sudden death reported following the rapid parenteral administration of chloroquine for the treatment of malaria in children. Pharmacokinetic-pharmacodynamic assessments found that toxicity resulted from transiently very high plasma concentrations following bolus administration. This effect was circumvented by using a slow, continuous, rate-controlled infusion or smaller, more frequent intramuscular or subcutaneous doses to administer the drug[84].

The current first-line parenteral antimalarial recommended by the WHO for the treatment of severe malaria is artesunate, which has no such cardiovascular effects[30].

1.4 EVIDENCE SYNTHESIS FOR DRUG SAFETY ASSESSMENT

1.4.1 The Antidepressant Experience

In 2004, the US FDA published a controversial black box warning on the increased risk of suicide-related adverse events (suicidal thinking, feeling, and behaviour) in children and adolescents following a meta-analysis of 23 randomised controlled trials of selective serotonin reuptake inhibitors for depression conducted by the agency[156]. This meta-analysis was initiated in response to a report from a drug manufacturer which described a possible increase in paroxetine-related suicidality in paediatric patients, particularly those with major depressive disorder[156].

Significant relative reductions in antidepressant use in the two years after the FDA advisory was issued – 31.0%, 24.3%, and 14.5% among adolescents, young adults, and adults respectively[157] – were attributed to the warning and the media coverage which ensued. Concerns that the warning had discouraged depressed patients from seeking treatment and doctors from prescribing antidepressants of a range of drug classes to patients of all ages[158,159], and may therefore have contributed to an increase in completed suicide rates in young people in the US and the Netherlands[160] stoked the debate.

To address these concerns, the FDA updated the black box warning in 2007 to state that depression was itself associated with an increased risk of suicide. However, criticism of the risk assessment methodology used by the FDA did not abate[161]. In the decade after, the agency embarked on a comprehensive review of drug safety evaluation[162,163], reporting[164], and communication[165] methodology which culminated in a series of guidelines[166,167] with this experience as a major case study. This thesis is based on the recommendations of the related Council for International Organizations of Medical Sciences (CIOMS) Report on Evidence Synthesis and Meta-analysis for Drug Safety[167].

1.4.2 Postmarket Drug Safety Assessment

It is now recognised that postmarket assessment of drug safety should include a review of the totality of evidence available with appropriate consideration of data quality and the broader therapeutic landscape which the drug under study is part of, as further detailed in Figure 1.8[168]. The Grading of Recommendations Assessment, Development and Evaluation Working Group (GRADE) framework[169] for rating quality of evidence and strength of recommendations is widely used in clinical guideline development, including in the WHO Guidelines for the Treatment of Malaria[30].

Clinical evidence would include spontaneous case reports from individual case safety report databases and the medical literature as well as pharmacoepidemiological studies of routinely collected data such as medical claims databases. The WHO has the largest international database of case reports of spontaneous adverse events with increasing engagement from resource-limited countries[170]. These evidence sources would be in addition to randomised controlled trials which are generally not designed or powered for prospective assessment of drug safety[171], and often have limited generalisability[172].

Clarity in outcome definition is essential and composite outcomes which are open to misinterpretation, such as cardiotoxicity or suicidality, should be used with caution. When a composite outcome makes biological sense, analysis of the individual components should still be performed. Surrogate outcomes, such as QT interval prolongation, are discouraged when sufficient information about clinical outcomes is available as they add another layer of variability and are often difficult to validate[167].

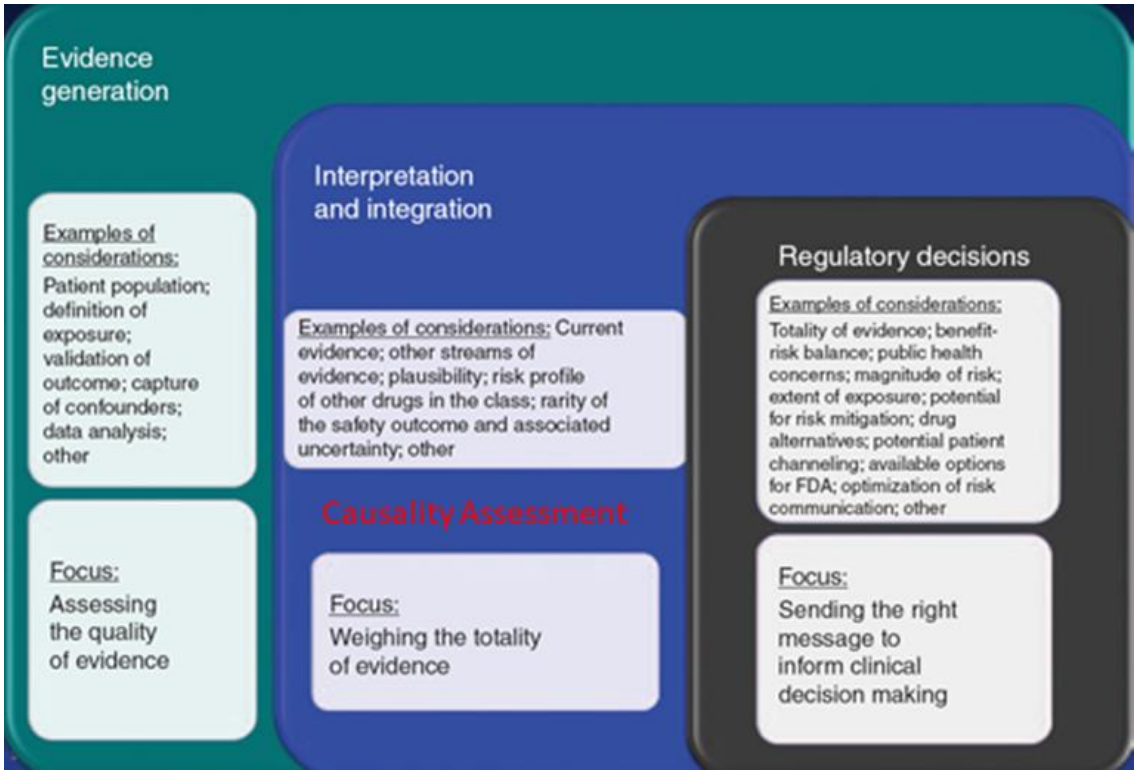
Causality assessment is another important consideration. The Bradford-Hill criteria[173] provide a useful framework and independent adjudication by an expert committee is recommended when adverse events typically treated by a medical specialist or sub-specialist are being assessed[167].

The treatment indication for the drug exposure would usually determine the dosage regimens and populations of interest, as is the case for antimalarial therapy. Combining data from different drugs within a drug class may be needed to investigate rare adverse events but relies on the assumption that the risk of the adverse event is similar within that class[167]. Comparing drugs within a drug class where relevant data are available is required to characterise clinically significant heterogeneity between drugs particularly where an outlier drug may dominate the effect observed.

Meta-analyses of rare adverse events also pose methodological and statistical challenges for which Bayesian methods are promising[167]. Individual patient data (IPD) meta-analyses have a number of advantages over aggregate data meta-analyses, including allowing the possibility of data standardisation and improved detection of subgroup effects[167].

Ultimately, coherence of results across a range of databases, experimental designs, and statistical methodologies is key[161,168].

Figure 1.8: Dimensions of Postmarket Drug Safety Assessment



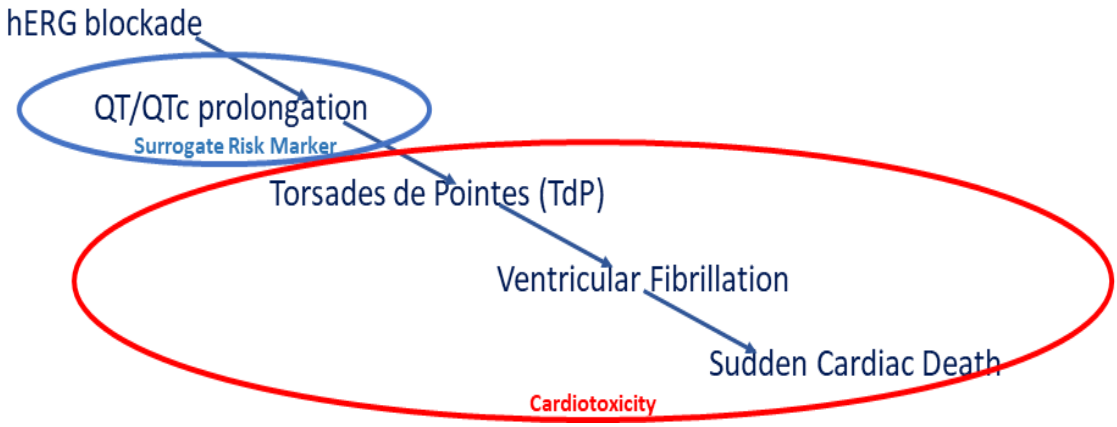
Adapted from a United States Food and Drug Administration review[168].

1.5 THESIS OUTLINE

This thesis aims to define the cardiovascular toxicity of the quinoline and structurally related antimalarials used most widely in malaria treatment and prevention in order to inform antimalarial drug choice and dosage for deployment and development. It proposes to achieve this aim through synthesis of all available clinical data to provide risk estimates of the different clinical levels of repolarisation-related cardiotoxicity after antimalarial treatment (Figure 1.9), and to determine whether malaria itself affects these risks.

It builds on my work as the technical resource person and rapporteur of the WHO Global Malaria Programme Evidence Review Group on the Cardiotoxicity of Antimalarials[47], co-chaired by the cardiologist Professor Josep Brugada and my supervisor Professor Nicholas White, during the course of this thesis.

Figure 1.9: Levels of Cardiac Ventricular Repolarisation-related Cardiotoxicity



Clinical levels of cardiac ventricular repolarisation-related cardiotoxicity are highlighted with coloured ellipses: the blue ellipse contains the electrocardiographic QT interval, the most commonly used if non-specific surrogate risk marker for development of torsade de pointes; the red ellipse contains torsade de pointes and the life-threatening ventricular tachyarrhythmias it can degenerate into which can lead to sudden cardiac death.

In **Chapter 2**, I describe the data identification, gathering, standardisation, and analysis methods I used.

In **Chapter 3**, I investigate the risk of sudden unexplained death potentially resulting from lethal iatrogenic ventricular arrhythmias in individuals treated with the ACT dihydroartemisinin-piperaquine (DHA-PPQ), a leading candidate for mass drug administration and intermittent preventive therapy for malaria. DHA-PPQ has since been added to the WHO Essential Medicines List and Essential Medicines List for children.

In **Chapter 4**, I present the results of an extensive literature search for clinical cardiovascular adverse effects and ECG-documented arrhythmias occurring after amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, quinine, and sulfadoxine-pyrimethamine (SP) in malaria clinical trials. GlaxoSmithKline (GSK) have since withdrawn halofantrine (Halfan®) from market.

In **Chapter 5**, I investigate the effects of malaria on the pre-treatment QT interval in addition to heart rate and known demographic factors of age and sex through an individual patient data meta-analysis of the studies identified through the systematic review begun in Chapter 4.

In **Chapter 6**, I compare the cardiovascular and electrocardiographic effects of amodiaquine, the WHO-recommended quinoline antimalarial (in combination with SP) for seasonal malaria chemoprevention and a proposed partner drug for triple ACTs, with other front-line antimalarials, focusing on bradycardia and corrected QT interval prolongation.

In **Chapter 7**, I summarise the output and wider implications of this thesis as well as discuss ongoing and possible future investigations for further clinical and molecular definition of the cardiovascular effects identified.

2 Methods

2.1 CHAPTER 3 METHODS

2.1.1 Research Question

The research question was summarised using the PICOTS framework (Table 2.1).

Table 2.1: PICOTS Framework for Systematic Review of Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine for Malaria

Population	Individuals of all ages, including pregnant women
Intervention	DHA-PPQ in at least one 3-day course, with or without single low-dose primaquine, for the treatment or prevention of malaria
Comparison	Global rates of sudden cardiac death in the young (<35 years)
Outcome	Sudden unexplained death as adjudicated by the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials
Timeframe	Within the 30-day risk window from DHA-PPQ administration
Study Type	Primary or secondary analyses of prospective randomised controlled trials or cohort studies with at least 3 days of active follow-up

This framework was applied to define the literature search strategy and study eligibility criteria for this systematic review.

2.1.2 Search Strategy

2.1.2.1 Published Studies

An electronic literature search was conducted of the following nine clinical bibliographic databases for journal articles and conference abstracts on 20 September 2016 (last updated on 24 May 2017):

1) MEDLINE
2) EMBASE
3) Web of Science
4) CINAHL
5) Cochrane Database of Systematic Reviews

6) Cochrane Central Register of Controlled Trials
7) Database of Abstract of Reviews of Effects
8) Global Health
9) WHO Global Health Library, and
10) WWARN Clinical Trials of Uncomplicated Malaria Publication Library

I searched for "dihydroartemisinin-piperaquine" as title, abstract, and subject heading keywords, using synonyms and variant spellings as additional search terms. I excluded animal studies, but did not apply language or publication date limits.

E.g. MEDLINE search on 24 May 2017:

#	Searches
1)	((dihydroartemisinin adj2 piperaquine) or dihydroartemisinin?piperaquine or dhappq or dha-ppq).mp.
2)	((Artekin or Eurartesim or Diphos or Timequin or Duocotecxin or Malacur) and (malaria* or antimalaria* or anti-malaria* or plasmodium*)).mp.
3)	1 or 2
4)	exp animals/ not humans.sh.
5)	3 not 4

All references were de-deduplicated and screened against eligibility criteria by myself and another independent reviewer using the Covidence platform.

2.1.2.2 Unpublished Studies

Unpublished studies were identified through expert consultation as part of the work of the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials[47].

2.1.3 Data Extraction

I extracted the following information from study reports and trial database records, and where necessary, requested further details from study investigators:

1) Study and participant characteristics: study year of publication, study location, study design, treatment indication, inclusion and exclusion criteria, participant mean or median age with standard deviation or interquartile range
2) Drug dosing: regimen including dosing tables and DHA-PPQ brand name, frequency of courses if repeated, directly observed therapy and adherence checks
3) Pharmacovigilance: adverse event monitoring system, any other follow-up, number lost to follow-up after DHA-PPQ and comparators (if any) with reasons
4) Exposures: number of individuals treated with DHA-PPQ and comparators (if any), number of courses of DHA-PPQ administered with adherence figures (if available)
5) Adverse events: deaths after DHA-PPQ and comparators (if any), any other cardiovascular adverse events

If a death was identified, I sought the following information about the deceased individual from study investigators:

1) Demographics: age and sex
2) Medical history: syncope or non-fatal cardiac arrest, other comorbidities, comedications
3) Family history: syncope, sudden cardiac death
4) Drug dosing: dose round, dose number, dose date, dose time
5) Death: death date, death time, autopsy report
6) Further investigator input: assessment of relatedness to drug, serious adverse event report

2.1.4 Risk of Bias Assessment

2.1.4.1 Risk of Bias in Individual Studies

Risk of bias assessment of individual studies at the outcome level was conducted using the PROTECT checklist for systematic reviews on drug adverse events[174].

2.1.4.2 Risk of Bias Across Studies

Publication bias was minimised through inclusion of data from large unpublished studies as well as the incorporation of estimates from the non-malaria literature as clinical priors as part of Bayesian meta-analysis.

2.1.5 Data Analysis

2.1.5.1 Standardisation of Population Rates into 30-Day Risks

Global surveillance rates of sudden cardiac death in the population under 35 years of age and drug-induced torsade de pointes in the general population from the literature were scaled to 30-day risks for the analysis (Table 2.2).

Table 2.2: Standardisation of Population Rates of Sudden Cardiac Death and Drug-Induced Torsade de Pointes to 30-day Risks

Reference	Type of Estimate	Reported Risk/Rate	Scaled 30-day Risk
Sarganas 2014[140]	Drug-induced torsade de pointes in the general population	3.2 in 1,000,000 person-years	1 in 3,750,000 individuals
Molokhia 2008[141]	Drug-induced torsade de pointes in the general population	10.9 in 1,000,000 person-years	1 in 1,100,912 individuals
Darpo 2001[142]	Drug-induced torsade de pointes in the general population	13.3 in 1,000,000 person-years	1 in 902,250 individuals
Roden 2016[138]	Drug-induced torsade de pointes after non-cardiovascular drugs	1 in 1,000,000 exposures	1 in 1,000,000 individuals
Ackerman 2016[175]	Sudden cardiac death in the young (<35 years) [lower bound of 18 studies from Europe, North America, and East Asia]	7 in 1,000,000 person-years	1 in 1,714,280 individuals
Ackerman 2016[175]	Sudden cardiac death in the young (<35 years) [upper bound of 18 studies from Europe, North America, and East Asia]	101 in 1,000,000 person-years	1 in 118,806 individuals
Bagnall 2016[176]	Sudden cardiac death in the young (<35 years) [Australasia – Australia and New Zealand]	13 in 1,000,000 person-years	1 in 923,071 individuals
Bonny 2017[177]	Sudden cardiac death in the young (<35 years) [Sub-Saharan Africa – Cameroon]	119 in 1,000,000 person-years	1 in 100,835 individuals

Incidence rates reported in person-years were scaled to monthly 30-day risks with an actuarial approximation using the following formula:

$$P_{month} = 1 - \left[\left(1 - P_{year}\right)^{\frac{1}{12}}\right]$$

where P_{month} is the risk of repolarisation-related cardiotoxicity over a month of 30 days and P_{year} is the risk of repolarisation-related cardiotoxicity over a year of 12 months.

2.1.5.2 Statistical Modelling

The overall likelihood was modelled as jointly independent binomial processes in view of these characteristics of the data:

1) The outcome of interest being binary, with each individual receiving the drug being an independent trial which resulted in one of in two outcomes, either sudden unexplained death or no sudden unexplained death within one terminal elimination half-life of DHA-PPQ
2) The number of individual participants receiving DHA-PPQ within each study, i.e. the number of trials within each unit of repeated trials, being fixed
3) Each study being conducted independently of all other studies, i.e. independence across units

Of related distributions, such as the Poisson, the binomial distribution was thought to be most appropriate for this dataset in consideration of the above characteristics.

A complete pooling intercept-only model, i.e. a fixed-effects model assuming the true risk of sudden unexplained death after DHA-PPQ in all studies was the same, was used to estimate the risk of sudden unexplained death after DHA-PPQ. As the regression software I used utilises a sampling algorithm for parameter estimation, the extremely low event count in the data precluded the use of a partial pooling hierarchical generalised linear model with covariate levels for mixed-effects modelling.

Posterior distributions were estimated using Hamiltonian Monte Carlo. Convergence of the Hamiltonian algorithm was done by running four independent chains and computing the Gelman-Rubin statistic ($\hat{R}$) which was below the threshold of tolerance (1.01). Visual posterior predictive checks were satisfactory.

2.1.5.3 Sensitivity Analyses

I conducted sensitivity analyses using weakly informative alternative priors centred on probabilities about half an order of magnitude higher and lower than the prior used for the main model in keeping with the range of observed risks of drug-related repolarisation-related cardiotoxicity in the literature from 2.1.5.1.

I also performed sensitivity analyses to consider the effects of excluding studies with less than 28 days of follow-up and the additional inclusion of any studies with more than 10,000 participants identified after the literature search was concluded.

2.1.5.4 Software

All statistical analysis and data visualisation was performed in R[178] (version 3.4.3), with the meta-analysis conducted using the rstanarm[179] package (version 2.13.1) for Bayesian applied regression modelling.

2.2 CHAPTER 4 METHODS

2.2.1 Research Question

The research question was summarised using the PICOTS framework (Table 2.3).

Table 2.3: PICOTS Framework for Systematic Review of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring

Population	Individuals of all ages, including pregnant women, who were either patients with *P. falciparum* and/or *P. vivax* malaria or healthy participants
Intervention	Amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, or quinine monotherapy or as part of an ACT
Comparison	Sulfadoxine-pyrimethamine
Outcome	Clinically significant cardiovascular adverse events (sudden deaths, seizures) Clinically significant arrhythmias (ventricular tachyarrhythmias) QT interval prolongation (absolute values & change from baseline)
Timeframe	Clinical and ECG assessment before and after drug administration
Study Type	Prospective randomised controlled trials or cohort studies

This framework was applied to define the literature search strategy and study eligibility criteria for this systematic review.

2.2.2 Search Strategy

2.2.2.1 Published Studies

An electronic literature search was conducted of the following three clinical bibliographic databases for journal articles last on 22 October 2015:

1) MEDLINE
2) EMBASE
3) Global Health

The quinoline and structurally related antimalarial drugs of interest were amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, and quinine. Sulfadoxine-pyrimethamine was included as a non-structurally related comparator.

The search was designed to identify prospective clinical studies of these antimalarials for malaria-related indications in healthy participants or malaria patients (*P. falciparum* and/or *P. vivax* mono- or mixed infection) which recorded ECGs before and after drug administration.

Animal studies were excluded, but language or publication date limits were not applied. Review articles, pooled analyses, case reports, commentary or correspondence articles, and conference abstracts were also excluded.

E.g. MEDLINE search on 22 October 2015

#	Searches
1)	Malaria/
2)	Malaria, Cerebral/
3)	Malaria, Falciparum/
4)	Malaria, Vivax/
5)	plasmodium falciparum/
6)	plasmodium vivax/
7)	malaria.ti,ab.
8)	falciparum.ti,ab.
9)	vivax.ti,ab.
10)	plasmodium.ti,ab.
11)	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12)	piperaquine.ti,ab.
13)	chloroquine.ti,ab.
14)	quinine.ti,ab.
15)	amodiaquine.ti,ab.
16)	lumefantrine.ti,ab.

17) benflumetol.ti,ab.

18) coartem.ti,ab.

19) halofantrine.ti,ab.

20) mefloquine.ti,ab.

21) primaquine.ti,ab.

22) sulfadoxine.ti,ab.

23) pyrimethamine.ti,ab.

24) Amodiaquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

25) Mefloquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

26) Chloroquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

27) Quinine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

28) Primaquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

29) Sulfadoxine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

30) Pyrimethamine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

31) Drug Administration Schedule/

32) 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31

33) Electrocardiography/

34) Electrocardiography, Ambulatory/

35) Cardiotoxicity/

36) Arrhythmias, Cardiac/ci, co, di, pp

37) Heart Conduction System/ab, de, pp

38) Long QT Syndrome/ci

39) Torsades de Pointes/ci

40) Cardiovascular Diseases/ci, co

41) Heart/de

42) Heart rate/de, ph, pd

43) Blood Pressure/co, de, pd, ph, th

44) (QT or QTc).ti,ab.

45) (electrocardiogra$ or ECG).ti,ab.

46) cardiotoxicity.ti,ab.

47) toxic$.ti,ab.

48) safety.ti,ab.

49) (adverse adj effect$).ti,ab.

50) (blood adj pressure).ti,ab.

51) pharmacokinetic$.ti,ab.

52) 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51

53) 11 and 32 and 52

All references were de-duplicated and screened against eligibility criteria by myself and another independent reviewer.

2.2.2.2 Unpublished Studies

Unpublished studies were not included in this systematic review

2.2.3 Data Extraction

I extracted the following information from study publications:

1) Study characteristics: study design, year of publication, recruitment period, location, participant population (including presence or absence of children and pregnant women), antimalarial treatment indication, number of days of follow-up, participant inclusion and exclusion criteria, number of participants enrolled and who had ECG monitoring
2) Cardiovascular adverse events: clinical (sudden cardiac death, syncope, palpitations), life-threatening ventricular tachyarrhythmias (ventricular fibrillation, ventricular tachycardia, torsade de pointes), other cardiac arrhythmias or cardiovascular adverse events
3) Antimalarial dosing regimen: route of administration, drug manufacturer, level of supervision, food intake around time of dosing
4) Antimalarial drug concentration measurement: drug measurement timepoints, biological matrix (capillary whole blood or venous plasma), assay type
5) ECG measurement methodology: ECG measurement timepoints, centralised or study site-based, manual or automated, cardiologist or other physician reader, intermittent or continuous, QT interval heart rate correction used, QT interval prolongation definition, other relevant details

2.2.4 Risk of Bias Assessment

2.2.4.1 Risk of Bias in Individual Studies

Risk of bias assessment of individual studies was based on the following criteria:

1) Blinding of participants, study personnel, and ECG readers
2) Selective outcome reporting and completeness of outcome data
3) ECG measurement methodology

2.2.4.2 Risk of Bias Across Studies

The proportion of studies with a low, unclear, or high risk of bias for each criterion was calculated for comparison across antimalarial drugs.

2.2.5 Data Analysis

A narrative synthesis was undertaken. I pooled quantitative data to obtain proportions and percentages without further meta-analysis.

2.3 CHAPTER 5 METHODS

2.3.1 Research Question

The research question was summarised using the PICOTS framework (Table 2.4).

Table 2.4: PICOTS Framework for Updated Systematic Review of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring

Population	Individuals of all ages, including pregnant women
Intervention	Amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, pyronaridine, or quinine as monotherapy or as part of an ACT but not in combination with another QT-prolonging medication for the treatment of patients with clinical *P. falciparum* and/or *P. vivax* malaria
Comparison	Drugs as for intervention but given to healthy individuals without clinical malaria
Outcome	Clinically significant cardiovascular adverse events (sudden deaths, seizures) Clinically significant arrhythmias (ventricular tachyarrhythmias) QT interval prolongation (absolute values & change from baseline)
Timeframe	Clinical and ECG assessment before and after drug administration
Study Type	Prospective randomised controlled trials or cohort studies

This is similar to Table 2.3 in section 2.3.1, but with the removal of sulfadoxine-pyrimethamine and the addition of pyronaridine to the list of antimalarials of interest. This framework was applied to define the literature search strategy and study eligibility criteria for this systematic review.

2.3.2 Search Strategy

2.3.2.1 Published Studies

An electronic literature search was conducted of the following three clinical bibliographic databases for journal articles last on 21 August 2017:

1) MEDLINE
2) EMBASE
3) Global Health

I searched for studies of the quinoline and structurally related antimalarials amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, pyronaridine, and quinine for malaria-related indications in human participants with and without clinical *Plasmodium falciparum* and/or *P. vivax* malaria in which ECGs were recorded at documented timepoints before and after drug administration.

I searched for malaria type, antimalarial drug names, and levels of repolarisation-related cardiovascular toxicity as title, abstract, and subject heading keywords, using synonyms and variant spellings as additional search terms.

I excluded animal studies, but did not apply language or publication date limits. Review articles, pooled analyses, case reports, commentary or correspondence articles, and conference abstracts were also excluded.

E.g. MEDLINE search on 21 August 2017

#	Searches
1)	Malaria/
2)	Malaria, Cerebral/
3)	Malaria, Falciparum/
4)	Malaria, Vivax/
5)	plasmodium falciparum/
6)	plasmodium vivax/
7)	malaria.ti,ab.
8)	falciparum.ti,ab.
9)	vivax.ti,ab.
10)	plasmodium.ti,ab.
11)	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10

12) piperaquine.ti,ab.

13) chloroquine.ti,ab.

14) quinine.ti,ab.

15) amodiaquine.ti,ab.

16) lumefantrine.ti,ab.

17) benflumetol.ti,ab.

18) coartem.ti,ab.

19) halofantrine.ti,ab.

20) mefloquine.ti,ab.

21) primaquine.ti,ab.

22) (pyronaridine or pyramax).ti,ab.

23) Amodiaquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

24) Mefloquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

25) Chloroquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

26) Quinine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

27) Primaquine/ad, ae, ct, pk, pd, po, to, tu, me, ur, bl, aa

28) Drug Administration Schedule/

29) 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28

30) Electrocardiography/

31) Electrocardiography, Ambulatory/

32) Cardiotoxicity/

33) Arrhythmias, Cardiac/ci, co, di, pp

34) Heart Conduction System/ab, de, pp

35) Long QT Syndrome/ci

36) Torsades de Pointes/ci

37) Cardiovascular Diseases/ci, co

38) Heart/de

39) Heart rate/de, ph, pd

40) Blood Pressure/co, de, pd, ph, th

41) (QT or QTc).ti,ab.

42) (electrocardiogra$ or ECG).ti,ab.

43) cardiotoxicity.ti,ab.

44) toxic$.ti,ab.

45) safety.ti,ab.

46) (adverse adj effect$).ti,ab.

47) (blood adj pressure).ti,ab.

48) pharmacokinetic$.ti,ab.

49) 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48

50) 11 and 29 and 49

All references were de-deduplicated and screened against eligibility criteria by myself and another independent reviewer using the Covidence platform.

2.3.2.2 Unpublished Studies

Unpublished studies were identified through expert consultation as part of the work of the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials[47].

2.3.3 Data Gathering, Extraction, and Standardisation

2.3.3.1 Ethics

All included individual patient-level data were obtained in accordance with appropriate ethical approvals from countries and institutions of origin. Additional ethical approval for this systematic review and meta-analysis of fully anonymised individual patient data was not deemed necessary in keeping with University of Oxford Central University Research Ethics Committee guidance.

2.3.3.2 Study-Level Data Extraction

I extracted the following information from study publications, reports, and protocols, and where necessary, requested further details from study investigators:

1) Study characteristics: year of publication, recruitment period, location, antimalarial treatment indication, participant inclusion and exclusion criteria, number of study participants who had ECG monitoring
2) ECG measurement methodology: centralised or study site-based, manual or automated, cardiologist or other physician reader, intermittent or continuous, any other relevant details
3) Cardiovascular adverse events: sudden cardiac death, life-threatening ventricular tachyarrhythmias (ventricular fibrillation, ventricular tachycardia, torsade de pointes), other clinically significant arrhythmias or cardiovascular adverse events

2.3.3.3 Individual Patient-Level Data Standardisation

Once digitised, individual patient-level datasets were converted into a standard file format using Stat/Transfer®[180] (version 13.3), then standardised via a bespoke Application Programming Interface in Python (version 3.6.3).

ECG Intervals

Where the same ECG recording was measured by more than one set of readers, the measurements from the more specialist set[181] of ECG readers were selected. Measurements from triplicate ECG recordings were averaged. Only measurements from intermittent ECG readings were used.

RR Interval

Heart rates in beats per minute were converted into RR intervals in milliseconds:

- RR interval = 60000/heart rate

RR intervals were then transformed with power functions:

- sqrtRR = $\sqrt{RR}$ (Bazett's correction-like)
- cbrtRR = $\sqrt[3]{RR}$ (Fridericia's correction-like)

QT/QTc Interval

Where only corrected QT intervals were available, uncorrected QT intervals were calculated as follows:

- $QT = QTcB * \sqrt{RR}$ as $QTcB = \frac{QT}{\sqrt{RR}}$ (Bazett's correction formula)
- $QT = QTcF * \sqrt[3]{RR}$ as $QTcF = \frac{QT}{\sqrt[3]{RR}}$ (Fridericia's correction formula)

where RR intervals are in units of seconds

Demographics

Age

Age was extracted as standardised to years, and otherwise calculated based on the number of years between the individual's date of birth and the date of the start of the study.

Weight

Weight was extracted as standardised to kilogrammes.

Vital Signs

Body Temperature

Oral and tympanic body temperatures were extracted as documented in the original data[182], and converted to degrees Celsius as required. Axillary body temperatures were extracted, converted to degrees Celsius as required, then standardised by the addition of 0.5°C to original readings.

Body temperature was standardised to degrees Celsius using the following formula:

- Temperature (°C) = [Temperature (°F) – 32] / 1.8

Temperature recordings documented to be >30 minutes apart from ECG recordings were not considered to be from the same timepoint and not extracted into the pooled dataset.

Laboratory Parameters

Parasitaemia

The highest parasite density available for each timepoint was extracted.

Malaria parasite count measurements were standardised as parasite density per microlitre of blood according to the following formulae before being logarithmically transformed:

- Parasitaemia = (parasite count per 500 WBC / 500) * WBC count [if WBC count available]
- Parasitaemia = (parasite count per 500 WBC / 500) * 8000 [if WBC count missing]

where WBC counts are in units of mm^3 of blood

- Parasitaemia = parasite count per 1000 RBC * 125.6 * haematocrit [if haematocrit available]
- Parasitaemia = parasite count per 1000 RBC * 125.6 * 33 [if haematocrit missing]

where haematocrit is in units of %

Haemoglobin

For studies in which only haematocrit was measured, haemoglobin was calculated as follows:

- Haemoglobin (g/dl) = [haematocrit (%) – 5.62] / 2.6 as

  Haematocrit (%) = 5.62 + 2.60 x haemoglobin (g/dl)[183]

2.3.3.4 Individual Patient-Level Data Integrity Checks

Individual patient data were checked for completeness, as well as for invalid, out-of-range, or inconsistent entries. Values incompatible with what would be observed in malaria clinical trials were considered missing. Queries were raised with study investigators and resolved where possible.

2.3.4 Risk of Bias Assessment

2.3.4.1 Risk of Bias in Individual Studies

Risk of bias assessment of individual studies at the outcome level was conducted using the PROTECT checklist for systematic reviews on drug adverse events[174].

2.3.4.2 Risk of Bias Across Studies

Risk of bias across studies was assessed by comparing the characteristics of included studies with those of the studies for which individual patient-level data were not available.

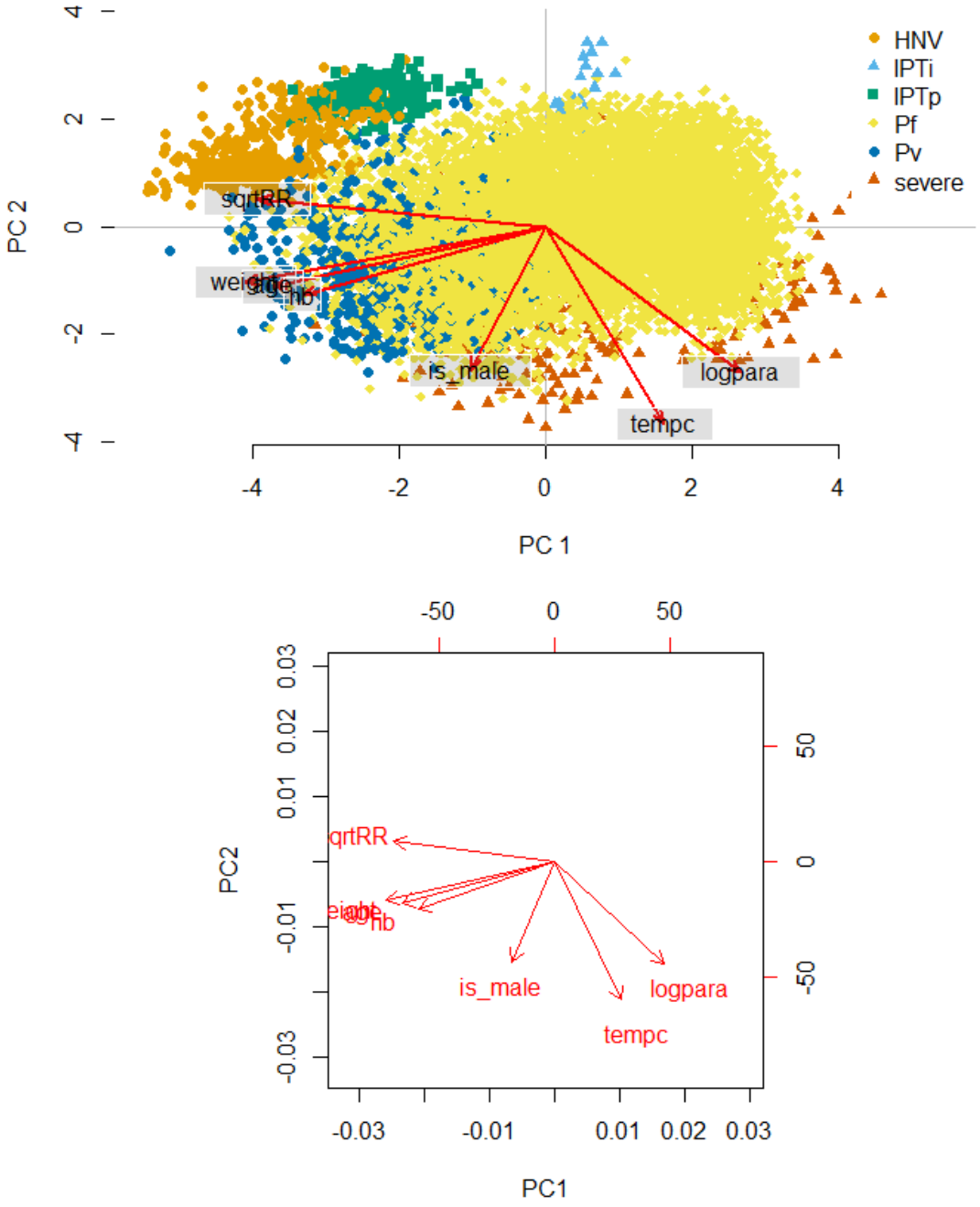
2.3.5 Data Analysis

2.3.5.1 Variable Selection

Pairwise relationships among collected variables were visualised using scatterplot matrices. I also summarised correlations among individual-level variables with principal component analysis biplots to identify potential redundancy (Figure 2.1).

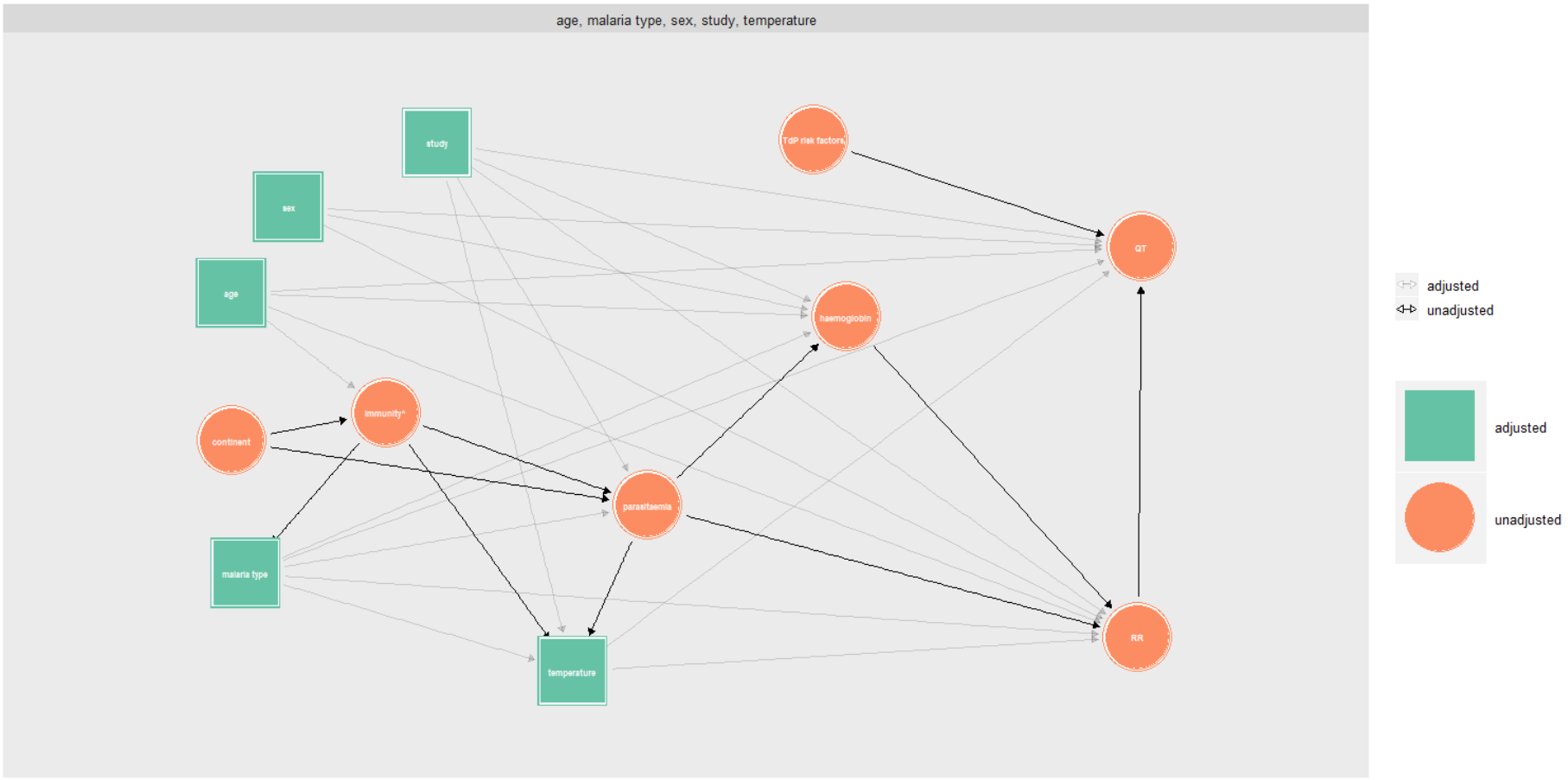
Variable selection was based on directed acyclic graphs of proposed causal relationships among collected variables informed by literature review and expert consultation[47] used to determine minimal sufficient adjustment sets for regression modelling (Figure 2.2).

Figure 2.1: Principal Component Analysis Biplots of Factors Affecting the QT Interval in Malaria



PC = principal component, HNV = healthy volunteers, IPTi = intermittent preventive treatment in infants, IPTp = intermittent preventive treatment in pregnant women, Pv = *P. vivax* malaria, Pf = uncomplicated *P. falciparum* malaria, severe = severe *P. falciparum* malaria, sqrtRR = $\sqrt{RR}$, is_male = sex, tempc = body temperature, logpara = log(parasitaemia), hb = haemoglobin.

Figure 2.2: Directed Acyclic Graph of Factors Affecting the QT Interval in Malaria



Directed acyclic graph describing proposed causal relationships among factors affecting the QT interval in malaria showing the minimal sufficient covariate adjustment set (facet label & green squares) in addition to the RR interval. The minimal adjustment set disease and demographic variables of malaria type, body temperature, age, and sex, as well as the RR interval were used as predictors and individual study ID as a varying intercept for Bayesian hierarchical multivariable regression analyses of the QT interval.

2.3.5.2 Statistical Modelling

Model Formulation

m1: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + (1 | study)

m2: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + (1 | study)

m3: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + (1 | study)

m4: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt{RR}$:indication + (1 | study)

where s() denotes a smooth term and : denotes an interaction between variables

Model Priors

I used weakly informative normal prior distributions summarised in Table 2.5.

Table 2.5: Model Prior Predictive Distributions

Description	Parameter Class	Prior Distribution
Coefficients of population-level effects/predictor variables	Coefficient	Normal (0, 50)
Standard deviations of group-level/varying effects and splines	Standard deviation	Normal (0, 100)
Standard deviation of residuals	Sigma	Normal (0, 30)

Model Diagnostics and Posterior Predictive Checks

Posterior distributions were estimated using Hamiltonian Monte Carlo. Convergence of the Hamiltonian algorithm was done by running four independent chains.

For each parameter:

- Trace plots were inspected for stationarity and mixing of chains
- Effective sample size computed to be more than 10% of total sample size
- Gelman-Rubin statistic ($\hat{R}$) checked to be 1 at convergence

In addition, the following Hamiltonian Monte Carlo diagnostics were checked in ShinyStan[184] (version 2.5.0):

- Tree depth information
- Energy Bayesian Fraction of Missing Information
- Divergence information

Visual posterior predictive checks were also performed.

Model Checking and Comparison

Comparing two models on Pareto smoothed importance-sampling leave-one-out cross-validation, if the absolute estimated difference in log predictive density is larger than twice the estimated standard error, this suggests one model is expected to have better predictive performance over the other. A negative estimated difference in log predictive density favours the first model, while a positive estimated difference in log predictive density favours the second.

2.3.5.3 Sensitivity Analyses

For all participants – addition of potential confounder variables

- Addition of binary variable for whether individual was enrolled in a study with one or more TdP risk factors as exclusion criteria: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt{RR}$:indication + TdPriskexclusion + (1 | study)
- Addition of haemoglobin as a continuous variable: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt{RR}$:indication + haemoglobin + (1 | study)

where s() denotes a smooth term and : denotes an interaction between variables

In the subgroup of malaria patients only – addition of parasitaemia as a potential confounder

- Addition of log parasitaemia as a continuous variable only: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt{RR}$:indication + log parasitaemia + (1 | study)
- Further addition of interaction term for log parasitaemia and treatment indication: QT ~ $\sqrt{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt{RR}$:indication + log parasitaemia + log parasitaemia:indication + (1 | study)

where s() denotes a smooth term and : denotes an interaction between variables

For all participants – alternative approaches for the non-linear QT-RR relationahips

- Alternative RR interval transformation into cube root instead of square root term: QT ~ $\sqrt[3]{RR}$ + s(age:sex) + sex + temperature + indication + $\sqrt[3]{RR}$:indication + (1 | study)
- Log-log linear model with base 10 logarithmic transformation of QT and RR: logQT ~ logRR + s(age:sex) + sex + temperature + indication + logRR:indication + (1 | study)

where s() denotes a smooth term and : denotes an interaction between variables

2.3.5.4 Software

All statistical analyses and data visualisation were done in R[178] (version 3.5.0). Bayesian regression was done using the brms[185] package (version 2.6.0) and the probabilistic programming language Stan[186] (version 2.18.0).

2.4 CHAPTER 6 METHODS

2.4.1 Research Question

The research question was summarised using the PICOTS framework (Table 2.6).

Table 2.6: PICOTS Framework for Systematic Review of Studies of Amodiaquine with Electrocardiographic Monitoring

Population	Individuals of all ages, including pregnant women, who were either patients with *P. falciparum* and/or *P. vivax* malaria or healthy participants
Intervention	Amodiaquine as monotherapy or as part of an ACT, but not in combination with another QT-prolonging medication, in a full 3-day treatment course
Comparison	Non-amodiaquine quinoline and structurally related antimalarials as monotherapy or as part of an ACT, but not in combination with another QT-prolonging medication, in a full 3-day treatment course
Outcome	QT interval prolongation (absolute values & changes from baseline) Heart rate changes (absolute values & changes from baseline) Blood pressure changes (absolute values & changes from baseline)
Timeframe	Clinical and ECG assessment before and after drug administration
Study Type	Prospective randomised controlled trials or cohort studies

This is similar to Table 2.3 in section 2.3.1 and Table 2.4 in section 2.4.1, but with a focus on amodiaquine in comparison to other front-line quinoline and structurally related antimalarials, including pyronaridine, all in a full 3-day treatment course. This framework was applied to define study eligibility criteria for this systematic review.

2.4.2 Search Strategy

2.4.2.1 Published Studies

As in 2.3.2.1.

2.4.2.2 Unpublished Studies

Unpublished studies were identified through expert consultation as part of the work of the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials[47].

2.4.3 Data Gathering, Extraction, and Standardisation

2.4.3.1 Ethics

All included individual patient-level data were obtained in accordance with appropriate ethical approvals from countries and institutions of origin. Additional ethical approval for this systematic review and meta-analysis of fully anonymised individual patient data was not deemed necessary in keeping with University of Oxford Central University Research Ethics Committee guidance.

2.4.3.2 Study-Level Data Extraction

I extracted the following information from study publications, reports, and protocols, and where necessary, requested further details from study investigators:

1) Study characteristics: study design, year of publication, recruitment period, location, participant population, antimalarial treatment indication, number of days of follow-up, participant inclusion and exclusion criteria, number of participants who had ECG monitoring
2) Antimalarial dosing regimen: route of administration, course length, dosing frequency, drug formulation, drug manufacturer, dosing table, level of supervision, food intake around time of dosing
3) Antimalarial drug concentration measurement: drug measurement timepoints, units of measurement, biological matrix (capillary whole blood or venous plasma), assay type, assay limit of quantification
4) ECG measurement methodology: ECG measurement timepoints, centralised or study site-based, manual or automated, cardiologist or other physician reader, intermittent or continuous, other relevant details

5) Cardiovascular adverse events: sudden cardiac death, life-threatening ventricular tachyarrhythmias (ventricular fibrillation, ventricular tachycardia, torsade de pointes), other clinically significant arrhythmias or cardiovascular adverse events

2.4.3.3 Individual Patient-Level Data Standardisation

This was implemented via a bespoke Application Programming Interface in Python (version 3.7.2) as described in 2.4.3.3 but updated for repeated measures databasing.

ECG Intervals

Where the same ECG recording was measured by more than one set of readers, the measurements from the more specialist set[181] of ECG readers were selected. Measurements from triplicate ECG recordings were averaged. Only measurements from intermittent ECG readings were used.

RR Interval

RR intervals were standardised to units of milliseconds and transformed into heart rate based on the following formula as necessary:

- Heart rate = 60000/RR interval

QT/QTc Interval

QT interval measurements were extracted as standardised to units of milliseconds.

Where only corrected QT intervals were available, uncorrected QT intervals were calculated as follows:

- $QT = QTcB * \sqrt{RR}$ as $QTcB = \frac{QT}{\sqrt{RR}}$ (Bazett's correction formula)
- $QT = QTcF * \sqrt[3]{RR}$ as $QTcF = \frac{QT}{\sqrt[3]{RR}}$ (Fridericia's correction formula)

where RR intervals are in units of seconds

QRS Interval

QRS interval measurements were extracted as standardised to units of milliseconds, where available.

PR Interval

PR interval measurements were extracted as standardised to units of milliseconds, where available.

Demographics

Age

Age was extracted as standardised to years, and otherwise calculated based on the number of years between the individual's date of birth and the date of the start of the study.

Weight

Individual body weight was extracted as standardised to units of kilograms.

Vital Signs

Pulse Rate

Peripheral pulse rate, i.e. heart rate as measured from the peripheral pulse rather than the RR interval on the ECG, was extracted as standardised to units of beats per minute.

Blood Pressure

Supine systolic and diastolic blood pressure measurements were extracted as standardised to units of mmHg, where these were available.

Erect systolic and diastolic blood pressure measurements were also extracted as standardised to units of mmHg, where these were available.

Body Temperature

Oral and tympanic body temperatures were extracted as documented in the original data[182], and converted to units of degrees Celsius as required. Axillary body temperatures

were extracted, converted to units of degrees Celsius as required, then standardised by the addition of 0.5°C to original readings.

Body temperature was standardised to units of degrees Celsius as follows:

- Temperature (°C) = [Temperature (°F) – 32] / 1.8

Laboratory Parameters

Parasitaemia

The highest parasite density available for each timepoint was extracted.

Malaria parasite count measurements were standardised as parasite density per microlitre of blood according to the following formulae before being logarithmically transformed:

- Parasitaemia = (parasite count per 500 WBC / 500) * WBC count [if WBC count available]
- Parasitaemia = (parasite count per 500 WBC / 500) * 8000 [if WBC count missing]

where WBC counts are in units of mm^3 of blood

- Parasitaemia = parasite count per 1000 RBC * 125.6 * haematocrit [if haematocrit available]
- Parasitaemia = parasite count per 1000 RBC * 125.6 * 33 [if haematocrit missing]

where haematocrit is in units of %

Haemoglobin

For studies in which only haematocrit was measured, haemoglobin was calculated using the following formula:

- Haemoglobin (g/dl) = [haematocrit (%) – 5.62] / 2.6 as
  Haematocrit (%) = 5.62 + 2.60 * haemoglobin (g/dl)[183]

Antimalarial Drug-Related Parameters

Milligram per Kilogram Dose

The total milligram per kilogram (mg/kg) dose of quinoline or structurally related antimalarial received by each individual was extracted where available but was otherwise derived from available drug dosing data and study-specific weight-based dosing charts:

- mg/kg dose = total dose received (mg) / weight (kg)
- mg/kg dose = total number of doses * number of tablets per dose * dose per tablet (mg) / weight (kg)

Vomiting and Repeated Doses

Vomiting after dosing and whether the treatment dose was repeated after vomiting were extracted as 'present' or 'absent'. Where a dose was repeated, the date and time of the repeated dose were used to calculate time from dosing.

Concomitant Medications

Concomitant medications were extracted as a list of drug names as recorded in the original data if available.

Antimalarial Pre-treatment

Antimalarial pre-treatment was extracted as 'present' or 'absent' with the name of the pre-treatment drug extracted as free text into a separate column where available.

2.4.3.4 Individual Patient-Level Data Integrity Checks

Individual patient data were checked for completeness, as well as for invalid, out-of-range, or inconsistent entries. Values incompatible with what would be observed in malaria clinical trials were considered missing. Queries were raised with study investigators and resolved where possible.

2.4.4 Risk of Bias Assessment

2.4.4.1 Risk of Bias in Individual Studies

Risk of bias assessment of individual studies at the outcome level was conducted using the PROTECT checklist for systematic reviews on drug adverse events[174].

2.4.4.2 Risk of Bias Across Studies

Risk of bias across studies was assessed by comparing the characteristics of included studies with those of the studies for which individual patient-level data were not available.

2.4.5 Data Analysis

2.4.5.1 Age Group Categories for Heart Rate and its QT Interval Correction Factor

Table 2.7: Bradycardia Thresholds by Age Group

	Heart Rate (beats/minute)	
Age Group (years)	**Lower Limit of Normal (10^{th} Percentile)**	**Lower Limit (5^{th} Percentile)**
0.5 to 2	100	90
2 to <5	80	70
5 to <8	75	65
8 to <12	70	60
12 to <15	65	55
≥15	60	50

Adapted from systematic review of normal range of heart rate in 143,346 healthy children between birth and 18 years of age from 69 observational studies[187]

Table 2.8: Study-Specific Correction Exponents by Age Group

	Study Reference			
Age Group (years)	**Ndiaye 2011[188]**	**Ogutu 2014[189]**	**Siqueira 2017[190]**	**WANECAM 2018[122]**
0.5 to <5	N/A	N/A	N/A	0.46
5 to <10	N/A	N/A	N/A	0.43
10 to <15	0.455	N/A	0.42	0.43
≥15	0.435	0.42	0.35	0.395

The correction exponent β_{age} is the coefficient of $agegroup * logRR$ from log-log linear regression:

$$logQT \sim agegroup * logRR + sex + temperature + ECGday * drug + (1 \mid patient)$$

In addition to antimalarial drug, the malaria disease and demographic variables included are those identified in Chapter 5 to have independent effects on the QT interval in malaria

2.4.5.2 Study-Level Analyses

Means were compared with Welch's unequal variances *t*-test, one-way ANOVA, or the Kruskal-Wallis test (if Levene's test was significant); proportions were compared with Fisher's exact or Pearson's chi-squared test with Yates' continuity correction; and for medians, distributions were compared with the Wilcoxon rank sum test with continuity correction or the Kruskal-Wallis test as appropriate.

2.4.5.3 Individual Patient-Level Data Meta-analyses

Variable Selection

Variable selection was based on directed acyclic graphs of proposed causal relationships among collected variables informed by literature review and expert consultation used to determine minimal sufficient adjustment sets for regression modelling (Figures 2.3-5).

Model Formulation

Heart Rate Models

HR_adult ~ drug*ECGday + temperature + sex + (1 | study/site/patient) [age ≥12 years]

HR_child ~ drug*ECGday + temperature + age + (1 | study/site/patient) [age <12 years]

Corrected QT Interval Model

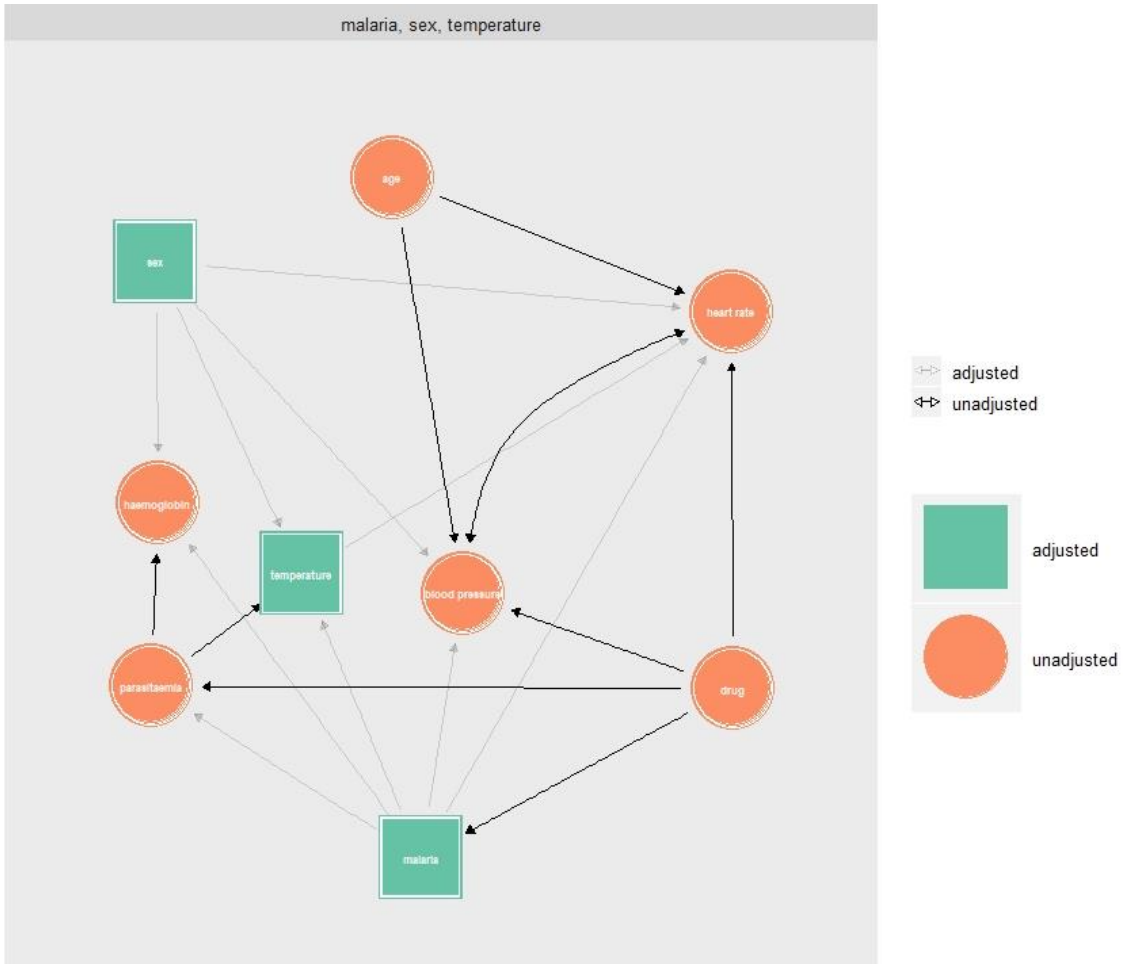
QTcS ~ drug*ECGday + temperature + agegroup*sex + (1 | study/site/patient)

where $QTcS = \frac{QT}{\beta_{age}\sqrt{RR}}$ from 2.4.5.1 and RR is in units of seconds

Model Diagnostics and Comparison

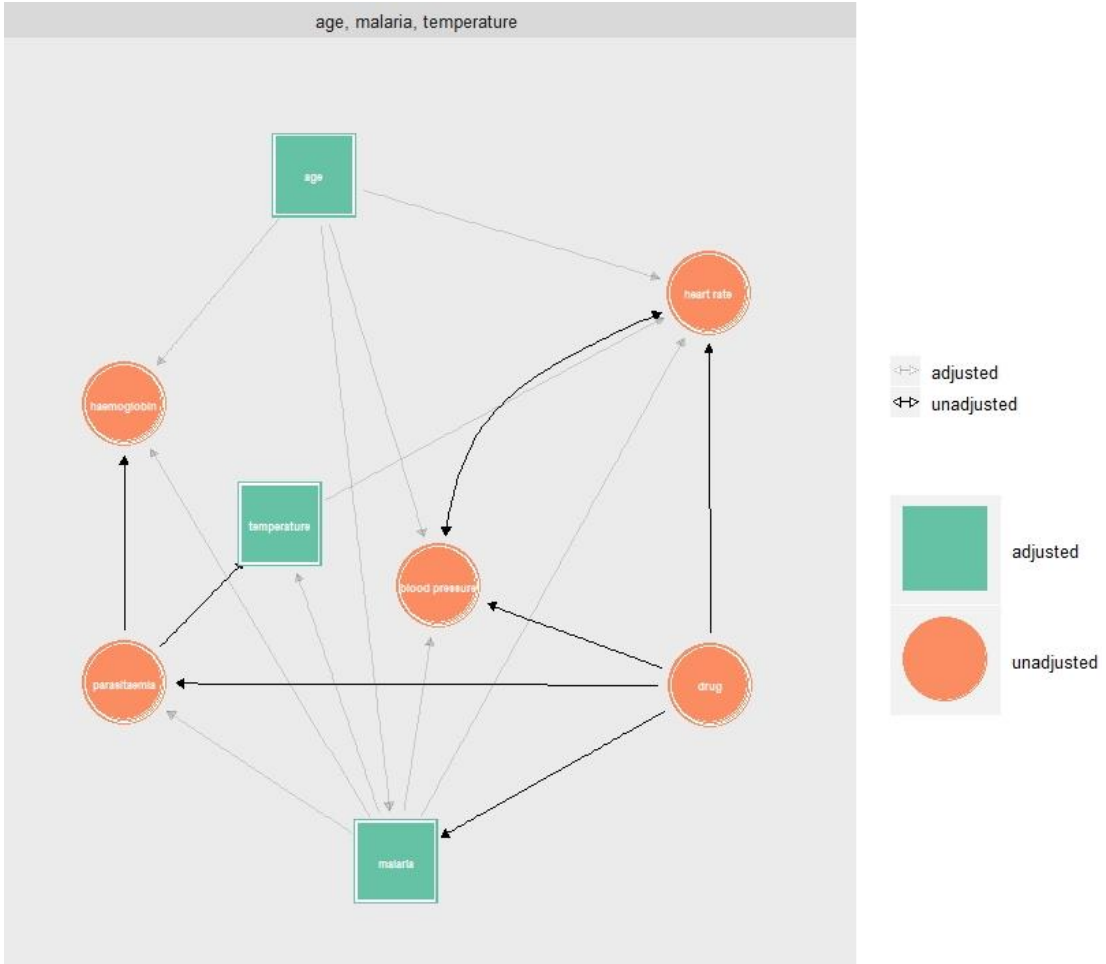
Model fit was assessed by visual inspection of residuals while model discrimination was on the basis of likelihood ratio tests with p <0.05 as the threshold for statistical significance.

Figure 2.3: Directed Acyclic Graph of Factors Affecting the Heart Rate in Malaria in Individuals Aged ≥12 Years after Amodiaquine Treatment



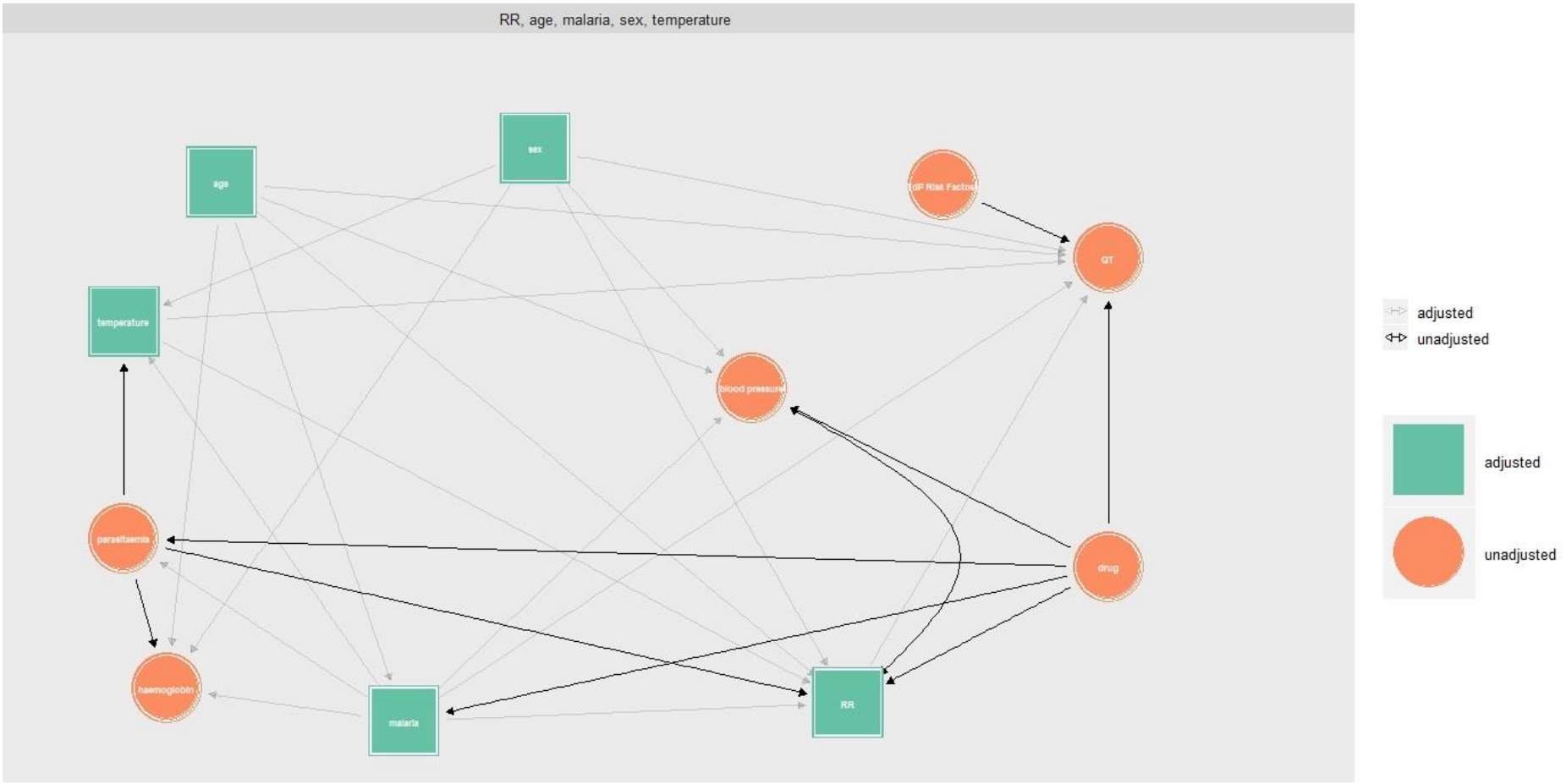
Directed acyclic graph describing proposed causal relationships among factors affecting the heart rate in malaria in adolescents and adults after antimalarial treatment with amodiaquine showing minimal sufficient covariate adjustment set (facet label & green squares). The minimal adjustment set consisting of disease variables of malaria and body temperature along with the demographic covariate of sex were included as fixed effects in multivariable linear mixed effects analyses.

Figure 2.4: Directed Acyclic Graph of Factors Affecting the Heart Rate in Malaria in Individuals Aged <12 Years after Amodiaquine Treatment



Directed acyclic graph describing proposed causal relationships among factors affecting the heart rate in malaria in children after antimalarial treatment with amodiaquine showing minimal sufficient covariate adjustment set (facet label & green squares). The minimal adjustment set consisting of disease variables of malaria and body temperature along with the demographic covariate of age were included as fixed effects in multivariable linear mixed effects analyses.

Figure 2.5: Directed Acyclic Graph of Factors Affecting the QT Interval in Malaria after Amodiaquine Treatment



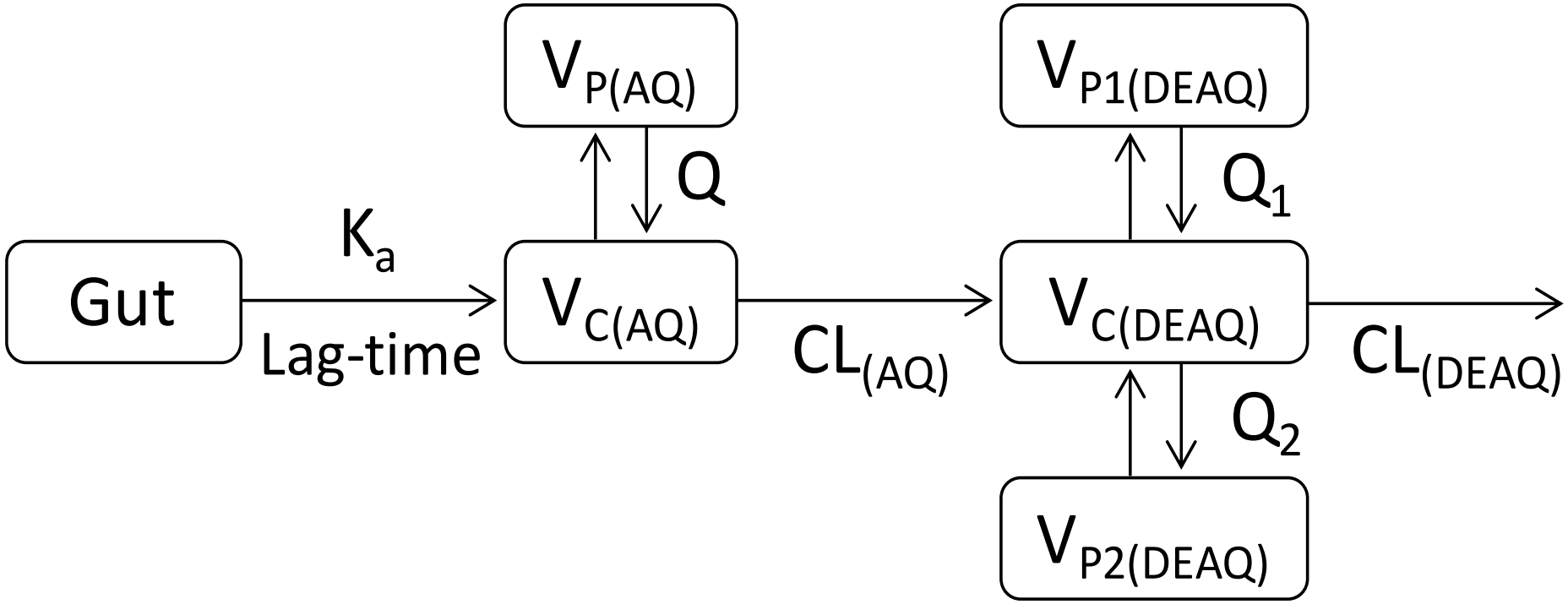
Directed acyclic graph describing proposed causal relationships among factors affecting the electrocardiographic QT interval in malaria after antimalarial treatment with amodiaquine showing minimal sufficient covariate adjustment set (facet label & green squares). The minimal adjustment set consisting of malaria disease variables of malaria and body temperature along with demographic covariates of age and sex as well as the RR interval were included as fixed effects in multivariable linear mixed effects analyses.

2.4.5.4 Pharmacokinetic Analyses

Observed amodiaquine and desethylamodiaquine concentrations from the available study[189,191], transformed into their natural logarithms, were analysed with non-linear mixed-effects modelling using the first-order conditional estimation with interactions method. Concentrations below the lower limit of quantification were omitted.

The structural pharmacokinetic model of amodiaquine and desethylamodiaquine used was based on one developed from another study of amodiaquine monotherapy for treatment of *P. vivax* malaria in pregnant women with rich (i.e. more intensive) pharmacokinetic sampling[192]. Amodiaquine concentrations were described by lagged first-order absorption with a two-compartment distribution model followed by a three-compartment distribution model of desethylamodiaquine (Figure 2.6). Amodiaquine was assumed to be completely metabolised into desethylamodiaquine as the drug-metabolite conversion fraction was not identifiable. The final population pharmacokinetic parameter and interindividual variability estimates with their parameter uncertainties from the previous study[192] were incorporated into the model developed for this study as prior estimates.

Figure 2.6: Population Pharmacokinetic Structural Model of Amodiaquine and Desethylamodiaquine[192]



K_a = absorption first-order rate constant; $V_{C(AQ)}$ = apparent volume of distribution of the central amodiaquine compartment; $V_{P(AQ)}$ = apparent volume of distribution of the peripheral amodiaquine compartment; Q = inter-compartmental clearance rate of amodiaquine between its central and peripheral compartments; CL_{AQ} = apparent elimination clearance rate of amodiaquine from its central compartment to form desethylamodiaquine in the metabolite's central compartment; $V_{C(DEAQ)}$ = apparent volume of distribution of the central desethylamodiaquine compartment; $V_{P1(DEAQ)}$ = apparent volume of distribution of the first peripheral desethylamodiaquine compartment; $V_{P2(DEAQ)}$ = apparent volume of distribution of the second peripheral desthylamodiaquine compartment; Q_1 = inter-compartmental clearance rate of desethylamodiaquine between its central and first peripheral compartments; Q_2 = inter-compartmental clearance rate of desethylamodiaquine between its central and second peripheral compartments; CL_{DEAQ} = apparent elimination clearance rate of desethylamodiaquine from its central compartment

Pharmacokinetic parameters were assumed to be log-normally distributed. Interindividual variability was added to all parameters according to the following equation:

$$P_i = \theta_p \times \exp(\eta_{i,p})$$

where P_i is the pharmacokinetic parameter estimate for the ith individual, θ_p is the population value of the investigated parameter, and $\eta_{i,p}$ is the deviation of the ith individual estimate from the population parameter value.

Interindividual variability was assumed to be normally distributed with mean zero and variance ω^2 (diagonal correlation matrix). Where estimates were less than 10% or had a residual standard error of more than 50%, interindividual variability was fixed to zero. The residual unexplained variability in concentration was described by an additive error on the individually predicted logarithmic concentrations which is equivalent to an exponential error for non-transformed concentrations on the arithmetic scale.

Individual body weight, scaled by the median body weight (48.0kg) of the previous study[192] population, was included as a fixed allometric function to all clearance (power of 0.75) and volume of distribution (power of 1) parameters.

Model discrimination was based on the objective function value (OFV) which is proportional to -2 times the log likelihood of the data and has a Chi-squared distribution. A likelihood ratio test with a reduction in OFV of 3.84 or more was considered significant at p = 0.05 for a nested model with a difference of one degree of freedom. Goodness-of-fit plots were used to identify potential model misspecification and systematic errors. Model robustness and parameter confidence intervals were evaluated using a sampling-importance-resampling procedure[193]. Predictive performance was assessed with prediction-corrected visual and numerical predictive checks (n = 2000)[194].

Predicted amodiaquine and desethylamodiaquine concentrations for the time points at which cardiovascular vital signs (pulse rate and blood pressure) and electrocardiographic intervals (QT, QRS, and PR) were measured in the available study[189,191] were used in the concentration-effect analysis.

2.4.5.5 Concentration-Effect Analyses

Variable Selection

Variable selection was based on directed acyclic graphs of proposed causal relationships among collected variables informed by literature review and expert consultation used to determine minimal sufficient adjustment sets for regression modelling (Figures 2.3, 2.5, 2.7 & 2.8).

Model Formulation

Change in Pulse Rate Model

$\Delta HR \sim$ total drug concentration + malaria + Δtemperature + sex + (1 | patient)

Change in Blood Pressure Models

$\Delta SBP \sim$ total drug concentration + malaria + (1 | patient) [supine]

$\Delta DBP \sim$ total drug concentration + malaria + (1 | patient) [supine]

$\Delta SBPe \sim$ total drug concentration + malaria + (1 | patient) [erect]

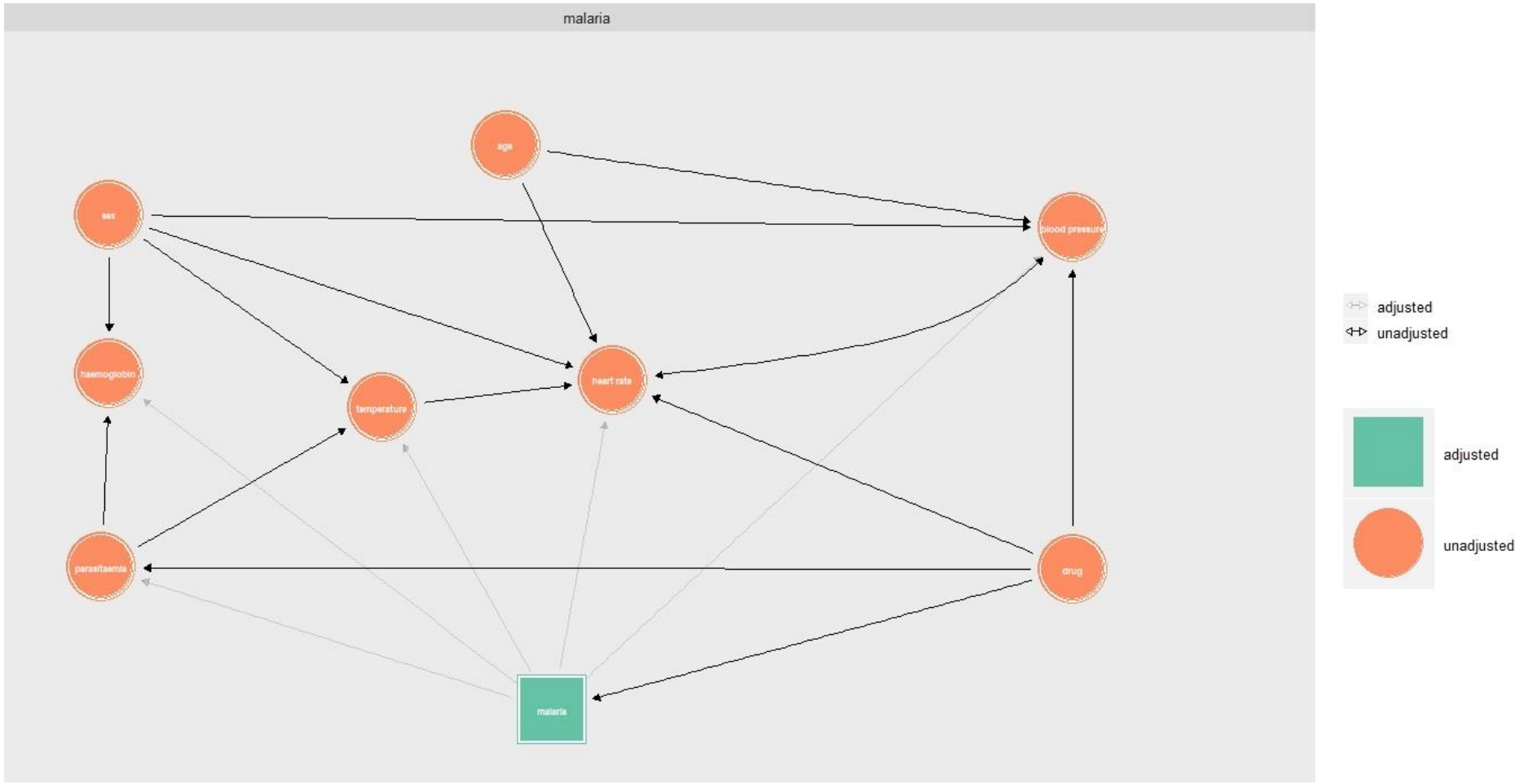
$\Delta DBPe \sim$ total drug concentration + malaria + (1 | patient) [erect]

$\Delta pSBP \sim$ total drug concentration + malaria + (1 | patient) [postural = supine - erect]

$\Delta pDBP \sim$ total drug concentration + malaria + (1 | patient) [postural = supine - erect]

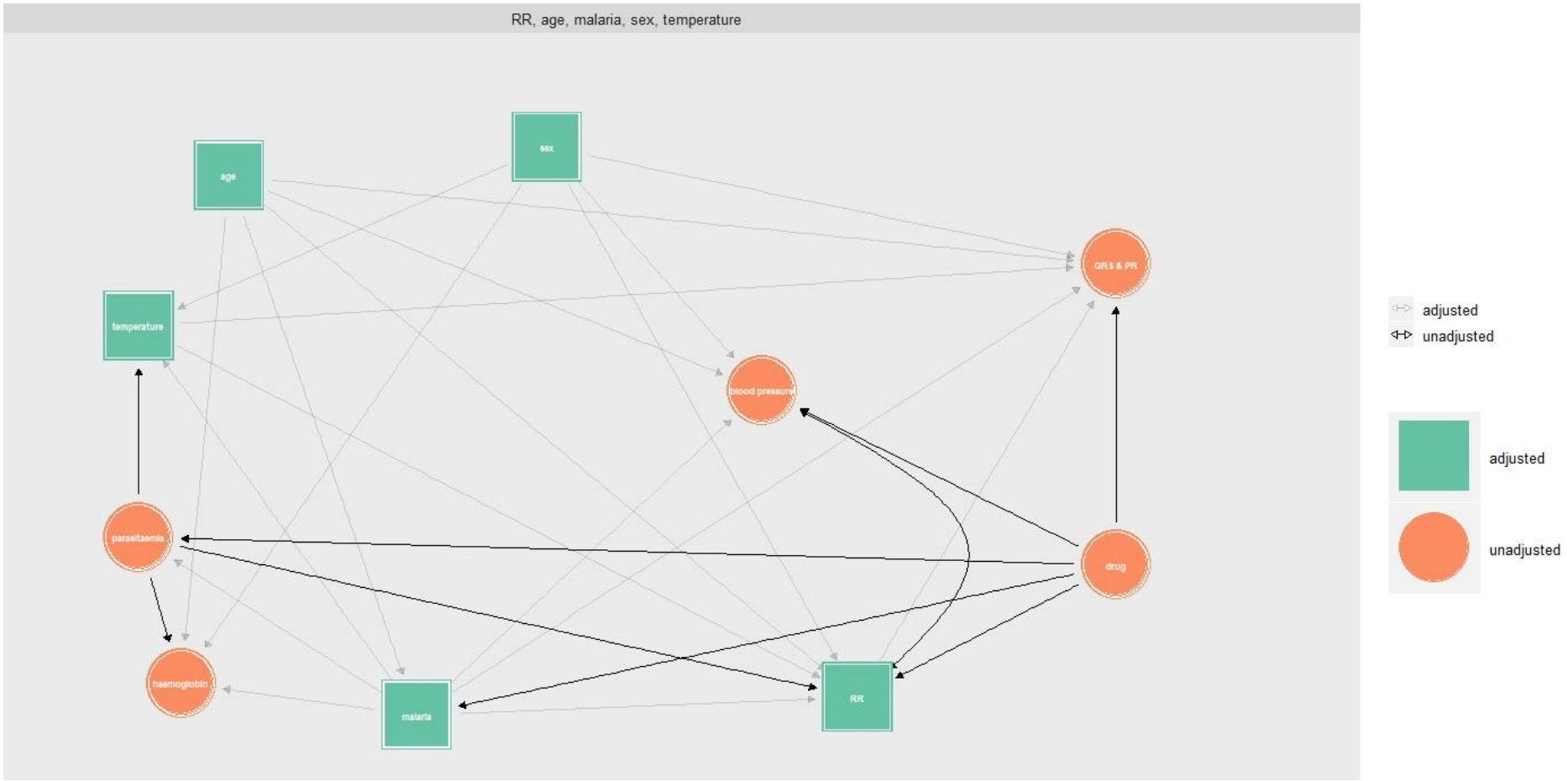
where SBP = systolic blood pressure and DBP = diastolic blood pressure

Figure 2.7: Directed Acyclic Graph of Factors Affecting Blood Pressure in Malaria after Amodiaquine Treatment



Directed acyclic graph describing proposed causal relationships among factors affecting blood pressure in malaria after antimalarial treatment with amodiaquine showing minimal sufficient covariate adjustment set (facet label & green squares). The minimal adjustment set consisting of the malaria disease variable was included as a fixed effect in multivariable linear mixed effects analyses.

Figure 2.8: Directed Acyclic Graph of Factors Affecting the QRS & PR Intervals in Malaria after Amodiaquine Treatment



Directed acyclic graph describing proposed causal relationships among factors affecting the electrocardiographic QRS and PR intervals in malaria after antimalarial treatment with amodiaquine showing minimal sufficient covariate adjustment set (facet label & green squares). The minimal adjustment set consisting of malaria disease variables of malaria and temperature along with demographic covariates of age and sex as well as the RR interval were included as fixed effects in multivariable linear mixed effects analyses.

Corrected QT Interval Models

QTcS~ total drug concentration + Δtemperature + age + sex + ΔRR interval + (1 | patient)

QTcF~ total drug concentration + Δtemperature + age + sex + ΔRR interval + (1 | patient)

QTcB~ total drug concentration + Δtemperature + age + sex + ΔRR interval + (1 | patient)

where $QTcS = \frac{QT}{RR^{0.42}}$ & $QTcF = \frac{QT}{\sqrt[3]{RR}}$ & $QTcB = \frac{QT}{\sqrt[2]{RR}}$, and RR is in units of seconds

QRS & PR Interval Models

QRS ~ total drug concentration + Δtemperature + age + sex + ΔRR interval + (1 | patient)

PR ~ total drug concentration + Δtemperature + age + sex + ΔRR interval + (1 | patient)

Model Diagnostics and Comparison

Model fit was assessed by visual inspection of residuals while model discrimination was on the basis of likelihood ratio tests with p <0.05 as the threshold for statistical significance.

2.4.5.6 Software

All statistical analyses and data visualisation were done in R[178] version 3.6.0, with linear mixed effects modelling conducted using the *nlme*[195] package.

Pharmacokinetic non-linear mixed-effects modelling was implemented in NONMEM®[196] (version 7.4). In addition to R, Pirana[197] (version 2.9.0) and Perl-speaks-NONMEM®[198] (version 4.8.0) were used for automation, model evaluation, and diagnostics during the modelling process. The NONMEM® $PRIOR functionality was used to stabilise model performance.

3 Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine

3.1 SUMMARY

Background

Dihydroartemisinin-piperaquine is a highly effective and well-tolerated artemisinin-based combination therapy which has been evaluated extensively for the prevention and treatment of malaria. Piperaquine, like several structurally related antimalarials currently deployed, can prolong cardiac ventricular repolarisation duration and the electrocardiographic QT interval, leading to concerns about its proarrhythmic potential. We aimed to assess the risk of potentially lethal iatrogenic ventricular arrhythmias in individuals receiving dihydroartemisinin-piperaquine for malaria-related indications.

Methods

We conducted a systematic review and Bayesian meta-analysis. We searched clinical bibliographic databases (last on 24 May 2017) for studies of dihydroartemisinin-piperaquine in humans. Further unpublished studies were identified with the World Health Organization Evidence Review Group on the Cardiotoxicity of Antimalarials. Eligible studies were prospective randomised controlled trials or cohort studies in which participants received at least one 3-day treatment course of dihydroartemisinin-piperaquine for mass drug administration, preventive therapy, or case management of uncomplicated malaria, with follow-up over at least three days. The risk of sudden unexplained death after dihydroartemisinin-piperaquine with 95% credible intervals was compared with the baseline rate of sudden cardiac death.

Findings

Our search identified 94 eligible primary studies including data for 197,867 individuals who had received dihydroartemisinin-piperaquine: 154,505 in mass drug administration

programmes, 15,188 in 14 studies of repeated courses in preventive therapies and case management of uncomplicated malaria, and 28,174 as single-course treatments of uncomplicated malaria in 76 case management studies. There was one potentially drug-related sudden unexplained death: a healthy woman aged 16 in Mozambique who developed heart palpitations several hours after the second dose of dihydroartemisinin-piperaquine in mass drug administration, then collapsed and died on the way to hospital. The median pooled risk estimate of sudden unexplained death after dihydroartemisinin-piperaquine was 1 in 757,950 (95% credible interval: 1 in 2,854,490 to 1 in 209,114).

Interpretation

Dihydroartemisinin-piperaquine is associated with a very low risk of sudden unexplained death that was not higher than the baseline rate of sudden cardiac death. Concerns about repolarisation-related cardiotoxicity need not limit its current use for the prevention and treatment of malaria.

3.2 INTRODUCTION

The artemisinin-based combination therapy dihydroartemisinin-piperaquine (DHA-PPQ) is an attractive choice for the treatment and prevention of malaria. DHA-PPQ has proved highly efficacious and safe[199,200] in the treatment of uncomplicated malaria and in repeated dosing[201] including in healthy adults[202,203] and children[204] receiving intermittent preventive therapy. DHA-PPQ is considered an ideal candidate for mass drug administration because it is well-tolerated and has a long post-treatment prophylactic effect. Piperaquine, a bisquinoline compound of the 4-aminoquinoline group, has a terminal elimination half-life of 20-30 days[113] and prevents patent reinfection for a month or more. Studies in the Greater Mekong Subregion[205-207] and early evidence from a large-scale pilot programme in Zambia[208] confirm that mass drug administration with DHA-PPQ has good tolerability and promising benefits in the short-term.

Before it was combined with dihydroartemisinin in the current ACT formulation, piperaquine was used extensively as monotherapy. Some 140 million piperaquine treatments were consumed in China for individual and mass treatments between 1978 and 1992 until the emergence of piperaquine resistance prompted a change in national treatment policy[112].

No adverse cardiovascular effects have been reported from this extensive early experience but the potential of piperaquine to produce repolarisation-related cardiotoxicity – manifest as prolongation of the QT interval of the surface electrocardiogram (ECG) – has received renewed interest in recent years. Dose-dependent QT interval prolongation was observed in healthy volunteers in a phase 1 'Thorough QT'[136] assessment[119] of the DHA-PPQ formulation approved by the European Medicines Agency[152] in 2011. Since then, DHA-PPQ has become the most intensively studied antimalarial in relation to repolarisation-

related cardiotoxicity. Particular attention has been paid to the effects of the highest plasma piperaquine levels which usually occur 4-6 hours after the third and final daily dose of DHA-PPQ in a 3-day treatment course[113]. Piperaquine has qualitatively similar effects to other antimalarials in the quinoline class in current widespread use, notably chloroquine, amodiaquine, and quinine. These antimalarials were introduced more than 50 years ago, when the potential risks of drug-induced repolarisation-related cardiotoxicity were not appreciated[48].

Prolongation of the QT interval is a sensitive but not specific indicator of increased risk of torsade de pointes (TdP), a polymorphic ventricular tachyarrhythmia which, if sustained, can degenerate in some cases into ventricular fibrillation and cause sudden cardiac death. However, the relationship between QT interval prolongation and TdP is not straightforward. Drugs which cause QT interval prolongation are inconsistently associated with life-threatening tachyarrhythmias[138] and only a very small proportion of patients with QT interval prolongation develop these very rare arrhythmic complications. The incidence of drug-induced TdP and life-threatening ventricular arrhythmias is reported as between 3.2 and 13 per million-person years in the general population in active surveillance studies conducted in Europe[140-142]. There are few comparable data available from tropical areas.

Quantifying the risk of sudden unexplained death potentially compatible with drug-induced repolarisation-related tachyarrhythmia and sudden cardiac death is an important but challenging part of antimalarial cardiotoxicity risk assessment. In malaria-endemic regions, there is very limited access to electrocardiographic monitoring for arrhythmia detection and post-mortem examination for ascertainment of cause of death. The rarity of this adverse drug reaction further necessitates a synthesis of all available clinical evidence.

To address this, I assessed the risk of sudden unexplained death in individuals treated with DHA-PPQ in mass drug administration, intermittent preventive therapy, and case management[209].

3.3 METHODS

3.3.1 Search Strategy and Selection Criteria

I conducted a systematic review and meta-analysis of published and unpublished data. I performed a systematic literature search on 20 September 2016 (updated on 24 May 2017) for clinical studies of DHA-PPQ in humans. I searched the bibliographic databases MEDLINE, EMBASE, Web of Science, CINAHL, the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, the Database of Abstracts of Reviews of Effects, Global Health, the WHO Global Health Library, and the WWARN Clinical Trials of Uncomplicated Malaria Publication Library (see 2.1.2). Further unpublished or ongoing studies were identified as part of the work of the Evidence Review Group on the Cardiotoxicity of Antimalarials convened by the World Health Organization[47]. I excluded animal studies, but did not apply limits on language or publication date.

Studies were eligible for inclusion if they were prospective randomised controlled trials or cohort studies administering in at least one arm or group a minimum of one full 3-day treatment course of DHA-PPQ, with or without single low-dose primaquine[125], for mass drug administration, intermittent preventive therapy, or case management of uncomplicated malaria, with active follow-up over at least the first three days from drug initiation when piperaquine levels and risk of drug-induced cardiotoxicity are highest. No ages or age groups were excluded from eligibility; however, malaria-endemic regions have

young populations with median ages in the teens to early 20s. I also included secondary analyses of such studies identified from systematic reviews of DHA-PPQ treatment. Both published and unpublished studies were eligible for inclusion if sufficient information was available for interpretation.

3.3.2 Data Extraction

I screened titles, abstracts, and full texts, and agreed study eligibility with another independent reviewer. I extracted information about study and participant characteristics, adverse event surveillance methodology, DHA-PPQ exposures, loss-to-follow up and any deaths after DHA-PPQ treatment into a standardised database (see 2.1.3). The number of individuals exposed to DHA-PPQ in each study was obtained from the as-treated (where available) or intention-to-treat populations. In studies with repeated rounds of drug administration, if it was unclear whether the same individuals were included in all rounds of treatment, then the number of individuals was extracted from the first round of dosing only. Data from primary and secondary analyses of studies were checked for consistency. Where required, trial registry records or investigators were consulted. Study investigators were contacted for further details about individual deaths identified, including for serious adverse event reports or autopsy reports if available (see 2.1.3). I conducted risk of bias assessments within and across studies with another independent reviewer (see 2.1.4).

3.3.3 Data Analysis

Initial results of the data extraction were reviewed at the WHO Evidence Review Group meeting on the Cardiotoxicity of Antimalarials in Geneva, Switzerland, on 13-14 October 2016 for which I was the WHO technical resource person and rapporteur. The panel was comprised of experts in cardiology or cardiac electrophysiology, pharmacovigilance, clinical pharmacology, and clinical malariology. Authors or investigators of primary and

secondary analyses contributing more than 2,500 participants to this study attended as participants of the Evidence Review Group or designated appropriate expert representatives[47].

The choice of number of individual participants rather than number of courses or doses as the main denominator of exposure reflects expert advice that, in the absence of acute illness, individual cardiotoxicity risk is unlikely to alter considerably during the period of repeated dosing in mass drug administration and preventive therapies. The 30-day window from initiation of DHA-PPQ was agreed to be the risk period for drug-induced adverse events as the terminal elimination half-life of piperaquine is between 20-30 days, and after this time the concentration of piperaquine in the blood is very low[47].

I selected a Bayesian approach to meta-analysis for its advantages in synthesising diverse types of evidence, such as expert opinion and surveillance data, in a formal, consistent, and coherent process while taking the uncertainty at different levels into account. This allows for the generation of more realistic pooled estimates especially where data are sparse. The use of informative clinical priors, where existing knowledge is incorporated in the form of prior probability distributions, is an important feature of this approach. Bayesian methods also allow direct probability statements about the parameter being estimated and provide better characterisation of very low event counts in meta-analysis whereas frequentist methods perform poorly[167].

On the basis of the numerator agreed at the meeting, the absolute risk of sudden unexplained death after DHA-PPQ was estimated as follows: each included study was treated as a unit, within which each individual participant was considered to be an independent trial with success probability equal to the absolute risk of sudden unexplained death after DHA-PPQ. A complete pooling intercept-only model parameterised by the log-odds of the absolute risk was used to generate a posterior

probability distribution of the absolute risk of sudden unexplained death after DHA-PPQ, and its 95% credible intervals. I used a weakly informative prior with a normal distribution, confined to the probability scale using an inverse logit link function, centred on the estimated risk of drug-induced TdP of non-cardiovascular drugs with QT prolonging potential provided from expert opinion (1 in 1,000,000 exposures[138]) (see 2.1.5.1 & 2.1.5.2). For comparison, 95% confidence intervals using frequentist methods were also calculated.

In recognition of the young population age structures of malaria-endemic regions, and that most individuals who have received DHA-PPQ in clinical studies to date did so as part of mass drug administration programmes with participant age structures similar to that of the general population, the posterior probability distribution of the risk of sudden unexplained death after DHA-PPQ and its 95% credible interval were compared with reported global surveillance rates of sudden cardiac death in persons aged younger than 35 years (0.7-11.9 per 100,000 person-years – scaled to 30-day risks) (see 2.1.5.1)[175-177]. Sensitivity analyses of alternative priors and study inclusion criteria were also conducted (see 2.1.5.3).

3.4 RESULTS

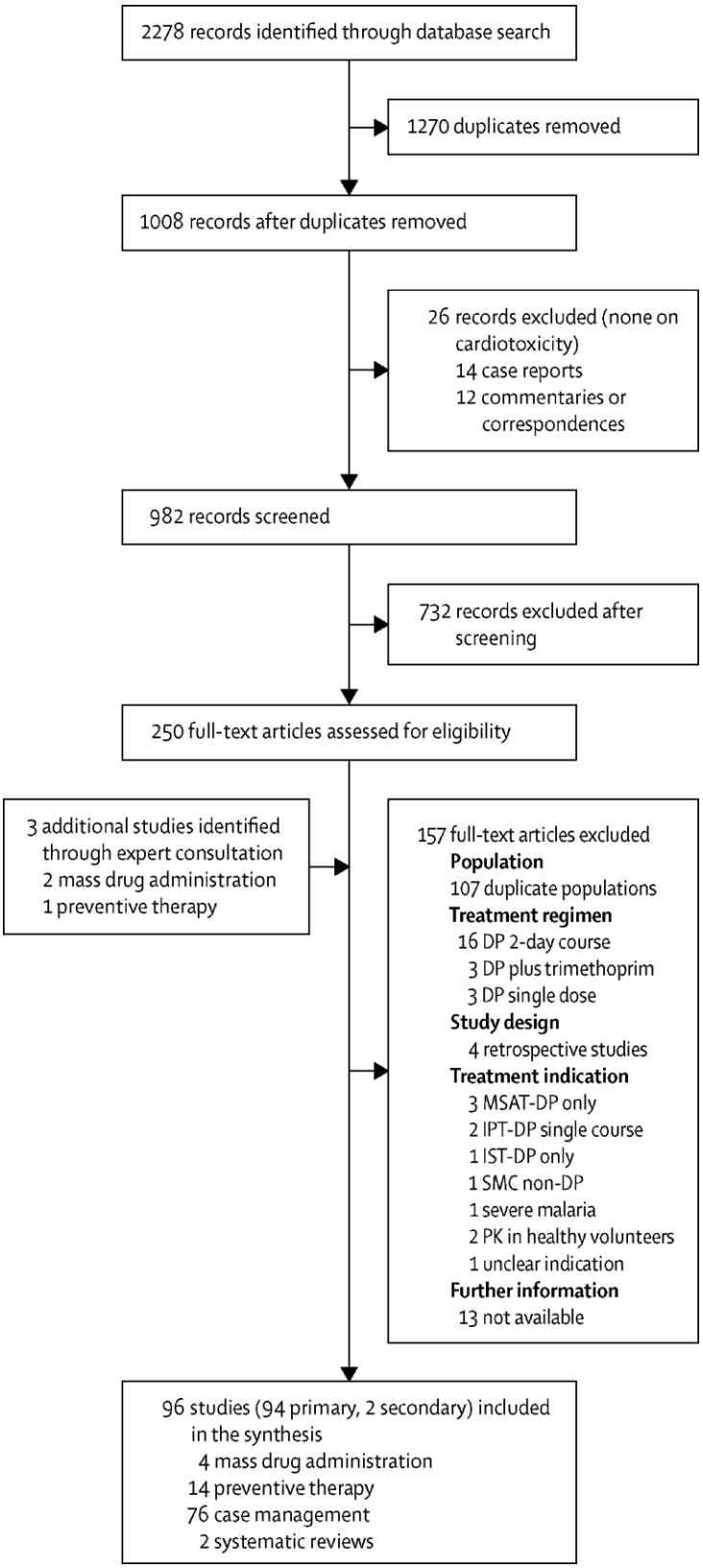
3.4.1 Studies and Exposures

3.4.1.1 Study Selection

My search identified 94 eligible primary studies including data for 197,867 individuals who had received 501,156 courses of DHA-PPQ in 94 independent studies (Figure 3.1). 154,505 received DHA-PPQ in four mass drug administration programmes, 15,188 in 14 studies of repeated courses in preventive therapies and case management of uncomplicated malaria, and 28,174 as single courses in 76 case management studies.

14 case reports and 12 commentary or editorial articles were excluded, but none of these was related to cardiotoxicity. The 13 full-text records which were excluded because further information was not available were conference abstracts from 2012 onwards of recently completed or ongoing studies for which corresponding journal articles could not be found and finalised results were not yet available from the investigators (Figure 3.1).

Figure 3.1: Selection of Studies of Dihydroartemisinin-Piperaquine



DP = dihydroartemisinin-piperaquine. MSAT = mass screening and treatment. IPT = intermittent preventive therapy. IST = intermittent screening and treatment. SMC = seasonal malaria chemoprevention. PK = pharmacokinetic.

3.4.1.2 Included Studies – Design and Demographics

A summary of characteristics of the 94 included primary studies, the number of individuals exposed to DHA-PPQ, as well as all-cause mortality figures at all time points and within 30 days from DHA-PPQ administration are provided in Table 3.1. All primary studies included in the systematic reviews of DHA-PPQ for case management of uncomplicated falciparum malaria[200] and repeated dosing for prevention and treatment of malaria[201] were included in the synthesis.

68 (72%) of the 94 studies were individually or cluster-randomised controlled trials. 59 (63%) were published after European Medicines Agency approval for Eurartesim® in 2011. Almost all studies were conducted in either the WHO African (42/94) or South East Asian and Western Pacific (47/94) regions. 173,583 (88%) of the 197,867 individuals exposed to DHA-PPQ were from the African region and 188,377 (95%) were in studies published between 2012 and 2017. 88 (99%) of the 89 studies for which age data were available, comprising 196,138 (99%) of all individuals who received DHA-PPQ, had a participant mean or median age of 35 years or younger.

A tenth of studies focused on pregnant women in the second and third trimesters: these were four studies of DHA-PPQ for intermittent preventive therapy in pregnancy[202,203,210,211] and five studies on malaria in pregnancy[212-216] in which 2,840 women received DHA-PPQ. The four mass drug administration programmes all excluded women in the first trimester of pregnancy at enrolment. Women in all trimesters of pregnancy were excluded in 81 (86%) of the 94 studies.

Table 3.1: Characteristics of Included Studies of Dihydroartemisinin-Piperaquine

	Studies	Exposed Individuals	Deaths	Deaths within 30 days
Total Number	*94*	*197867*	*61*	*31*
Indication for Study				
Mass Drug Administration	4	154505	30	11
Intermittent Preventive Therapy	14	15188	16	6
Seasonal Malaria Chemoprevention	7	12367	11	5
Intermittent Preventive Treatment in Pregnancy	4	1491	0	0
Repeated Treatment	2	562	4	1
Occupational Prophylaxis	1	768	1	0
Case Management	76	28174	15	14
P. falciparum mono-infection	57	25217	13	12
P. falciparum mixed infection	9	1864	2	2
P. vivax mono-infection	9	1063	0	0
Other (*P. falciparum*, *P.vivax*, *P. ovale*, or *P. malariae*)	1	30	0	0
Year of Publication				
2004-2011	35	9490	11	7
2012-2017	59	188377	50	24
WHO Region				
African Region	42	173583	26	16
South East Asian Region	26	9162	6	6
Eastern Mediterranean Region	3	455	0	0
Western Pacific Region	19	3281	1	0
Region of the Americas	1	252	0	0
Multiple Regions: South East Asian and West Pacific	2	10554	28	9
Multiple Regions: African, South East Asian and West Pacific	1	580	0	0
Study Type				
Randomised controlled trial	68	141890	53	23
Cohort	26	55977	8	8
Mean or Median Age Group (years)				
0 to ≤5	21	6971	15	8
>5 to ≤15	14	23505	10	8
>15 to ≤35	53	165662	35	15
>35	2	915	1	0
Not reported	4	814	0	0

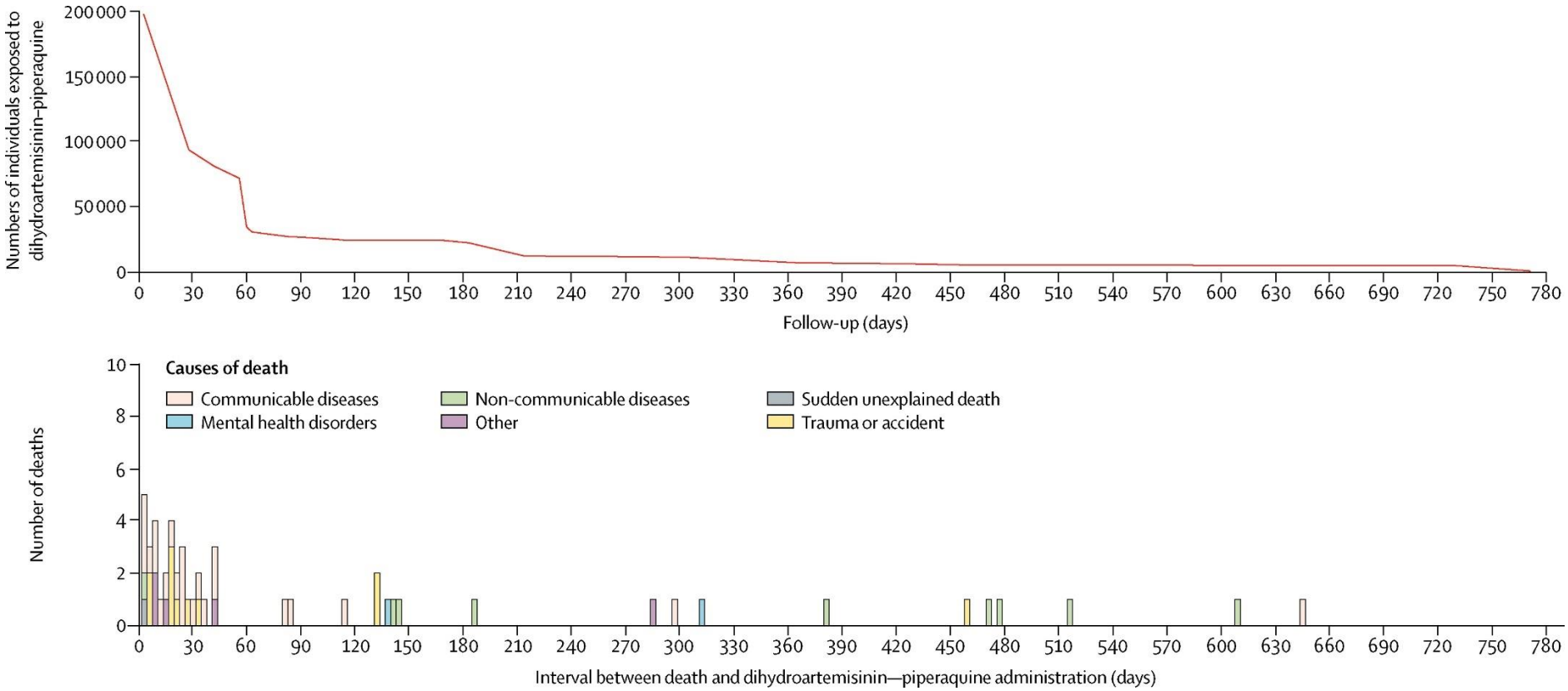
Pregnant Patients Included				
Yes - study on pregnant women in second and third trimesters of pregnancy	9	2840	0	0
Yes - study on general population excluding women in first trimester of pregnancy	4	154505	30	11
No	81	40522	31	20
Torsade de Pointes Risk Factors Excluded				
Yes	12	22547	9	8
No	82	175320	52	23
Directly Observed Therapy				
Yes - at least the first dose of medication per treatment course	89	197397	59	29
Yes - all doses of medication per treatment course	79	80519	48	20
No - drug intake documented by parents	2	145	2	2
Not reported	3	325	0	0
Length of Follow-up (days)				
3	2	104371	1	1
28	14	12537	7	7
42	39	9332	4	3
56	6	41322	1	1
63	12	3712	3	3
84	1	217	0	0
85- 181	8	4399	8	4
182-365	8	11893	4	1
>365	4	10084	33	11
Brand of Dihydroartemisinin-Piperaquine				
Duo-cotecxin®	64	24055	22	10
Eurartesim®	19	162552	11	11
D-ARTEPP®	2	602	0	0
Arterakine®	1	164	0	0
Mixture (Eurartesim®, D-ARTEPP®, or Arterakine®)	1	9785	28	9
Not reported	7	709	0	0
Use in Combination with Primaquine				
Yes - single dose gametocytocide in mass drug administration	2	13097	25	6
Yes - single dose gametocytocide in *P. falciparum* infection	6	1015	1	1
Yes - radical cure in *P. vivax* infection	6	487	0	0
No - primaquine use not documented	80	183268	35	24

3.4.1.3 Included Studies – Torsade de Pointes Risk Factors and Follow-up

Only 12 of the 94 (13%) of studies enrolling 22,547 of 197,867 (11%) of individual participants specifically excluded risk factors for TdP[217-226]. Almost all studies had directly observed therapy of DHA-PPQ for at least the first dose of each treatment course.

Apart from one large mass drug administration programme of 103,963 individuals[208] and one cohort study of malaria in pregnancy[213] with active follow-up over three days, all 92 other studies, which enrolled 93,496 (47%) of 197,867 individuals who received DHA-PPQ had follow-up for at least 28 days, covering the duration of one terminal elimination half-life of piperaquine (Figure 3.2).

Figure 3.2: Number of Individuals Exposed to and Deaths after Dihydroartemisinin-Piperaquine



3.4.1.4 Included Studies – Dosing Regimen

Information about food intake was available from only three studies, in which a small amount of fat (6.4 or 8.5mg) was co-administered with DHA-PPQ in at least one arm; this was reported in all three not to affect piperaquine exposure. These were two studies of DHA-PPQ in case management of uncomplicated falciparum malaria[116,117] and one study of DHA-PPQ for occupational prophylaxis[227].

Although 64 (68%) of the 94 studies utilised Duo-cotecxin®, Eurartesim® was administered to 167,384 (85%) of 197,867 individuals who were part of 20 studies including all four mass drug administration programmes of which one used a mixture of brands. Other studies for which information about brand of DHA-PPQ was available used D-ARTEPP® and Arterakine®. All four of these formulations contain either 320mg or 160mg of piperaquine phosphate per tablet.

DHA-PPQ was used in combination with primaquine in 14 studies: in two studies in Southeast Asia as a single dose gametocytocide in mass drug administration[205], in six studies in Southeast Asia as a single dose gametocytocide in case management of uncomplicated falciparum malaria[219,223,228-231], and in six studies in Africa and Asia as radical cure in vivax malaria[220,224,232-235].

3.4.2 Deaths

31 deaths occurred within 30 days of DHA-PPQ administration, and a further 30 deaths occurred beyond one terminal elimination half-life of piperaquine (Figure 3.2). Information on the number of deaths and all DHA-PPQ exposures extracted are summarised by treatment indication in Table 3.2.

Table 3.2: Exposures to and Deaths after Dihydroartemisinin-Piperaquine

Study Type	Individuals	Courses	Doses	Deaths	Deaths Within 30 Days	Sudden Unexplained Deaths
Mass Drug Administration	154,505	432,001*	1,189,029	30	11	1
Preventive Therapies & Case Management Repeated Courses	15,188	40,981*	122,943*	16	6	0
Case Management Single Courses	28,174	28,174*	84,522*	15	14	0
Total	**197,867**	**501,156**	**1,396,494**	**61**	**31**	**1**

*Derived from another denominator.

3.4.2.1 Sudden Unexplained Death

Only one of these deaths was thought to be consistent with sudden cardiac death and considered possibly causally related to drug exposure by cardiology experts at the WHO Evidence Review Group meeting[47]. This was the sudden death of an otherwise healthy 16-year-old female in Mozambique with no past medical or other medication history, who collapsed and died on the way to hospital after stating she had palpitations several hours after the second dose of her first course of DHA-PPQ in a mass drug administration programme. According to her stepmother, the girl had self-administered her second dose of DHA-PPQ about 20 minutes after eating rice, cooked salad, and bread. Both her malaria rapid diagnostic test and pregnancy test performed at enrolment the day before were negative. No autopsy or ECG was performed for this individual.

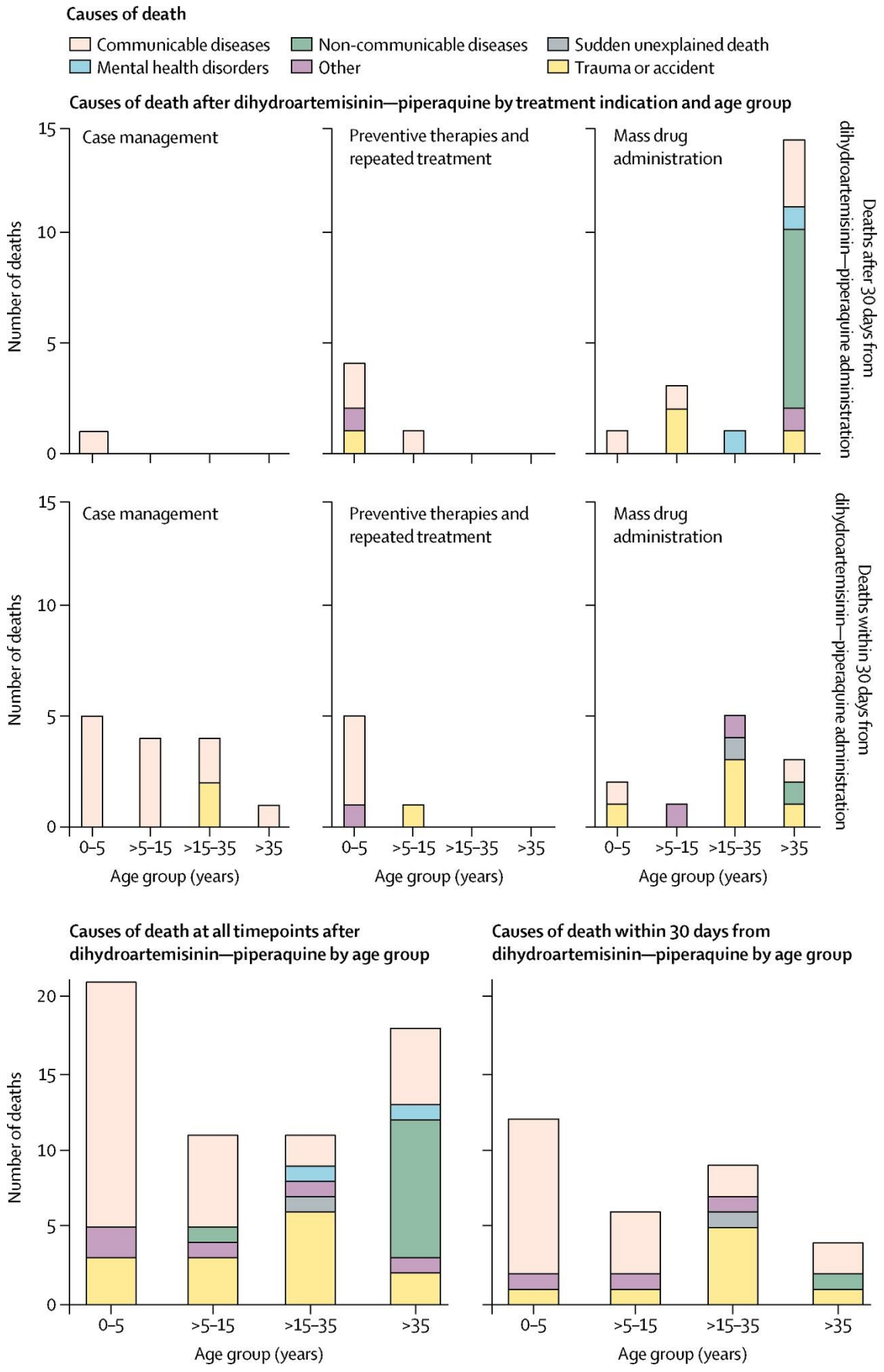
3.4.2.2 Other Deaths with Known Causes

All other deaths had alternative explanations (Figure 3.3). In keeping with the main causes of death in low- and middle-income countries, communicable diseases such as malaria, sepsis, pneumonia, and diarrhoea accounted for the majority of deaths overall, as well as in children aged 0-5 and 5-15 years. 13 (87%) of the 15 deaths in the case management group were attributed to infections.

Trauma or accidents were the next most frequent causes of death and were the dominant causes among young adults aged 15-35 years. Unexplained drownings and sudden deaths of drivers of vehicles could result from arrhythmias. Three deaths resulted from road traffic accidents: in two cases the individuals were identified to be passengers or pedestrians, while the third death occurred 459 days after treatment, far beyond the terminal elimination half-life of piperaquine. Similarly, four of the five drownings occurred towards the end or beyond one termination elimination half-life of piperaquine. The one case of drowning within a week of DHA-PPQ administration was of a 32-year-old man with both a personal and family history of poorly controlled epilepsy, who drowned while washing in the river after the first dose of his second course of DHA-PPQ with primaquine in a mass drug administration programme. He had not received the full course of DHA-PPQ as his wife had witnessed him drinking a large quantity of alcohol. His death was considered unrelated to DHA-PPQ.

Non-communicable diseases were the most common causes of death more than 30 days after DHA-PPQ administration in adults older than 35 years.

Figure 3.3: Causes of Death after Dihydroartemisinin-Piperaquine by Age Group and Treatment Indication



There were no deaths after DHA-PPQ among the 2,840 women who received it as part of studies of the antimalarial in pregnancy, nor among the 1,063 participants who were treated with DHA-PPQ and primaquine for *P. vivax* mono-infections.

Other than the death identified by the Evidence Review Group as possibly causally related to DHA-PPQ, the only other death reported by investigators as being probably causally related to the drug was of a 2-year-old boy who choked on his first dose of DHA-PPQ in mass drug administration and whose cause of death was confirmed on autopsy. His death was attributed directly to upper airway obstruction, and not to cardiac causes.

3.4.2.3 Reported Characteristics of Deaths and Safety Monitoring

Characteristics expected to be present for all deceased individuals were well-reported (Table 3.3). Data Safety Monitoring Board oversight was confirmed in all except six studies of which three had a Safety Monitor.

Table 3.3: Available Characteristics of Individual Deaths after Dihydroartemisinin-Piperaquine

Characteristics of All Deaths	**Deaths at any Timepoint (out of 61)**	**Deaths within 30 Days (out of 31)**
Age	60	30
Sex	59	30
Time of death relative to dosing	56	31
Assessment of relationship to drug	50	23
Data Safety Monitoring Board oversight (+ Safety Monitor only)	50 (+ 4)	25 (+ 3)
Characteristics of Specific Deaths only	**Deaths at any Timepoint (out of 61)**	**Deaths within 30 Days (out of 31)**
Comorbidities	9	6
Comedications (including alcohol)	2	2
History of fits, syncope, or cardiac arrest (personal and/or family)	1	1
Source of Additional Information	**Deaths at any Timepoint (out of 61)**	**Deaths within 30 Days (out of 31)**
Investigator contact	60	30
Serious adverse event report	16	8
Autopsy report	1	1

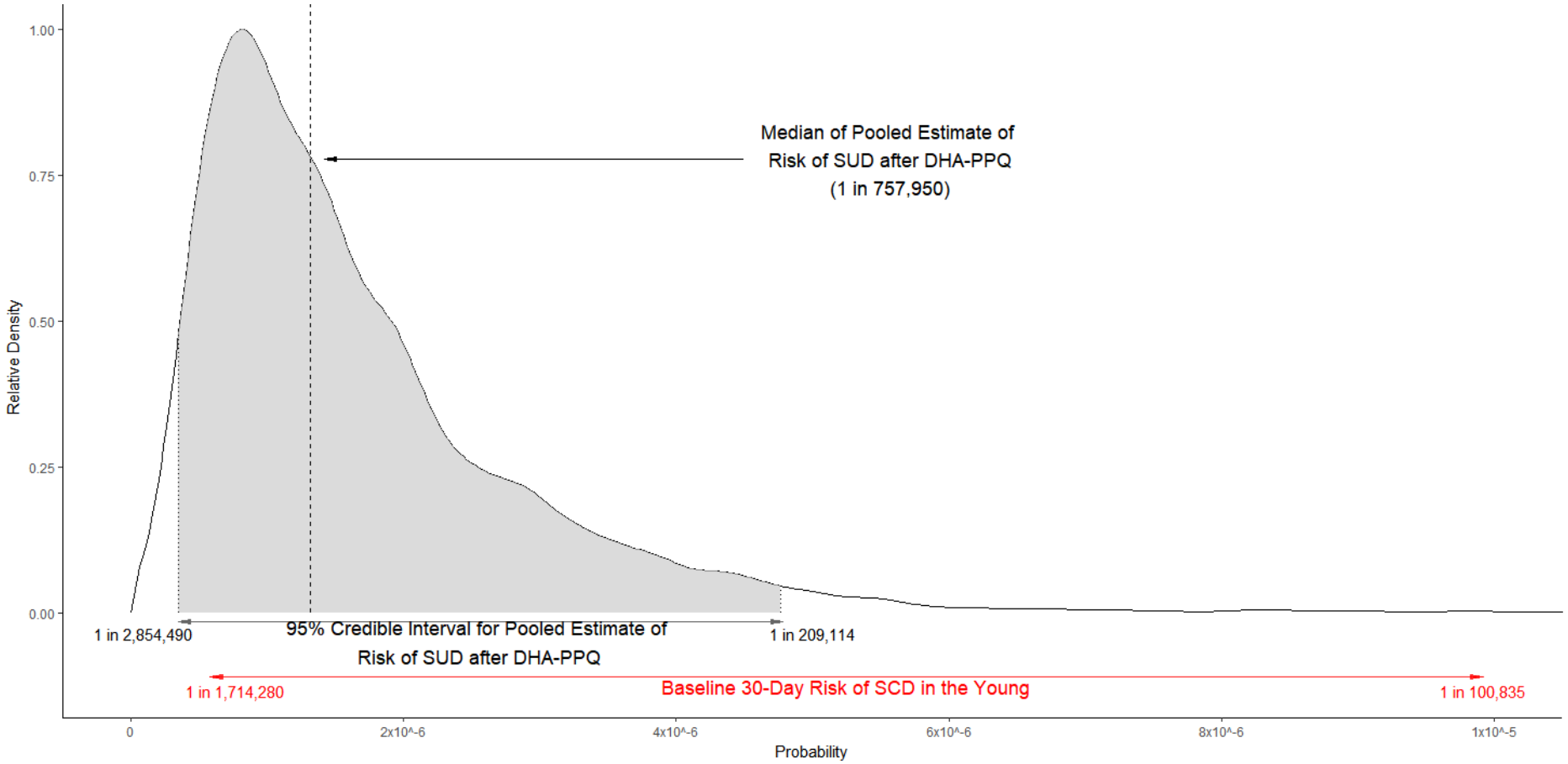
3.4.3 Risk of Bias Assessment

In keeping with death being a well-defined outcome with established reporting mechanisms[236] occurring at a low rate, risk of bias was low in most studies and unclear in a small minority (Appendix 11.1.1 – Table 11.1).

3.4.4 Aggregate Data Meta-analysis

I included all 94 primary studies in the meta-analysis of the risk of sudden unexplained death after DHA-PPQ. The mass of the posterior probability distribution function of the risk of sudden unexplained death after DHA-PPQ was concentrated very close to zero with a median pooled risk estimate of 1 in 757,950 (95% credible interval: 1 in 2,854,490 to 1 in 209,114). Almost all of this posterior probability distribution lay within or below the reference range of the baseline 30-day risk of sudden cardiac death in the young (1 in 1,714,280 to 1 in 100,835); the estimated risk of sudden unexplained death after DHA-PPQ was no higher than baseline (Figure 3.4).

Figure 3.4: Posterior Probability Distribution of the Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine Compared with the Baseline 30-Day Risk of Sudden Cardiac Death in the Young

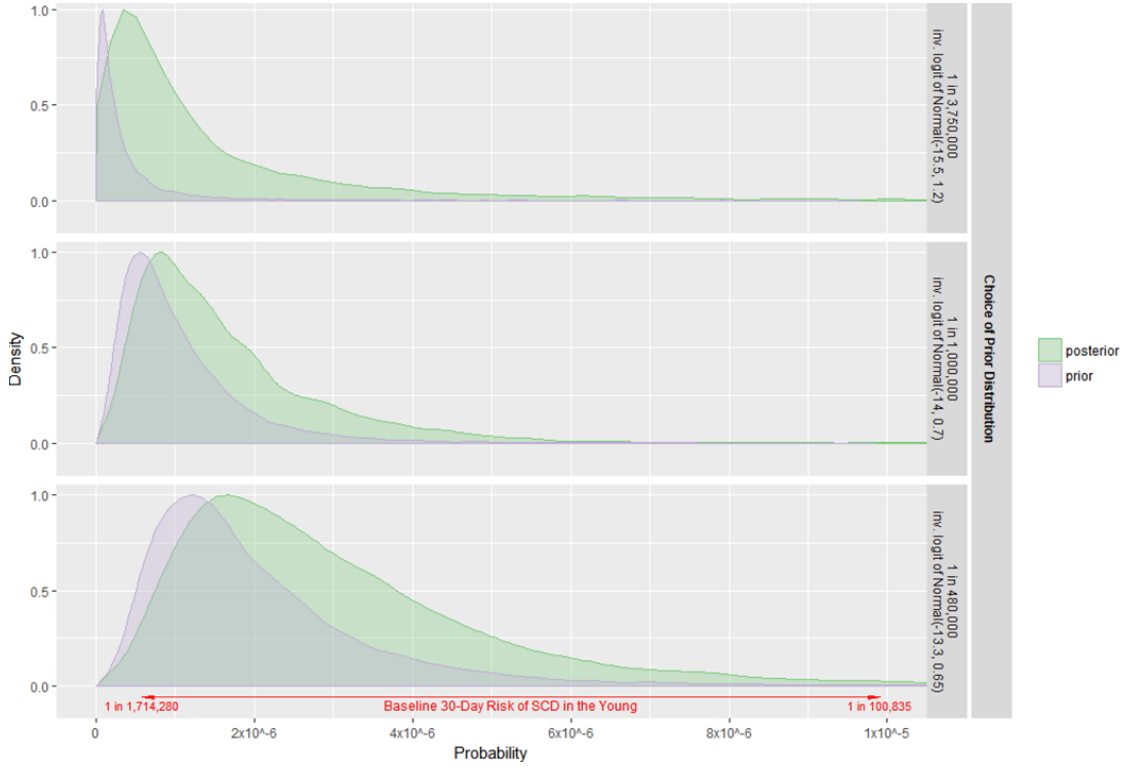


DHA-PPQ = dihydroartemisinin-piperaquine, SUD = sudden unexplained death, SCD = sudden cardiac death.

3.4.4.1 Sensitivity Analysis – Priors

I conducted sensitivity analyses using weakly informative alternative priors centred on probabilities about half an order of magnitude higher and lower than the prior used for the main model (Figure 3.5) in keeping with the range of observed risks of drug-related repolarisation-related cardiotoxicity in the literature (see 2.1.5.1).

Figure 3.5: Posterior Probability Distributions of the Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine with Alternative Prior Probability Distributions

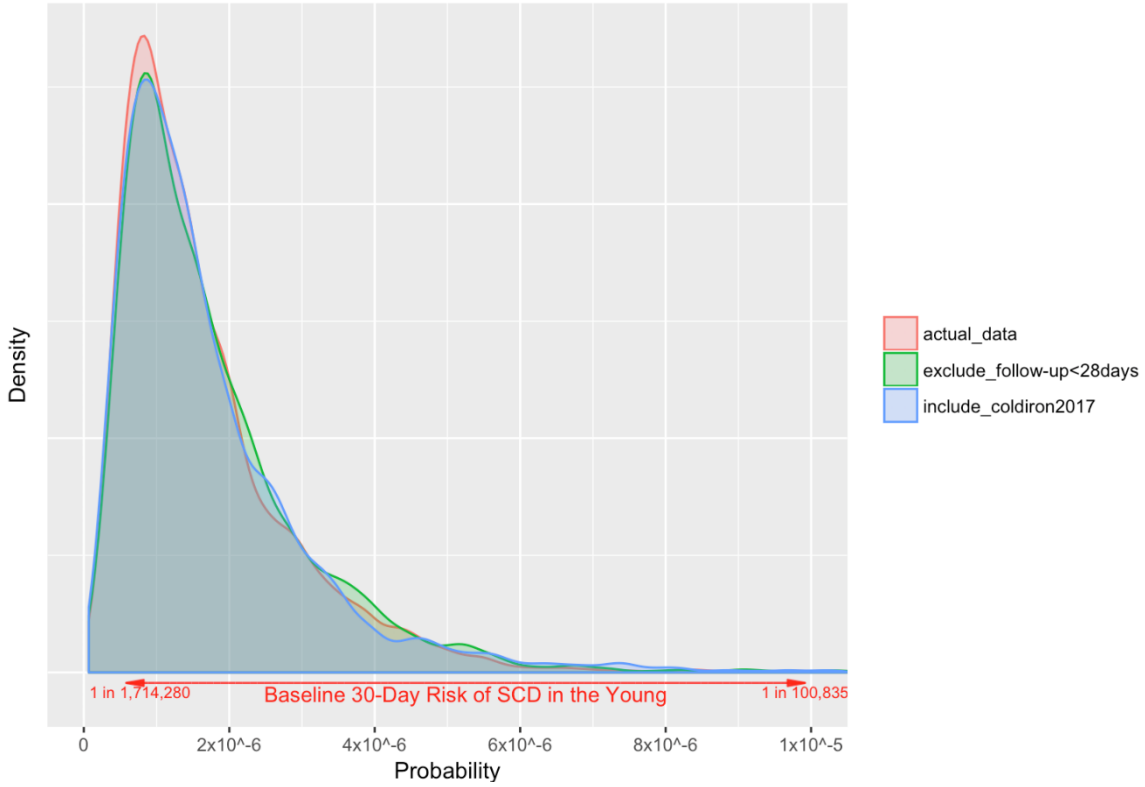


While the posterior probability distributions shifted slightly with the use of each different prior, all of these distributions were concentrated very close to zero and remained within or below the boundaries of the reference range of baseline 30-day risk of sudden cardiac death in the young. The conclusion that the risk of sudden unexplained death after DHA-PPQ was extremely low and not higher than the baseline rate of sudden cardiac death in the young appeared to be robust for this range of priors (Figure 3.5).

3.4.4.2 Sensitivity Analysis – Quality of Follow-up

I also performed sensitivity analyses to consider the effects of adding or removing studies with different levels of follow-up (Figure 3.6). I excluded studies which had under 28 days of follow-up: these were two studies with follow-up time of 3 days (n = 104,371). Separately, I added data from a large intermittent preventive therapy study (n = 40,166) published in 2017 by Coldiron and colleagues[237] which did not have active follow-up but had reinforced passive surveillance for adverse events in the contained setting of a refugee camp.

Figure 3.6: Posterior Probability Distributions of the Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine with Addition and Removal of Studies with Different Quality of Follow-up



The posterior probability distributions did not change noticeably with the addition of the Coldiron 2017 study or the exclusion of studies with follow-up of less than 28 days. The conclusion that the risk of sudden unexplained death after DHA-PPQ was extremely low and not higher than the baseline rate of sudden cardiac death in the young appeared to be robust to these changes to included studies.

3.4.4.3 Frequentist Confidence Intervals

Frequentist 95% confidence intervals were computed using the recommended asymptotic and Agresti-Coull methods for large sample sizes[238] (Table 3.4).

Table 3.4: Frequentist 95% Confidence Intervals for the Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine

Method	x	n	Mean	Lower bound of 95% confidence interval	Upper bound of 95% confidence interval
Asymptotic	1	197,867	0.000005053900	-0.00000485153680	0.00001495934
Agresti-Coull	1	197,867	0.000005053900	-0.00000216733337	0.00003168891

These were found to be unsatisfactory for estimating a proportion between zero and one as they had negative lower bounds allocating belief to impossible values below zero.

3.5 DISCUSSION

This study represents the most comprehensive attempt to consider the entirety of clinical evidence pertaining to risk of death after use of DHA-PPQ in the standard 3-day treatment course to prevent or cure malaria. This analysis included clinical studies for which there was confirmed follow-up over the three days from drug initiation, i.e. the window spanning the highest risk of drug-induced repolarisation-related cardiotoxicity when piperaquine drug concentrations are at their peak. It adds to previous reviews of case management[200] and intermittent preventive therapy[201] with DHA-PPQ by including data from cohort studies and large-scale pilot programmes of mass drug administration as well as incorporating risk estimates of drug-induced repolarisation-related cardiotoxicity from surveillance studies and experience with other non-cardiovascular drugs with QT prolonging potential. However, a meaningful comparison with another antimalarial drug was not possible because of the low quality of safety data reported in mass drug administration studies of other antimalarials[239], which reflects passive reporting from

routine pharmacovigilance systems. Without national or international prescription databases in malaria-endemic regions, it may be difficult to obtain more robust estimates.

This study showed that sudden unexplained death potentially caused by repolarisation-related tachyarrhythmia after treatment with DHA-PPQ is very rare. Only one possible sudden cardiac death associated with DHA-PPQ was reported among nearly 200,000 individuals with directly observed treatment and close follow-up, despite 87% of studies – including all four mass drug administration programmes – comprising 89% of individuals not specifically excluding TdP risk factors. Synthesising these findings with prior information from expert advice through Bayesian meta-analysis suggests this risk is even lower. In addition, since this search was concluded, DHA-PPQ has also been reported to have been safely administered to more than 56,000 further individual participants: 40,611 children as seasonal malaria chemoprevention in a refugee camp with reinforced surveillance in Uganda[237], 14,271 participants in two mass drug administration programmes in The Gambia[240] and Tanzania[241], as well as 1,731 individuals in repeated treatments for case management of uncomplicated *P. falciparum* malaria[122] and intermittent preventive therapy in pregnancy[242].

While sudden cardiac death may be precipitated by TdP, life-threatening tachyarrhythmia leading to sudden cardiac death is the final common pathway of a wide range of cardiac conditions. Furthermore, sudden unexplained death can result from non-cardiac causes. These are all generally impossible to exclude with certainty, particularly in tropical settings where resources for post-mortem examinations and genetic testing are scarce. For this meta-analysis, nearly all of the included studies had a participant mean or median age of 35 years or younger. Although information from tropical areas is limited, the overall incidence rate of sudden cardiac death, i.e. sudden death from any cardiac cause, is estimated to be less than 1 to 11.9 per 100,000 person-years in the population younger than

35 years[175-177]. The incidence of sudden cardiac death in the young is one to two orders of magnitude lower than that in older adults[175] and that in infants younger than 1 year (which is around 96 per 100,000 person-years when deaths attributed to the sudden infant death syndrome are included[243]). There is insufficient clinical or statistical evidence to suggest that this one case of sudden unexplained death identified represents a higher than expected rate of sudden cardiac death in this study population.

The main limitation of this study was the potential for underreporting of deaths such as those undetected after loss to follow-up. However, these figures are unlikely to be a substantial underestimate as the data are from well-conducted studies and deaths are serious adverse events which study investigators are obligated to report under Good Clinical Practice guidelines[236]. Furthermore, sudden deaths are notable events in the communities within which these studies were conducted, and have the potential to undermine confidence in the malaria treatment programme if they do occur. There was no evidence of such concerns, including from the four large-scale mass drug administration programmes. A previous meta-analysis[201] has also demonstrated that repeated dosing of DHA-PPQ is not associated with an increased risk of loss to follow-up relative to comparator antimalarials.

No further sudden unexplained deaths, sudden cardiac deaths, or cases of TdP were identified from searches of the WHO and drug manufacturer pharmacovigilance databases despite distribution of millions of doses of DHA-PPQ[47] (at least 2.8 million doses of Eurartesim® and 5.4 million doses of generic formulations). Given the considerable attention given to this potential adverse drug reaction since the drug was registered, the absence of further deaths potentially attributable to cardiotoxicity despite widespread use is reassuring for prescribers and malaria control programmes. This safety record contrasts starkly with the history of the structurally related antimalarial

halofantrine, which has been associated with more than 30 sudden cardiac deaths and documented TdP with progression to life-threatening ventricular tachyarrhythmias[47,69].

In view of the low rate of sudden unexplained deaths, it was not possible to determine from this study whether there was a difference in the risk of repolarisation-related cardiotoxicity between patients with uncomplicated malaria and healthy participants in mass drug administration or intermittent preventive therapy programmes. Nor was there sufficient information to assess this risk in vulnerable subgroups such as pregnant women and infants, or to assess any differential risk following co-administration of DHA-PPQ with fat. A large amount of co-administered fat has been found to increase piperaquine concentrations significantly[114,115] while a small amount of fat in normal meals does not[115-117]. This study did not suggest there should be any change to recommendations to avoid high-fat meals with DHA-PPQ to minimise the risk of repolarisation-related cardiotoxicity[30].

DHA-PPQ in a 3-day treatment course was associated with a very low risk of sudden unexplained death, which was not higher than the baseline rate for sudden cardiac death in the population younger than 35 years. While ongoing monitoring for adverse drug effects should continue to be strengthened, such as through Health and Demographic Surveillance System record linkage, cohort event monitoring, and targeted spontaneous reporting[244], concerns about the repolarisation-related cardiac safety need not limit the use of DHA-PPQ for the treatment and prevention of malaria.

3.6 AUTHORSHIP STATEMENT

I designed this study with Professor Nicholas White, conducted the systematic review, carried out the data extraction and additional data gathering from individual study investigators, performed the meta-analysis, made all the figures and tables, and wrote all the text. I published the paper[209] this chapter is based on with input from Professor Nicholas White and Professor Josep Brugada of the Hospital Clinic of Barcelona who were the co-chairs of the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials. Nia Roberts of the Bodleian Libraries assisted me with the literature search. Dr Yan Naung Win and Dr Laura Mawer were the additional independent reviewers in the systematic review and data extraction. Jireh Tan provided support with statistical programming and methodology.

4 Arrhythmia after the Quinoline and Structurally Related Antimalarials

4.1 SUMMARY

Background

Several of the quinoline and structurally related antimalarial medicines are associated with cardiovascular adverse effects, particularly hypotension and electrocardiographic (ECG) QT interval prolongation. A prolonged QT interval is a sensitive but not specific risk marker for the development of torsade de pointes, a potentially lethal polymorphic ventricular tachyarrhythmia. The increasing use of QT-prolonging quinoline and structurally related antimalarials in mass treatments to eliminate malaria rapidly highlights the need to review their arrhythmogenic potential.

Methods

The primary objective of this systematic review was to describe the documented clinical and electrocardiographic cardiovascular side effects of the quinoline and structurally related antimalarials amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, and primaquine. Sulfadoxine-pyrimethamine was included as a non-structurally related comparator. Prospective studies in healthy participants or patients with *P. falciparum* or *P. vivax* infection were included if electrocardiograms were conducted before and after drug administration. Secondary outcomes were the methods adopted by trials for measuring and reporting the QT interval.

Findings

A total of 177 trials met the inclusion criteria. 35,448 participants received quinoline antimalarials in these trials, of which 18,436 participants underwent ECG evaluation. Participants with co-medication use or comorbidities including cardiovascular disease were excluded from the majority of trials. Dihydroartemisinin-piperaquine was the drug

most studied (5,083 participants). Despite enormous use over the past 60 years, only 1,076, 452, and 150 patients had ECG recordings reported in studies of chloroquine, amodiaquine, and primaquine respectively. Transiently high concentrations of quinine, quinidine, and chloroquine following parenteral administration have all been associated with hypotension, but there were no documented reports of death or syncope attributable to a cardiovascular cause, nor of electrocardiographic recordings of ventricular arrhythmia in these studies. The large volume of missing outcome information along with the heterogeneity of ECG interval reporting and measurement methodology prevented pooled quantitative analyses of QT interval changes.

Interpretation

No serious cardiovascular adverse effects were recorded in malaria clinical studies of 35,548 participants who received quinoline and structurally related antimalarials with close follow-up including 18,436 individuals who underwent ECG evaluation. While these findings provide further evidence of the rarity of serious cardiovascular events after treatment with these drugs, they also underscore the need for continued strengthening of pharmacovigilance systems for robust detection of rare drug adverse events in real-world populations.

4.2 INTRODUCTION

The quinoline and structurally related antimalarials have long been known to cause cardiovascular side effects. Many of these antimalarial drugs cause hypotension, partly through alpha blockade, and they affect both depolarisation and repolarisation of cardiac and skeletal muscle[48]. The dangers of rapid intravenous injection of quinine[61], the marked prolongation of the electrocardiographic (ECG) QT interval caused by quinidine[245-247], and the lethality of chloroquine in overdose[47] have each caused considerable concern over their use in the treatment of malaria. The discovery in 1993, after its registration, that halofantrine was associated with marked QT prolongation and sudden death augmented these concerns[147]. More recently, there has been uncertainty over the potential risks associated with the QT prolongation following use of piperaquine and the fixed-dose combination dihydroartemisinin-piperaquine, the latest addition to the artemisinin-based combination therapies (ACTs) recommended for treatment of malaria by the World Health Organization[30]. Consideration of this well-tolerated antimalarial drug for use in population-based treatments such as intermittent preventive therapy and mass drug administration as part of malaria control interventions underscores the urgent need to clarify the cardiovascular safety profile of piperaquine and structurally related antimalarials[201].

Characterising the electrophysiological effects of a drug, particularly during the treatment of acute illness, is not straightforward. It is not a problem unique to antimalarials and is a major consideration during drug development[136]. A prolonged QT interval, reflecting a delay in ventricular repolarisation, is a sensitive but not specific risk marker for ventricular tachyarrhythmia development, notably torsade de pointes (TdP). This rhythm if sustained can degenerate into ventricular fibrillation and result in sudden cardiac death. The relationship between QT prolongation and the risk of developing ventricular

tachyarrhythmias is incompletely understood, although many factors are known to contribute. These include the presence of underlying genetically determined QT prolongation, electrolyte abnormalities, structural heart disease, female sex, and co-administration with other drugs which also prolong the QT interval or increase drug levels[138]. Without detailed investigation, it is therefore difficult to assess an individual's risk of a drug precipitating life-threatening ventricular tachyarrhythmias.

To clarify the cardiovascular safety profile of piperaquine and structurally related antimalarials, I assessed, by systematic review of published clinical trials, the frequency of reported clinical and electrocardiographic cardiovascular adverse effects after use of quinoline and structurally related antimalarials for malaria-related indications[248].

4.3 METHODS

4.3.1 Search Strategy and Selection Criteria

A systematic literature search was performed on 22 October 2015 of the databases MEDLINE, EMBASE and Global Health, for primary clinical studies of the quinoline and structurally related antimalarials for malaria-related indications in which electrocardiograms were recorded before and after drug administration (see 2.2.2.1). Unpublished studies were not included.

Studies were eligible for inclusion in the review if they were prospective randomised controlled trials or cohort studies which administered amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, quinine, or sulfadoxine-pyrimethamine to healthy participants or patients with *P. falciparum* or *P. vivax* mono- or mixed infections. All ages and populations, including children and pregnant women, were included.

4.3.2 Data Extraction

I screened titles, abstracts, and full texts, and agreed study eligibility with another independent reviewer. From study publications, I extracted variables including study year and location, study design, participant population, antimalarial drug(s) assessed, ECG measurement methodology and time points, drug concentration measurement methodology, as well as clinical and electrocardiographic cardiovascular adverse events (see 2.2.3). I compared patient series with those of other included articles to minimise data duplication.

Primary outcomes were the number and character of clinical cardiovascular adverse events (sudden cardiac death, syncope, or palpitations) and ECG-documented arrhythmias (including ventricular tachycardia, ventricular fibrillation, and TdP). Secondary outcomes were the features of ECG methodology and QT interval analysis adopted by each trial.

Risk of bias assessments were conducted using standardised criteria (see 2.2.4).

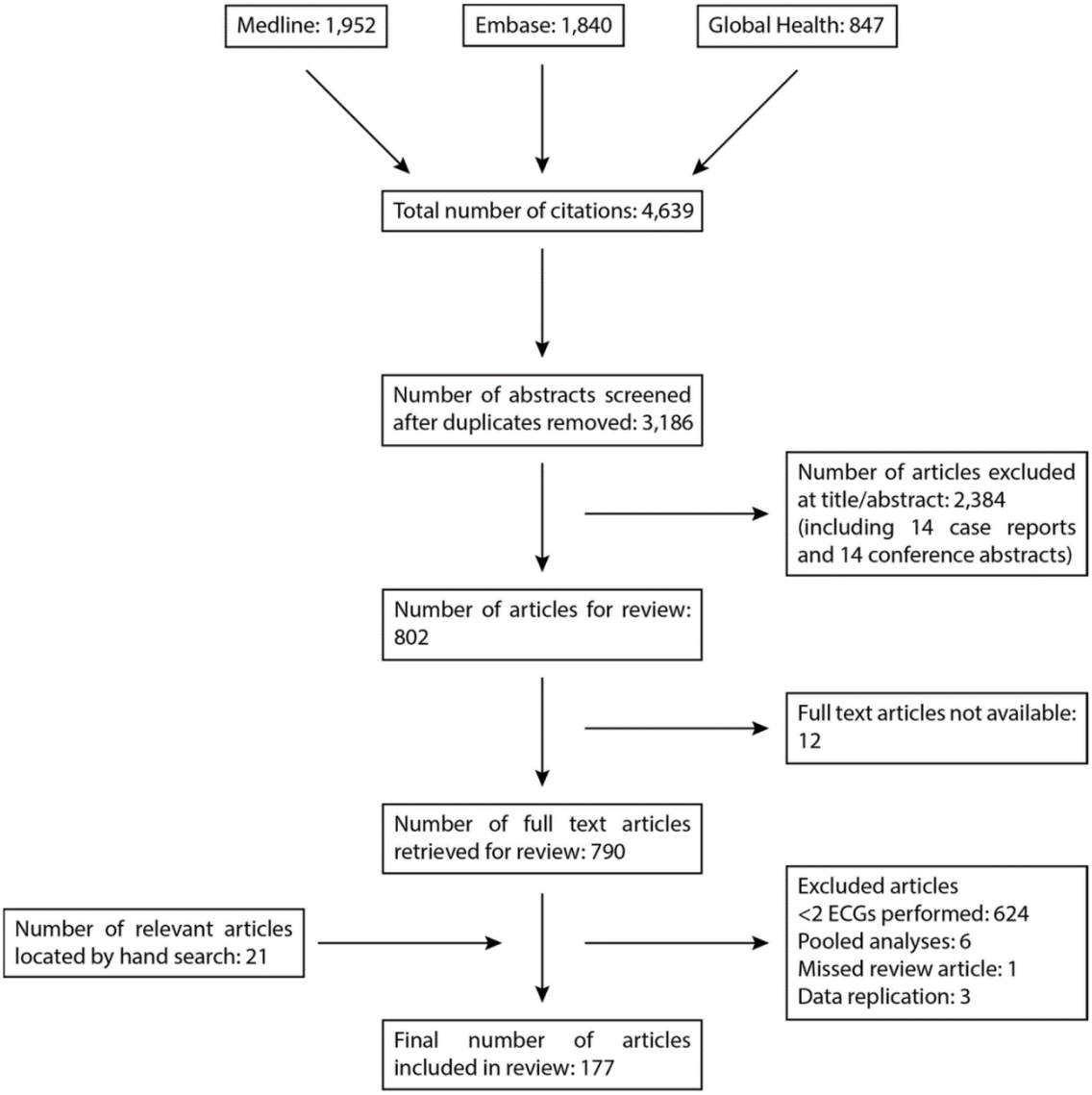
4.4 RESULTS

4.4.1 Studies

4.4.1.1 Study Selection

In total, 177 articles (Figure 4.1) enrolling a total of 39,960 participants were included in this review. Of these participants, 35,448 received at least one of the antimalarial drugs of interest and 18,436 underwent ECG evaluation (Table 4.1).

Figure 4.1: Selection of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring



4.4.1.2 Included Studies – Antimalarial Drug

Quinine, mefloquine, and lumefantrine were the most intensively studied drugs by number of published studies (51, 45, and 39 studies respectively), while piperaquine and lumefantrine had the largest numbers of participants undergoing ECG investigation (5,083 and 4,787 participants respectively) (Table 4.1). Despite vast use of chloroquine over the last six decades with hundreds of tonnes consumed annually, only 1076 participants in 17 studies of chloroquine underwent ECG investigation. Amodiaquine and primaquine have been the least intensively investigated (10 and 7 studies respectively) with 452 and 150 participants respectively undergoing ECG investigation. 59 studies included children, most of which were trials of quinine, mefloquine, or lumefantrine. Seven trials enrolled pregnant women (1,232 participants) (Table 4.1).

Table 4.1: Total Number of Studies and Participants with Electrocardiographic Monitoring by Antimalarial Drug

Drug	Total Number of Studies	Total Number of Participants who had Drug	Total Number of Participants who had ECGs	Total Number of Studies including Children	Total Number of Studies including Pregnant Women	Total Number of Studies including Pharmacokinetic Analysis
Quinine	51	2611	2320	18	1	19
Mefloquine	45	7874	3099	13	2	19
Lumefantrine	39	5703	4787	19	3	19
Piperaquine	24	15,224	5083	7	1	12
Halofantrine	22	822	774	7	0	10
Chloroquine	17	1207	1076	6	0	10
Sulfadoxine-pyrimethamine	14	1288	695	4	2	5
Amodiaquine	10	569	452	2	1	8
Primaquine	7	150	150	0	0	4

49 studies evaluated more than one of the drugs included in this review. These articles have been included under each of the appropriate drugs for analysis

4.4.1.3 Included Studies – Antimalarial Treatment Indication

127 (72%) of the 177 studies enrolled participants with *P. falciparum* mono- or mixed infection. 16 trials specifically included patients with *P. vivax* infection, and only four of these investigated participants with *P. vivax* mono-infections. The four *P. vivax* studies trialled chloroquine[249-251] and halofantrine[252]. 106 (60%) of the 177 trials were of patients with uncomplicated malaria, while 27 (15%) studied severe or complicated malaria. Healthy participants were the patient population in 47 (27%) of included studies – these trials were pharmacokinetic or intermittent preventative therapy trials.

4.4.1.4 Included Studies – Torsade de Pointes Risk Factors

Participants with at least one medical comorbidity were excluded from 103 (58%) of the 177 studies. It was not specifically mentioned whether medical comorbidities were excluded in the remaining 74 studies. Cardiovascular comorbidities were stated to have been excluded from 52 (29%) of the 177 studies, with only three trials specifically detailing that cardiovascular comorbidities were not excluded. Similarly, in 112 (63%) of the 177 studies, participants who had co-medications (most commonly other antimalarials and other drugs which interfere with the cardiovascular system) were excluded and only one trial specifically did not exclude participants with co-medications. 79 (45%) of the 177 trials excluded participants with both comorbidities and co-medication use, and there were no trials which included both groups of participants. 34 studies (19%) did not detail any exclusion criteria.

4.4.2 Cardiovascular Adverse Events

4.4.2.1 Deaths and Life-threatening Arrhythmias

There were no reports of death attributable to a cardiovascular cause in any trial included in this review. There were no electrocardiographic recordings of ventricular tachycardia, ventricular fibrillation, or TdP.

4.4.2.2 Other Arrhythmias

Other ECG abnormalities were described, the most common of these being bradycardia and first-degree atrioventricular block.

Bradycardia was reported in 49 (10.8%) of 452 participants after amodiaquine[253,254], 121 (3.9%) of 3,099 participants after mefloquine[147,255-259], and 11 (1.4%) of 774 after halofantrine[147,260]. Halofantrine was also associated with atrioventricular block: two children had second-degree Mobitz type 1 (Wenkebach) block after treatment for falciparum malaria[147,261], and 25 (3.2%) of 774 individuals had first-degree block[147,260,262]. Following quinine[263,264] (with azithromycin), amodiaquine[265], and mefloquine[257,259] (with sulfadoxine-pyrimethamine), 57 (2.5%) of 2,320 participants, 2 (0.4%) of 452 participants, and 7 (0.2%) of 3,099 participants respectively developed first-degree heart block.

Other ECG abnormalities were described in patients undergoing treatment for cerebral malaria although investigators attributed these to severity of malaria illness rather than drug treatment[256,266,267]. A 20-year-old male developed Wolff-Parkinson-White syndrome following piperaquine which was not detected on baseline ECGs[268]. The significance of this apparent revelation of an accessory conduction pathway is uncertain.

4.4.3 QT Interval Assessment Methodology

The heterogeneity of ECG measurement, analysis, and reporting methodology, along with the large volume of missing data, did not allow pooled analysis of antimalarial drug effects on the QT interval. Key examples of this heterogeneity were: measurement of ECGs at different time points across studies, inconsistency in the use of absolute values or changes from baseline to define QT interval prolongation and the thresholds chosen, as well as presentation of different summary statistics (means or proportions) which could not be pooled. Others are detailed below. Many studies, despite performing ECGs as part of the trial, either did not report ECG findings or only stated that none were clinically relevant.

4.4.3.1 ECG Measurement Time Points in Relation to Drug Concentrations

87 (49%) of the 177 studies measured antimalarial drug concentrations, with quinine, mefloquine, and lumefantrine having had the largest number of pharmacokinetic evaluations (19 trials each). 74 (41%) of the studies specified food intake of participants around the time of drug dosing. Most trials did not specify whether ECGs recorded after the start of antimalarial treatment were taken before (likely to be trough level of drug) or after dosing (higher drug levels which could include peak concentrations).

4.4.3.2 Heart Rate Correction

The majority of trials did not specify which heart correction formula was used, although every trial which reported QT intervals presented corrected QT values. 15 trials used two or more correction formulae. 48 (27%) of the 177 trials used the Bazett correction alone. QT intervals corrected with the Bazett and Fridericia formulae were reported in 12 studies.

4.4.4 Risk of Bias Assessment

Risk of bias assessment highlighted how many studies were limited by incomplete reporting of cardiovascular outcomes and ECG methodology (Appendix 11.2.1 – Figure 11.1).

4.5 DISCUSSION

I assessed, by systematic review of available published prospective studies, the incidence and severity of clinical and electrocardiographic cardiovascular adverse effects of antimalarial drugs. The primary focus was arrhythmic cardiotoxicity after use of the quinoline and structurally related antimalarials for malaria.

There were no sudden deaths attributed to cardiac arrhythmias recorded in the more than 35,000 individuals who received amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, primaquine, piperaquine, and quinine in the 177 clinical trials included in this review. Among the more than 18,000 subjects who underwent ECG evaluation, a variety of generally non-serious self-limiting cardiac rhythm abnormalities were described usually without contextual information, making interpretation of causation difficult. Balanced against the clear life-saving benefits of giving effective antimalarials promptly in malaria, with the exception of halofantrine, concerns over cardiotoxicity have not limited the current use of the quinoline and structurally related antimalarial drugs.

These findings provide further evidence of the rarity of serious cardiovascular events after treatment with the quinoline and structurally related antimalarials, although the precise estimation of risk is limited, because of this rarity, by the total size of the source data available despite an inclusive search strategy. In this review, the median number of participants per trial ranged from 16 to 165 among the nine antimalarials studied. Such individual study sample sizes are designed to evaluate drug efficacy and are too small, even when pooled, to characterise the risk of very rare (<1/10,000) drug adverse events such as TdP[105,167]. The representativeness of the clinical trial population of potential recipients of population-based drug administration interventions, for example, in terms of age, gender, ethnicity, and cardiovascular risk factors, is another potential limitation.

58% (103/177) and 63% (112/177) of included studies listed medical comorbidities and co-medications as exclusion criteria, while healthy volunteer studies, often of adult males from non-malaria endemic countries, comprised 27% (47/177) of the included studies.

The importance of robust detection and evaluation of extremely rare and serious adverse events such as sudden unexplained death in real-world populations and the implications of such findings for population-based drug administration strategies underscore the need for ongoing synthesis of all available clinical evidence, as also described in Chapter 3. Post-marketing pharmacovigilance approaches such as spontaneous individual case safety reporting are especially important in signal detection of very rare adverse events despite challenges in assessing causality[167]. For example, the two sentinel cases of sudden death and collapse with extreme QT interval prolongation after halofantrine given for the treatment of clinical malaria were important in stimulating the accumulation of further evidence which confirmed the arrhythmogenic effects of the drug[69,147]. The findings of the review in this chapter should therefore be interpreted in the context of this wider evidence base and the intended treatment indication(s) for each antimalarial drug.

The ECG QT interval is the most frequently used clinical biomarker for assessing the potential for development of TdP and related ventricular tachyarrhythmias. However, it is a surrogate marker with limited specificity[138]. Its interpretation is further compromised by the extensive heterogeneity of the methods used in its measurement, analysis, and reporting, as noted in this review. The QT interval is technically difficult to measure, even by experts, and the process of measurement introduces further confounding factors and systematic error[181]. More comprehensive reporting of food intake and time of ECG measurement relative to drug dosing along with better characterisation of antimalarial drug absorption profiles would improve future assessments, with peak drug levels being more relevant in the evaluation of potential cardiovascular toxicity than trough levels.

Another major source of potential confusion is the heart rate correction. This is necessary because the QT interval has an inverse relationship with heart rate. Several correction formulae are used, but the choice of the best formula is the subject of ongoing debate. The most widely used are the Bazett[269] and Fridericia[270] formulae. In a healthy population, the Bazett formula overestimates at higher heart rates (lengthens the QTc interval) and underestimates at lower heart rates (shortens the QTc interval). In healthy subjects, the Fridericia formula provides better, although still imperfect correction at heart rates <60 and >100 beats/minute. Where possible, the correction formula used should be derived for the study population, but this requires a large study and sufficient heart rate variation[136].

Several of the quinoline and structurally related antimalarial drugs are associated with electrocardiographic QT prolongation, but the only drug clearly associated with harm when used for the treatment of malaria is halofantrine. There have been no reports of death or syncope attributable to a cardiovascular cause nor electrocardiographic traces recording ventricular arrhythmia captured during malaria clinical therapeutic trials of other quinoline or structurally related antimalarial drugs which included systematic ECG assessment. While these findings add to existing evidence from individual case report databases[47] in supporting the rarity of these adverse events, they also underscore the need for continued strengthening of pharmacovigilance systems for robust detection of such rare drug adverse events in real-world populations.

Pooled analyses of individual patient clinical trial data including from intermittent preventive therapy and mass drug administration studies are important next steps to determine the effect of the quinoline antimalarials on the QT interval. Further evidence on the cardiotoxicity of the extensively deployed 4-aminoquinoline antimalarial

amodiaquine is needed. I go on to present my findings from these related analyses in Chapters 5 and 6.

4.6 AUTHORSHIP STATEMENT

I conducted the systematic review and carried out the data extraction as an independent reviewer with Dr Ilsa Haeusler. I made the figure and table as well as wrote all the text presented in this chapter. Dr Ilsa Haeusler and I published the paper[248] this chapter is based on with input from Professor Nicholas White and Professor Philippe Guérin of the WorldWide Antimalarial Resistance Network. Dr Ilsa Haeusler designed this study with Professor Nicholas White and Professor Philippe Guérin. Shona Kirtley of the EQUATOR Network and Nia Roberts of the Bodleian Libraries assisted with the literature search.

5 The Electrocardiographic QT Interval and Malaria

5.1 SUMMARY

Background

Electrocardiographic QT interval prolongation is the most widely-used risk marker for ventricular arrhythmia potential and thus an important component of drug cardiotoxicity assessments. Several antimalarial medicines are associated with QT interval prolongation. However, interpretation of electrocardiographic changes is confounded by the coincidence of peak antimalarial drug concentrations with recovery from malaria. We therefore reviewed all available data to characterise the effects of malaria disease and demographic factors on the QT interval in order to improve assessment of electrocardiographic changes in the treatment and prevention of malaria.

Methods

We conducted a systematic review and meta-analysis of individual patient data. We searched clinical bibliographic databases (last on August 21, 2017) for studies of the quinoline and structurally related antimalarials for malaria-related indications in human participants in which electrocardiograms were systematically recorded. Unpublished studies were identified by the World Health Organization Evidence Review Group on the Cardiotoxicity of Antimalarials. Bayesian hierarchical multivariable regression with generalised additive models was used to investigate the effects of malaria and demographic factors on the pre-treatment QT interval.

Findings

The meta-analysis included 10,452 individuals (9,778 malaria patients and 674 healthy participants) from 43 studies. None developed ventricular arrhythmia after antimalarial treatment. Compared to healthy participants, patients with uncomplicated falciparum

malaria had shorter QT intervals (-61.77 milliseconds; 95% CI: -80.71 to -42.83) and increased sensitivity of the QT interval to heart rate changes. These effects were greater in severe malaria (-110.89 milliseconds; 95% CI: -140.38 to -81.25). Body temperature was associated independently with clinically significant QT shortening of 2.80 milliseconds (95% CI: -3.17 to -2.42) per 1°C increase.

Interpretation

Adjustment for malaria and fever recovery-related QT lengthening is necessary to avoid misattributing malaria disease-related QT changes to antimalarial drug effects. This would improve risk assessments of antimalarial-related cardiotoxicity in clinical research and practice. Similar adjustments may be indicated for other febrile illnesses for which QT interval-prolonging medications are important therapeutic options.

5.2 INTRODUCTION

The artemisinin-based combination therapies (ACTs) are the gold standard oral treatment for malaria and the first-line antimalarial treatment in >80 malaria-endemic countries[30]. ACTs contain a rapidly acting artemisinin derivative combined with a more slowly eliminated partner drug. Most of the partner drugs in use and several of those in development are structurally related quinoline or quinoline-like compounds, some of which prolong the electrocardiographic (ECG) QT interval.

Drug-related QT interval prolongation is a widely-used[136] yet non-specific surrogate risk marker for repolarisation-related cardiotoxicity in the form of torsade de pointes (TdP), a potentially fatal polymorphic ventricular tachycardia. QT interval prolongation has been the most common reason for drug withdrawal and relabelling[134]. As part of ongoing safety assessments of population-based use of ACTs and other quinoline or structurally related compound-containing combinations for malaria control and elimination in both acutely unwell patients and healthy people at risk of symptomatic disease, there has been renewed interest in the evaluation of antimalarial effects on the ECG to guide antimalarial selection and dosage[47,209,248].

Malaria is characterised by red blood cell parasitisation, fever, and anaemia[11]. Malaria illness itself may affect the heart and in particular the QT interval[60,271], although these disease effects and their possible interaction with other factors known to affect the QT interval[272] are not well-understood[48]. As malaria illness and antimalarial drug concentrations change over the course of malaria treatment, it is important to characterise the independent contributions of disease and demographic factors on the QT interval in order to avoid misattributing changes solely to drug effects[48].

To address this, I conducted a systematic review and meta-analysis of individual patient data from malaria clinical trials to characterise the disease and demographic factors which affect independently the electrocardiographic QT interval in malaria.

5.3 METHODS

5.3.1 Search Strategy and Selection Criteria

I performed a systematic literature search on 22 October 2015 (updated on 21 August 2017) of the databases MEDLINE, EMBASE, and Global Health, for primary clinical studies of the quinoline and structurally related antimalarials for malaria-related indications in which electrocardiograms were recorded before and after drug administration (see 2.3.2.1). These published and additional unpublished studies were identified as part of the as part of the work of the Evidence Review Group on the Cardiotoxicity of Antimalarials convened by the World Health Organization[47] (see 2.3.2.2).

Studies were eligible for inclusion in the review if they were prospective randomised-controlled trials or cohort studies published from 1988 onwards in which five or more participants were given a quinoline or structurally related antimalarial – amodiaquine, chloroquine, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, pyronaridine, or quinine – either as monotherapy or as part of an artemisinin-based combination therapy. Studies which co-administered other drugs with QT-prolonging potential (e.g. azithromycin) as part of the trial intervention were excluded.

I contacted study authors with a request for clinical study reports and protocols as well as anonymised individual patient-level datasets of the following pre-specified variables identified from expert consultation[47]: age, weight, sex, body temperature, parasitaemia, haemoglobin or haematocrit, heart rate or RR interval duration, uncorrected QT interval

duration, ECG abnormalities, and other cardiovascular adverse events. Studies were included in this meta-analysis if individual patient-level data were available for all requested variables from the screening or a baseline timepoint before antimalarial drug administration. Data gathering was in line with existing ethical approvals (see 2.3.3.1).

5.3.2 Data Extraction and Standardisation

I screened titles, abstracts, full texts, trial documentation, and anonymised datasets, and agreed study eligibility with another independent reviewer. From study publications, reports, and protocols I extracted study-level characteristics including location, antimalarial treatment indication, inclusion and exclusion criteria, temperature measurement method, and ECG measurement methodology into a standardised database (see 2.3.3.2). Where required, trial registry records and study investigators were consulted for further information.

Manual data entry was undertaken for datasets available only in printed format. Once digitised, individual patient-level datasets were standardised and checked according to a pre-specified data dictionary (see 2.3.3.3). For studies of repeated treatments, only data from the first treatment episode were extracted. Individual patient records were excluded if data for any requested variables were missing at the selected timepoint before drug administration (see 2.3.3.4). I conducted risk of bias assessments within and across studies with another independent reviewer (see 2.3.4).

5.3.3 Data Analysis

I performed Bayesian hierarchical multivariable regression with generalised additive models. The QT interval was the response variable and individual study ID was the varying intercept. The square root transformed RR interval ($\sqrt{RR}$), sex, body temperature, and malaria type/antimalarial treatment indication were the linear predictors. Age was

modelled with separate smooths for females and males because of the known sex hormone-related QT interval changes around puberty[272]. Weight was omitted because of its collinearity with age in a predominantly paediatric population (see 2.3.5.1). Haemoglobin was considered an intermediate variable and omitted. Variable selection was based on directed acyclic graphs of proposed causal relationships among collected variables (see 2.3.5.1) identified from literature review and expert consultation[47].

Four models were fitted to the data where different combinations of malaria disease-related terms were added to known factors affecting the QT interval: the first contained only heart rate and demographic terms, the second added to the first a term for body temperature, the third included a further term for malaria type (species, severity), and the fourth added to the third an interaction term for malaria type and$\sqrt{RR}$. I carried out Pareto smoothed importance-sampling leave-one-out cross-validation for model comparison (see 2.3.5.2).

As sensitivity analyses, I compared the model with the best expected predictive performance to a model with an additional binary variable for whether a participant was in a study which excluded at screening individuals with one or more TdP risk factors, a model with a linear predictor for haemoglobin, as well as to another model with a cube root (Fridericia-like) instead of square root (Bazett-like) transformation of the RR interval. In the subgroup of malaria patients only, I added a linear predictor for log parasitaemia to the best model (see 2.3.5.3).

5.4 RESULTS

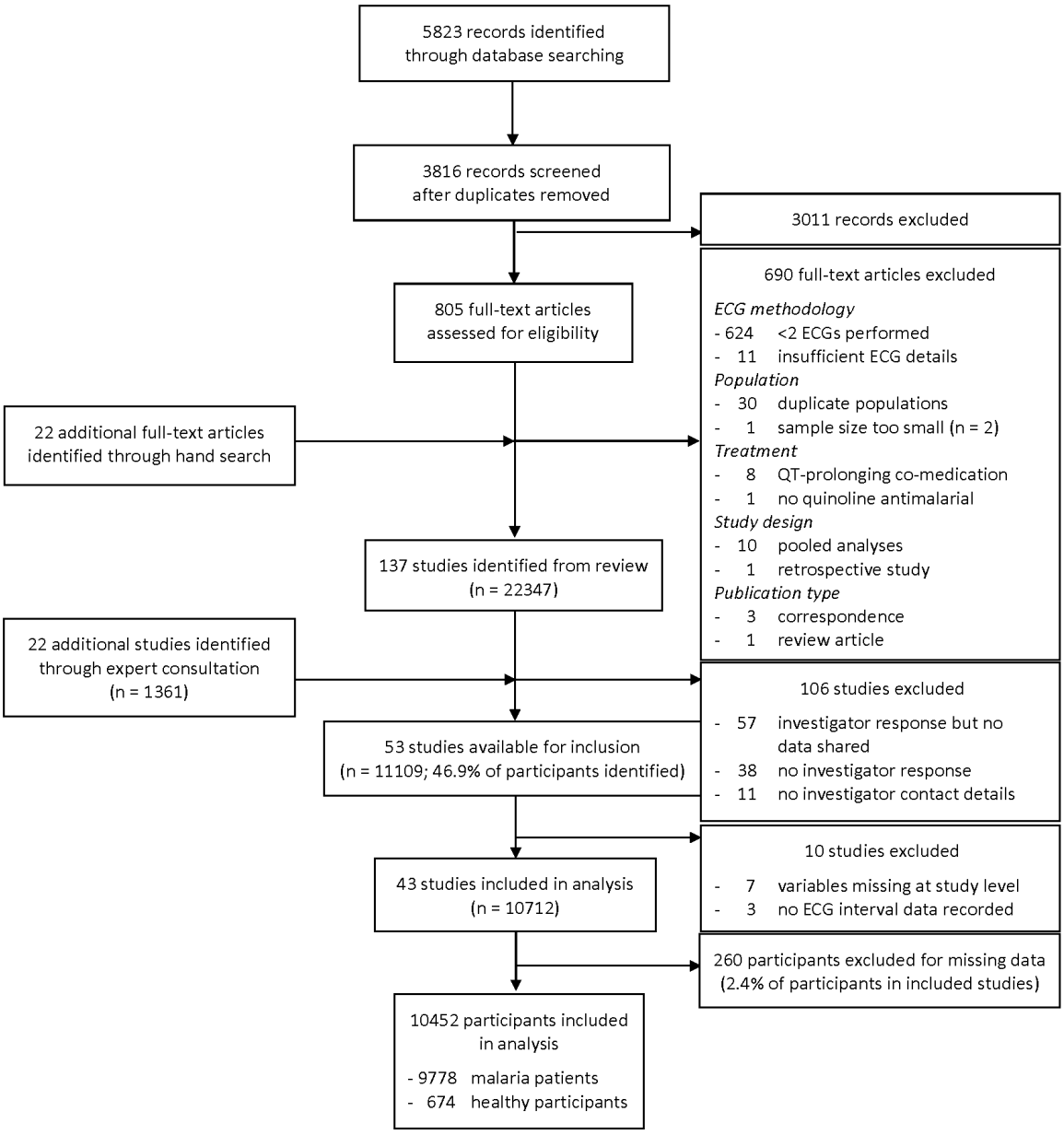
5.4.1 Studies and Populations

5.4.1.1 Study and Participant Selection

Individual patient-level data were sought from 159 clinical studies (137 published and 22 unpublished at the time of the literature search). There were no cases of sudden cardiac death, life-threatening ventricular tachyarrhythmias (ventricular fibrillation or ventricular tachycardia), or TdP documented for any of the 23,708 participants in the 159 studies from which individual patient-level data were sought.

Data from 11,109 participants in 53 studies were shared, of which data from 10,452 participants in 43 studies (28 published[84,147,188-190,211,217,273-293], 5 subsequently published[119,202,203,294,295], and 10 unpublished) were suitable for inclusion in the meta-analysis (Figure 5.1).

Figure 5.1: Selection of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring for IPD Meta-analysis



5.4.1.2 Included Population – Malaria Disease Characteristics and Demographics

Overall, 9,778 (93.6%) of 10,452 included individuals had microscopy-confirmed *P. falciparum* or *P. vivax* malaria, of whom 8,769 (89.7%) of 9,778 had uncomplicated *P. falciparum* mono- or mixed infection (Table 5.1). The remaining 674 individuals were healthy participants, the majority of whom (78.8%; 531/674) were enrolled in healthy volunteer pharmacokinetic studies (Table 5.1). The median age of the 10,452 included individuals was 13.3 years (IQR 4.6-26; range 6 months to 84 years) with 2,803 (26.8%) aged 5 to under 15 years, and 2,751 (26.3%) aged younger than 5 years. The healthy group were almost entirely adult participants from Europe, North America, and urban Asia and Africa, while malaria patients were more likely to be children or adolescents from rural Africa, Asia, and South America, and to have fever (temperature 37.5°C or higher), anaemia, and tachycardia (Table 5.1 & Appendix 11.3.1 – Figures 11.2-4).

5.4.1.3 Included Population – Torsade de Pointes Risk Factors

26 (60.5%) of the 43 included studies listed as exclusion criteria one or more risk factors for TdP, such as a personal or family history of clinically significant arrhythmias, pre-existing conditions or concomitant medications which prolong the QT interval or increase antimalarial drug concentrations, a baseline corrected QT interval of more than 450 milliseconds, and electrolyte imbalances including hypokalaemia and hypomagnesaemia. These 26 studies which excluded patients with risk factors for TdP enrolled 7,633 (73%) of the 10,452 individuals providing data (Table 5.1).

Table 5.1: Characteristics of Included Population from Antimalarial Studies with Electrocardiographic Monitoring

	Healthy Participants (n = 674)	Malaria Patients (n = 9778)	Overall (n = 10452)
Antimalarial Treatment Indication			
Severe/complicated malaria		343 (3.5%)	343 (3.3%)
Uncomplicated malaria		9435 (96.5%)	9435 (90.3%)
P. falciparum mono- or mixed infection		8769 (89.7%)	8769 (83.9%)
P. vivax mono-infection		666 (6.8%)	666 (6.4%)
Intermittent preventive therapy (IPT)	143 (21.2%)		143 (1.4%)
Pregnancy (IPTp)	125 (18.5%)		125 (1.2%)
Infancy (IPTi)	18 (2.7%)		18 (0.2%)
Healthy volunteer pharmacokinetics	531 (78.8%)		531 (5.1%)
Age (years)			
Median (IQR)	28.9 (23.0-37.0)	12.1 (4.2-24.5)	13.3 (4.6-26.0)
<15	18 (2.7%)	5536 (56.7%)	5554 (53.1%)
<1	18 (2.7%)	193 (2.0%)	211 (2.0%)
1-<5	0	2540 (26.0%)	2540 (24.3%)
5-<15	0	2803 (28.7%)	2803 (26.8%)
≥15	656 (97.3%)	4242 (43.4%)	4898 (46.9%)
≥35	209 (31.0%)	1296 (13.3%)	1505 (14.4%)
≥50	0	40 (0.41%)	40 (0.38%)
Weight (kg)			
Median (IQR)	63.6 (57.0-72.2)	33.0 (15.0-52.0)	36.9 (15.1-54.0)
Sex			
Female	343 (50.9%)	3909 (40.0%)	4252 (40.7%)
Pregnant	125 (18.5%)	9 (0.09%)	134 (1.3%)
Male	331 (49.1%)	5869 (60.0%)	6200 (59.3%)
Temperature (°C)			
Mean (SD)	36.8 (0.4)	38.2 (1.1)	38.2 (1.1)
≥37.5	23 (3.4%)	7147 (73.1%)	7170 (68.6%)
Parasitaemia (parasites/µL)			
Median (IQR)	N/A	14080 (2851-45219)	14080 (2851-45219)
≥10,000	N/A	5500 (56.2%)	5500 (52.6%)
≥50,000	N/A	2191 (22.4%)	2191 (20.9%)
≥100,000	N/A	905 (9.3%)	905 (8.7%)
≥250,000	N/A	175 (1.8%)	175 (1.7%)
Haemoglobin (g/dL)			
Mean (SD)	13.5 (1.7)	11.0 (2.3)	11.2 (2.3)
<11	51 (7.6%)	4856 (49.7%)	4907 (46.9%)
<8	0	844 (8.6%)	844 (8.1%)
<5	0	43 (0.4%)	43 (0.4%)
Heart Rate (beats/minute)			
Mean (SD)	68 (17)	108 (30)	106 (31)
≥140	0	1537 (15.7%)	1537 (14.7%)
120-139	16 (2.4%)	1603 (16.4%)	1619 (15.5%)
100-119	22 (3.3%)	2356 (24.1%)	2378 (22.8%)
80-99	79 (11.7%))	2560 (26.2%)	2639 (25.2%)
60-79	320 (47.4%)	1537 (15.7%)	1857 (17.8%)
<60	237 (35.2%)	185 (1.9%)	422 (4.0%)

Torsade de Pointes Risk Factors			
Excluded from the individual study	669 (99.3%)	6964 (71.2%)	7633 (73.0%)
Not excluded from the individual study	5 (0.7%)	2814 (28.8%)	2819 (27.0%)
ECG Measurement Methodology			
Location of ECG interpretation			
Centralised and study site-based	392 (58.2%)	6778 (69.3%)	7170 (68.6%)
Study site-based only	282 (41.8%)	3000 (30.7%)	3282 (31.4%)
ECG reader			
Cardiologist	449 (66.6%)	7423 (75.9%)	7872 (75.3%)
Other physician or trained personnel	154 (22.8%)	2355 (24.1%)	2509 (24.0%)
Machine only	71 (10.5%)	0	71 (0.7%)
Temperature Measurement Method			
Axillary	99 (14.7%)	7771 (79.5%)	7870 (75.3%)
Oral	305 (45.3%)	613 (6.3%)	918 (8.8%)
Tympanic	174 (25.8%)	195 (2.0%)	369 (3.5%)
Rectal	0	57 (0.6%)	57 (0.5%)
Unknown	96 (14.2%)	1142 (11.7%)	1238 (11.8%)
Year of Enrolment			
2012-2017	247 (36.6%)	5316 (54.4%)	5563 (53.2%)
2007-2011	383 (56.8%)	906 (9.3%)	1289 (12.3%)
1997-2006	29 (4.3%)	2916 (29.8%)	2945 (28.2%)
1985-1996	15 (2.2%)	621 (6.4%)	636 (6.1%)
Not reported	0	19 (0.2%)	19 (0.2%)
Geographical Region			
Africa	172 (25.5%)	6363 (65.0%)	6535 (62.5%)
Asia	147 (21.8%)	3065 (31.3%)	3212 (30.7%)
Americas	15 (2.2%)	350 (3.6%)	365 (3.5%)
Europe	340 (50.4%)	0	340 (3.3%)

SD = standard deviation, IQR = interquartile range, ECG = electrocardiogram

5.4.1.4 Included Population – ECG Methodology and Characteristics

Almost all (99.3%; 10381/10452) participants, including all malaria patients, had ECG intervals measured manually by trained personnel, with 7,872 (75.3%) of the 10,452 participants having their ECGs evaluated by cardiologists. In addition, 7,170 (68.6%) of the 10,452 participants in 13 (30.2%) of the 43 studies had ECGs sent to a centralised facility where specialist staff read ECGs, and in the remainder, ECGs were read at the study site. Only two studies, both of piperaquine in healthy volunteers[119,278], had 24-hour continuous ECG recordings at baseline (Table 5.1).

None of the 10,452 participants included had a baseline uncorrected QT interval of more than 500 milliseconds.

5.4.2 Risk of Bias Assessment

Compared to included studies, a higher proportion of excluded studies were conducted before 2007, did not specifically exclude TdP risk factors, and had unclear or high risk of bias (Tables 5.2 & Appendix 11.3.2 – Table 11.4) reflecting quality of measurement and reporting methods of safety outcomes. The characteristics of included and excluded studies were otherwise comparable (Table 5.2).

As with the excluded studies, most of the 260 participants who were excluded for missing data were malaria patients in studies conducted before 2007 with available characteristics similar to that of the included population.

Table 5.2: Comparison of Characteristics of Included and Excluded Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring

	Included Studies (n = 43)	Excluded Studies (n = 116)
Antimalarial Treatment Indication, studies (%)		
Severe/complicated malaria	2 (4.7%)	17 (14.7%)
Uncomplicated malaria	25 (58.1%)	61 (52.6%)
P. falciparum mono- or mixed infection	23 (53.5%)	52 (44.8%)
P. vivax mono-infection	2 (4.7%)	3 (2.6%)
P. falciparum or *P. vivax* mono- or mixed infection	0	6 (5.2%)
Intermittent preventive therapy (IPT)	4 (9.3%)	8 (6.9%)
IPT in pregnancy (IPTp)	3 (7.0%)	3 (2.6%)
IPT in infancy (IPTi)	1 (2.3%)	1 (0.9%)
Seasonal malaria chemoprevention (SMC)	0	1 (0.9%)
Occupational prophylaxis	0	3 (2.6%)
Healthy volunteer pharmacokinetics	13 (30.2%)	30 (25.9%)
Healthy volunteers only	13 (30.2%)	27 (23.3%)
Healthy volunteers and uncomplicated malaria (*P. falciparum* or *P. vivax* infection)	0	3 (2.6%)
Geographical Region, studies (%)		
Asia-Pacific	25 (58.1%)	57 (49.1%)
Africa	11 (25.6%)	28 (24.1%)
Americas	2 (4.7%)	6 (5.2%)
Europe	2 (4.7%)	17 (14.7%)
Asia-Pacific & Africa	3 (7.0%)	4 (3.4%)
Others (Asia-Pacific & Americas, Africa & Europe, Americas & Europe)	0	3 (2.6%)
Not reported	0	1 (0.9%)
Year Enrolment Completed, studies (%)		
2007-2017	24 (55.8%)	25 (21.6%)
Pre-2007	19 (44.2%)	80 (69.0%)
Not reported	0	11 (9.5%)
Torsade de Pointes Risk Factors Excluded, studies (%)	26 (60.5%)	46 (39.7%)
Mean Age in Years, median (IQR)	26.2 (17.4-32.4)	26.6 (16.2-31.5)*
Percentage of Females, median (IQR)	41.0 (24.4-53.2)	28.7 (0-48.7)†
Risk of Bias Assessment, studies (%)		
Low	39 (90.7%)	75 (64.7%)
Unclear	4 (9.3%)	39 (33.6%)
High	0	2 (1.7%)

*Mean age not available from 8 studies, †Percentage not available from 14 studies

5.4.3 Individual Patient Data Meta-analysis

I present the results from the best model which had heart rate (as $\sqrt{RR}$), age, sex, body temperature, and malaria type (both as an independent term and an interaction term with heart rate) as predictors.

5.4.3.1 Main Analysis – Body Temperature, Malaria Type, and the RR Interval

From the meta-analysis of all included participants (n = 10452), body temperature had an independent effect on the QT interval, with a mean shortening of the QT interval by 2.80 milliseconds (95% credible interval: 2.42 to 3.17) per 1°C rise in temperature (Table 5.3 & Figure 5.2). When compared to healthy participants (n = 674) and adjusting for other predictors, QT shortening increased with malaria severity: patients with severe malaria (n = 343) had the shortest QT intervals (mean difference: -110.89 milliseconds; 95% CI: -140.38 to -81.25), followed by patients with uncomplicated falciparum malaria (n = 8769) (mean difference: -61.77 milliseconds; 95% CI: -80.71 to -42.83). Patients with uncomplicated vivax malaria (n = 666) also had shorter QT intervals than healthy participants but the 95% CI included zero (mean difference: -11.77 milliseconds; 95% CI -37.30 to 14.72) (Table 5.3).

Sensitivity of the QT interval to changes in heart rate also increased with malaria severity: the additional increase in the QT interval per unit increase of $\sqrt{RR}$ (i.e. with decreasing heart rate) was higher in severe malaria patients (mean difference: 4.89 milliseconds; 95% CI: 3.85 to 5.91) than patients with uncomplicated falciparum malaria (mean difference: 2.24 milliseconds; 95% CI: 1.65 to 2.83). These values compared with a mean increase of 9.16 milliseconds (95% CI: 8.59 to 9.73) in healthy participants. Again, uncomplicated vivax malaria patients had a slightly larger increase in the QT interval with decreasing heart rate than healthy participants but the 95% CI contained zero (mean difference: 0.62 milliseconds; 95% CI: -0.11 to +1.34) (Table 5.3 & Figure 5.3).

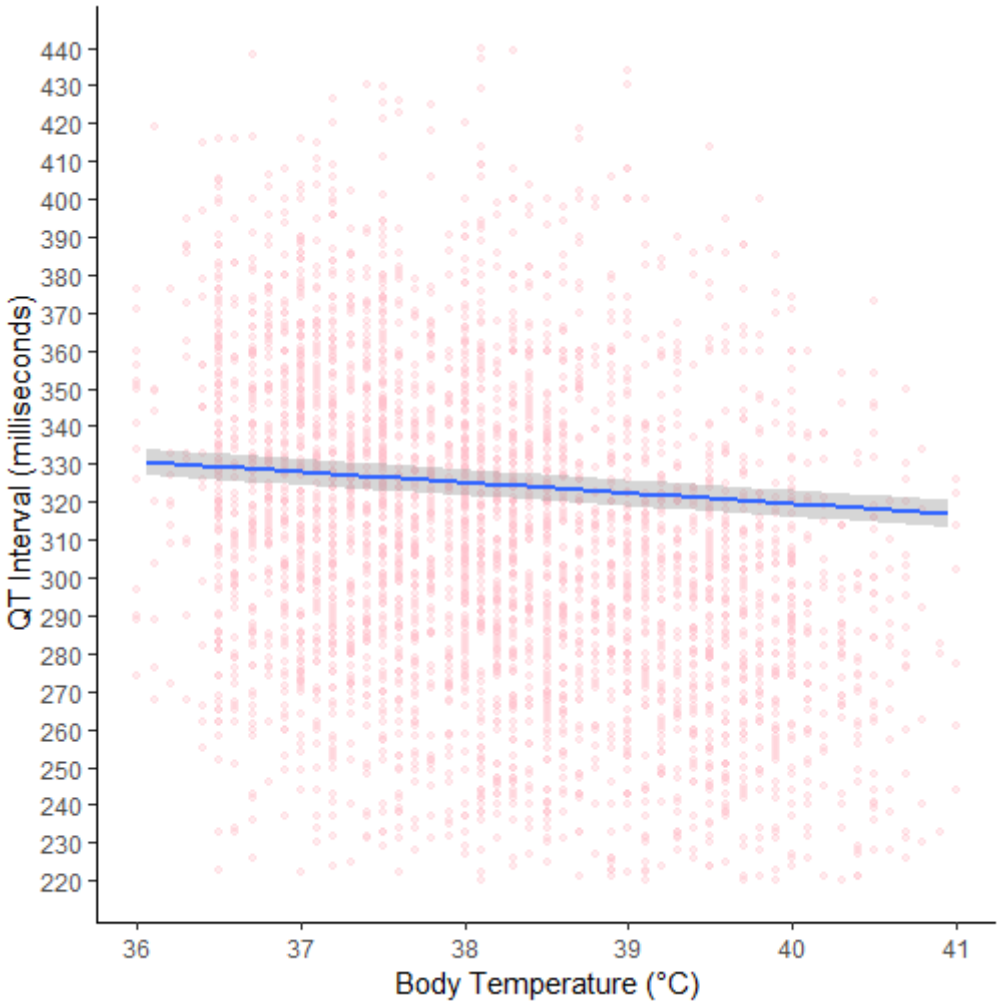
Table 5.3: Factors Affecting the QT Interval in Malaria

Predictor	Number of Participants	Estimate (95% Credible Interval) / Smooth Description	Clinically Significant?	Improved Model?†
$\sqrt{RR}$ interval, per √millisecond increase* (healthy participants)	10452	9.16 (8.59, 9.73) milliseconds	Yes	N/A
$\sqrt{RR}$ interval, per √millisecond increase* (by malaria type vs healthy participants)	10452		Yes	Yes
Healthy participants	674	Reference		
Uncomplicated vivax	666	0.62 (-0.11, 1.34) milliseconds		
Uncomplicated falciparum	8769	2.24 (1.65, 2.83) milliseconds		
Severe/complicated malaria	343	4.89 (3.85, 5.91) milliseconds		
Age	10452		Yes	N/A
Female	4252	Lengthens by ~8 milliseconds over childhood, then lengthens more gradually by another ~5 milliseconds in adulthood		
Male	6200	Lengthens by ~8 milliseconds over childhood, then shortens by ~10 milliseconds around puberty before gradually lengthening by ~10 milliseconds in adulthood		
Sex	10452		Yes	N/A
Female	4252	Reference		
Male	6200	-4.22 (-5.00, -3.43) milliseconds		
Body temperature, per 1°C increase	10452	-2.80 (-3.17, -2.42) milliseconds	Yes	Yes
Malaria Type	10452		Yes	Yes
Healthy participants	674	Reference		
Uncomplicated vivax	666	-11.77 (-37.30, 14.72) milliseconds		
Uncomplicated falciparum	8769	-61.77 (-80.71, -42.83) milliseconds		
Severe/complicated malaria	343	-110.89 (-140.38, -81.25) milliseconds		

*Electrocardiographic RR interval in milliseconds = 60000/(heart rate in beats per minute), †Improved expected predictive accuracy as estimated by the standard error of the difference in expected log predictive density

Multivariable regression results from hierarchical generalised additive model

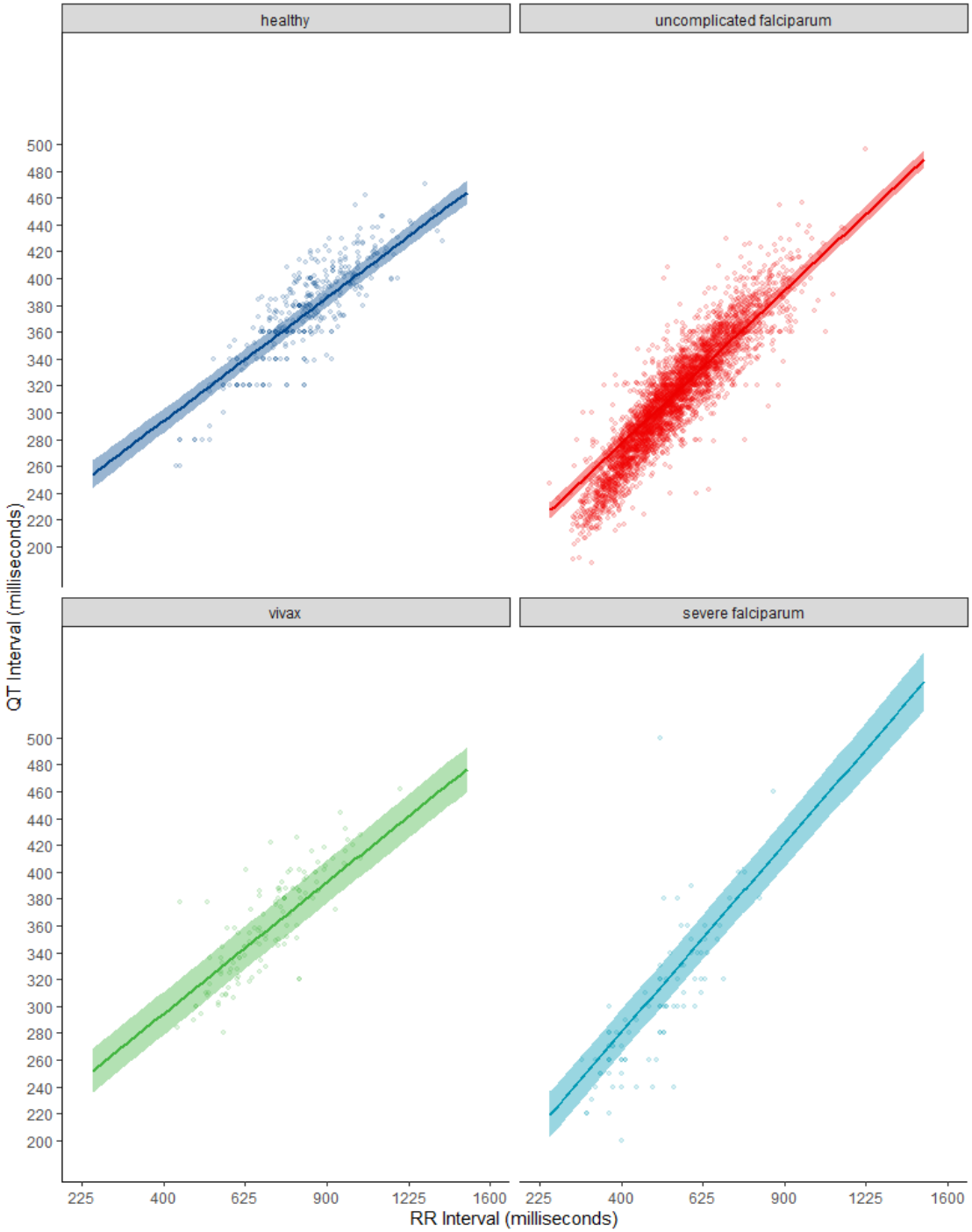
Figure 5.2: Body Temperature and the QT Interval in Malaria



Independent effect of body temperature on the QT interval from hierarchical generalised additive model adjusting for heart rate/RR interval (as $\sqrt{RR}$), age, sex, malaria type, and individual study.

Shaded area represents 95% credible intervals, circles represent original data points without adjustment.

Figure 5.3: Malaria Type, RR Interval, and the QT Interval



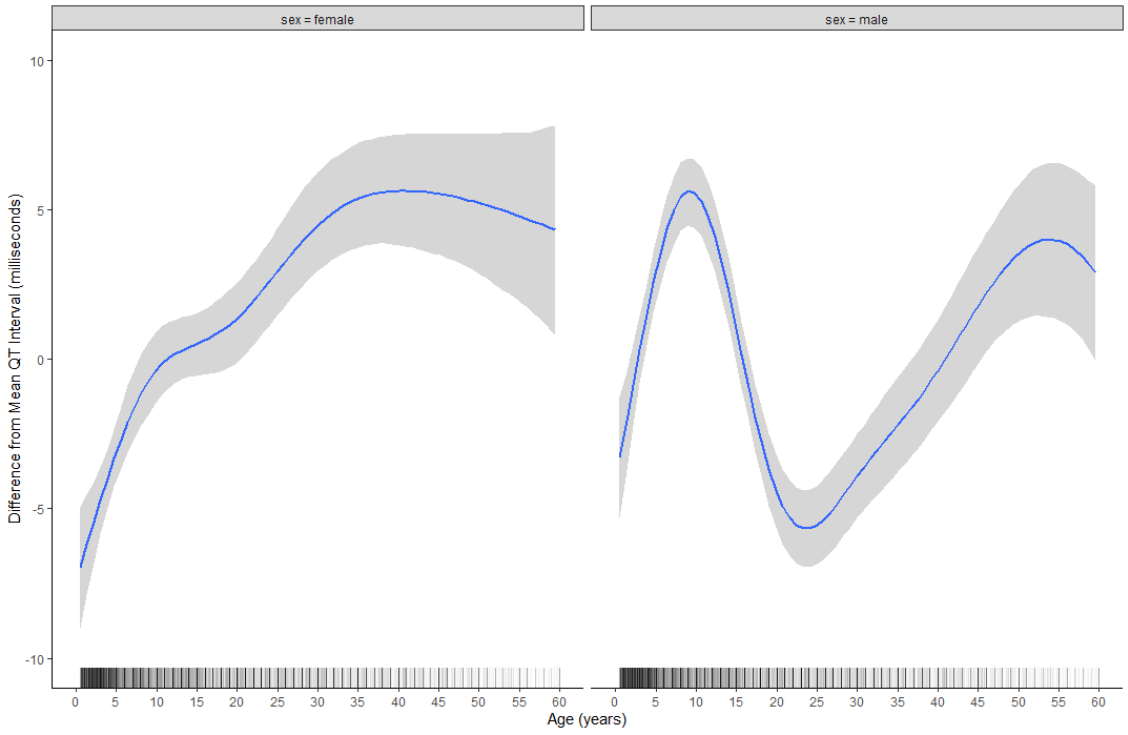
Interaction between malaria type and the RR interval (on the square root scale), and conditional effect on the QT interval (on the linear scale), from hierarchical generalised additive model adjusting for age, sex, body temperature, and individual study.

Shaded areas represent 95% credible intervals, circles represent original data points without adjustment.

5.4.3.2 Main Analysis – Age and Sex

The demographic variables of age and sex had clinically significant effects: the QT interval lengthened by a mean of ~8 milliseconds over childhood before shortening by ~10 milliseconds in males but not in females around puberty, then gradually lengthened by ~5-10 milliseconds in both sexes over adulthood although there were few data for participants aged older than 50 years (n = 40); males (n = 6200) also had overall shorter QT intervals than females (n = 4252) (mean difference: -4.21 milliseconds; 95% CI: -4.99 to -3.44) (Table 5.3 & Figure 5.4).

Figure 5.4: Age, Sex, and the QT Interval in Malaria



Interaction between age and sex, and conditional effect on the QT interval, from hierarchical generalised additive model adjusting for heart rate/RR interval (as $\sqrt{RR}$), malaria type, body temperature, and individual study.

Shaded areas represent 95% credible intervals, rug marks represent age distribution of original data points.

5.4.3.3 Main Analysis – Predicted QT Intervals at Baseline and Recovery

From this model, a 25-year-old male patient with uncomplicated falciparum malaria admitted with a heart rate of 100 beats per minute and a temperature of 38.5°C, whose heart rate slows to 60 beats per minute and who defervesces to a temperature of 36.5°C in recovery, would be predicted to have a 22 millisecond or 25% greater QT interval lengthening than an age- and sex-matched healthy afebrile participant with the same heart rate reduction independent of any drug treatment (Table 5.4).

Table 5.4: Predicted QT Intervals at Baseline and in Recovery from Malaria and Fever

	Healthy	Uncomplicated vivax	Uncomplicated falciparum	Severe malaria
QT interval at baseline, milliseconds (95% PI) [HR=100 beats/minute]	329 (285-371) [T=36.5°C]	327 (281-370) [T=38.5°C]	317 (273-358) [T=38.5°C]	332 (287-377) [T=38.5°C]
QT interval in recovery, milliseconds (95% PI) [HR=60 beats/minute]	394 (351-435) [T=36.5°C]	403 (356-446) [T=36.5°C]	404 (360-445) [T=36.5°C]	438 (392-484) [T=36.5°C]
QT lengthening from baseline, milliseconds	65	76	87	106
Additional QT lengthening from baseline compared to healthy participant, milliseconds	0	11	22	41
Malaria-related QT lengthening from baseline, %	0	14	25	39

PI = prediction interval, HR = heart rate, T = body temperature

Predicted values for a 25-year-old male from hierarchical generalised additive model adjusting for heart rate/RR interval (as $\sqrt{RR}$), age, sex, malaria type, body temperature, and individual study effects

5.4.3.4 Sensitivity Analyses – Adjustment for Additional Predictors

The QT intervals of participants in studies which screened for and excluded individuals with TdP risk factors (n = 7633) were not significantly different to those in studies without documented risk factor screening (n = 2819) (mean difference: -0.78 milliseconds; 95% CI -9.69 to 7.88) once other predictors had been adjusted for.

The small effects associated with haemoglobin (-0.51 milliseconds per g/dL increase; 95% CI: -0.72 to -0.30) and parasitaemia (0.65 milliseconds per 10-fold increase in parasite density; 95% CI: 0.17 to 1.14) were of unclear clinical significance, and these additional terms did not improve model performance.

5.4.3.5 Sensitivity Analyses – Alternative Approaches to the QT-RR Relationship

There were no clinically significant changes in predictor estimates when a cube root (Fridericia-like) instead of square root (Bazett-like) transformation of the RR interval was used (Tables 5.5 & 5.6). Similarly, log-log linear regression confirmed the relationships of increasing QT interval shortening and heart rate sensitivity with increasing malaria severity (Table 5.7).

Table 5.5: Factors Affecting the QT Interval in Malaria with Alternative RR Transformation

Predictor	Number of Participants	Estimate (95% Credible Interval) / Smooth Description
$\sqrt[3]{RR}$ interval, per ³√millisecond increase (healthy participants)	10452	43.36 (40.82, 45.81) milliseconds
$\sqrt[3]{RR}$ interval, per ³√millisecond increase (by malaria type vs healthy participants)	10452	
Healthy participants	674	Reference
Uncomplicated vivax	666	1.00 (-2.09, 4.10) milliseconds
Uncomplicated falciparum	8769	7.11 (4.57, 9.73) milliseconds
Severe/complicated malaria	343	16.87 (12.62, 21.16) milliseconds
Age	10452	
Female	4252	Lengthens by ~8 milliseconds over childhood, then lengthens more gradually by another ~5 milliseconds in adulthood
Male	6200	Lengthens by ~8 milliseconds over childhood, then shortens by ~10 milliseconds around puberty before gradually lengthening by ~10 milliseconds in adulthood
Sex	10452	
Female	4252	Reference
Male	6200	-4.23 (-4.99, -3.46) milliseconds
Body temperature, per 1°C increase	10452	-2.67 (-3.04, -2.30) milliseconds
Malaria Type	10452	
Healthy participants	674	Reference
Uncomplicated vivax	666	-3.08 (-35.47, 29.22) milliseconds
Uncomplicated falciparum	8769	-64.42 (-90.31, -39.01) milliseconds
Severe/complicated malaria	343	-130.84 (-169.47, -91.96) milliseconds

Table 5.6: Predicted QT Intervals at Baseline and in Recovery from Malaria and Fever with Alternative RR Transformation

	Healthy	Uncomplicated vivax	Uncomplicated falciparum	Severe malaria
QT interval at baseline, milliseconds (95% PI) [HR=100 beats/minute]	327 (283-395) [T=36.5°C]	328 (281-371) [T=38.5°C]	318 (275-358) [T=38.5°C]	333 (288-377) [T=38.5°C]
QT interval in recovery, milliseconds (95% PI) [HR=60 beats/minute]	396 (352-436) [T=36.5°C]	402 (356-446) [T=36.5°C]	403 (359-443) [T=36.5°C]	433 (386-477) [T=36.5°C]
QT lengthening from baseline, milliseconds	69	74	85	100
Additional QT lengthening from baseline compared to healthy participant, milliseconds	0	5	16	31
Malaria-related QT lengthening from baseline, %	0	7	19	31

PI = prediction interval, HR = heart rate, T = body temperature

Predicted values for a 25-year-old male from multivariable hierarchical generalised additive model adjusting for heart rate/RR interval (as $\sqrt[3]{RR}$), age, sex, malaria type, body temperature, and individual study effect

Table 5.7: Factors Affecting the QT Interval in Malaria from Log-Log Linear Regression

Predictor	Number of Participants	Estimate (95% Credible Interval) / Smooth Description
logRR interval, per log(millisecond) increase (healthy participants)	10452	0.36 (0.34, 0.39) log(milliseconds)
logRR interval, per log(millisecond) increase (by malaria type vs healthy participants)	10452	
Healthy participants	674	Reference
Uncomplicated vivax	666	0.014 (-0.018, 0.047) log(milliseconds)
Uncomplicated falciparum	8769	0.083 (0.055, 0.11) log(milliseconds)
Severe/complicated malaria	343	0.20 (0.15, 0.24) log(milliseconds)
Age	10452	
Female	4252	Lengthens over childhood, then lengthens more gradually in adulthood
Male	6200	Lengthens over childhood, then shortens around puberty before gradually lengthening in adulthood
Sex	10452	
Female	4252	Reference
Male	6200	-0.0055 (-0.0065, -0.0044) log(milliseconds)
Body temperature, per 1°C increase	10452	-0.0037 (-0.0042, -0.0032) log(milliseconds)
Malaria Type	10452	
Healthy participants	674	Reference
Uncomplicated vivax	666	-0.034 (-0.13, 0.064) log(milliseconds)
Uncomplicated falciparum	8769	-0.24 (-0.32, -0.15) log(milliseconds)
Severe/complicated malaria	343	-0.54 (-0.66, -0.41) log(milliseconds)

5.5 DISCUSSION

To my knowledge, this is the most extensive study to date of the factors affecting the electrocardiographic QT interval in malaria. I pooled individual patient data before treatment from 10,452 adults and children (9,778 malaria patients and 674 healthy participants) in antimalarial drug trials identified through a comprehensive systematic review and expert consultation. This allowed formal evaluation of the independent effects of malaria disease (type, temperature, and parasitaemia) and patient demographics (age and sex) on the QT interval without confounding from antimalarial drug effects through meta-analysis using hierarchical generalised additive models.

Malaria was found to have important effects on the QT interval which are proportional to disease severity: the marked QT interval shortening and increased sensitivity to changes in heart rate seen in malaria are greater in severe than uncomplicated disease, and greater in *P. falciparum* than in *P. vivax* infection. These effects occur independently of temperature and parasitaemia, suggesting further unmeasured disease factors may be responsible. Possible candidates include increasing stimulation of the sympathetic nervous system and acid-base abnormalities resulting from microvascular sequestration with increasing malaria severity. Parasite density is a poor predictor of sequestered parasite burden in falciparum malaria[11]. Higher levels of proinflammatory cytokines such as interleukin-6 during acute infection in less immune populations[296] and in severe malaria[297] may also contribute through inhibition of cardiomyocyte ion channel function[298,299]. Yet, for a disease with a wide range of systemic complications causing multiple organ dysfunction, the heart is relatively spared in acute malaria: clinically significant arrhythmias are rare[248], and even in severe malaria where there may be extensive sequestration of parasitised erythrocytes in the myocardial microvasculature, cardiac performance is maintained[14,266].

Fever, a cardinal sign of malaria illness, was found to have an independent effect on the QT interval: the QT interval shortens as body temperature increases and lengthens correspondingly as temperature decreases. In other words, fever shortens the QT interval, and recovery from fever lengthens it. This is supported by the only prospective study of fever and the QT interval[300] without confounding from drug treatment to my knowledge, which measured ECGs in 27 otherwise healthy young Finnish male soldiers before and after self-limiting uncomplicated febrile illness of bacterial, viral, or undefined aetiology. That study found that the QT interval was significantly shorter during fever than after recovery (measurement of QT intervals at specific heart rates of 60, 80, and 100 beats per minute obviated the need for heart rate correction). Further prospective evaluation of the QT interval in febrile illness would be useful to assess whether the pyrexial shortening of the QT interval extends to fevers of other aetiologies[301], particularly as *in vitro* evidence indicates that the risk of drug-induced long QT syndrome is temperature dependent[302]. This would be especially relevant for infectious diseases which present with fever and for which QT interval prolonging medications (e.g. macrolide and quinolone antibiotics) are also important therapeutic options.

Fever is known to unmask and trigger potentially life-threatening arrhythmias in individuals with inherited cardiac channelopathies. The arrhythmogenicity of fever is well established for Brugada syndrome[303], a leading cause of sudden unexplained death in young Southeast Asian men. It has also been observed in cases of type 2 congenital long QT syndrome with temperature-dependent phenotypes of human ether-à-go-go related gene potassium channel mutations where fever paradoxically prolonged instead of shortened the QT interval[304]. In this meta-analysis, there were no potentially life-threatening arrhythmias observed despite most participants with malaria being febrile. This may in part be because most malaria patients were enrolled in studies which excluded at screening individuals with TdP risk factors such as congenital channelopathies and

concomitant medications known to prolong the QT interval. It may also be that the QT interval shortening seen during malarial fever could have a protective effect against ventricular arrhythmias.

This meta-analysis further confirms established relationships between the QT interval and the demographic factors of age and sex are relevant in malaria. The QT interval does not exhibit a sex difference in childhood[271] until around puberty when it shortens in males but not females[189], then lengthens gradually in adulthood in males more than females[272]. This difference is thought to result from pubertal changes in sex hormone levels although the underlying mechanisms are not fully understood[272]. Post-pubertal females have a higher risk of TdP[305] but a lower risk of sudden cardiac death at all ages[306]. Adjustment for sex-related differences when evaluating the QT interval in post-pubertal individuals should be considered.

The flaws of commonly used QT correction factors adjusting for heart rate through proportional scaling with power functions (e.g. Bazett's[269] and Fridericia's[270] formulae) are well known and have been evaluated in large studies of healthy adults[307,308]. Reasons for their inadequacy in the healthy adult population include distorted correction, with substantial residual heart rate dependence of the corrected QT interval particularly at extreme heart rates, and failure to account for sex differences in QT interval dynamics[307]. Moreover, these correction factors do not address additional confounding from disease effects on the QT interval which are independent of heart rate. In this meta-analysis, I performed regression analyses[307] with heart rate (as the square root transformed RR interval), age, sex, and malaria disease variables (type, temperature, and parasitaemia) as predictors. This approach avoided the problems of proportional scaling for heart rate correction by retaining an intercept term and investigated any independent additive effects of malaria disease on the QT interval with multivariable regression. As malaria is

associated with high heart rates, the distortions produced from proportional scaling for heart rate correction seen in healthy participants would be even more pronounced in malaria patients. The effects of malaria severity and temperature seen in these analyses further suggest that meaningful comparison of corrected QT intervals between healthy participants and malaria patients, or even between repeated measurements from the same individual comparing acute malaria with recovery may be difficult without appropriate adjustment for these malaria disease effects.

This study has several potential limitations. First, data were available from only about half of the participants identified. However, included studies were more likely to have a low risk of bias than excluded studies, as most were conducted in the last decade and had more comprehensive measurement and reporting methods, reflecting the increased regulatory interest in the cardiac safety of antimalarials. Second, it was not possible to assess directly the effects of other factors known to contribute to the intrinsic variability of the QT interval such as circadian rhythm, activity level, postural changes, and food ingestion[136], as well as those which may alter cardiomyocyte electrophysiology during systemic illness such as inflammatory biomarkers[298], as these data are not usually collected in malaria clinical trials. Most hospitalised malaria patients are supine and anorexic. Third, I have considered interindividual measurements from a single timepoint before drug administration, an approach which allowed me to consider data from a large number of patients with malaria and a smaller number of healthy participants without confounding from drug therapy. While assessments of repeated measurements from patients undergoing treatment for malaria with drugs not known to prolong the QT interval would be valuable, these data are few[248] and were not available to me.

Evaluation of QT interval prolongation after treatment with the quinoline and structurally related antimalarials has been the major motivation for ECG monitoring in malaria. ECG

monitoring is an operational challenge in the resource-limited settings where malaria is endemic and would severely limit the use of any drug for which monitoring is mandatory. In this large study of the QT interval in malaria, I have found malaria shortens the QT interval and increases its sensitivity to changes in heart rate. These differences are greater in severe malaria[60]. In addition, fever shortens the QT and recovery from fever lengthens it. In acute uncomplicated malaria there is usually an irregular fever with an appropriate rise in heart rate. As the illness and fever resolve the heart rate declines to normal. This often coincides with the highest blood concentrations of slowly eliminated quinoline antimalarial drugs which usually reach peak concentrations on the third day of treatment. The QT interval lengthening seen with recovery from malaria after antimalarial therapy results from resolution of disease effects in addition to any drug effects. Comparisons of pre-drug QT interval measurements with those at peak drug concentrations in malaria studies should take into account malaria and fever recovery-related QT lengthening; this would avoid excessive attribution of QT prolongation to the antimalarial treatment and improve risk assessments of potential antimalarial-related cardiotoxicity. This could avoid unnecessary discontinuation of new drug development, and reduce the need for unnecessary adjustment or withdrawal of antimalarial treatment in response to malaria-related QT changes during research trials and clinical care. Similar adjustments may also be indicated for other febrile illnesses for which QT interval-prolonging medications are important therapeutic options.

5.6 AUTHORSHIP STATEMENT

I designed this study with Professor Nicholas White, conducted the systematic review, carried out the study-level data extraction and individual patient-level data gathering from study investigators, compiled the data dictionary, mapped each received study to the data dictionary, formatted received files for data standardisation, wrote the Python scripts for data standardisation, prepared the standardised data for statistical analysis, performed the statistical analysis, made all the figures and tables, and wrote all the text. Nia Roberts of the Bodleian Libraries assisted with the literature search. Dr Ilsa Haeusler, Dr Yan Naung Win, and Dr Shu Kiat Chan were the additional independent reviewers in the systematic review and data extraction. Dr Yan Naung Win assisted with manual data entry, standardisation of file formats, and mapping of individual studies. Jireh Tan engineered the bespoke Python Application Programming Interface used for data standardisation, wrote the R script for database assembly, and provided database programming support. Dr Yan Naung Win, Dr Shu Kiat Chan, and Dr Laura Mawer replicated additional Python scripts for data standardisation. Dr Sompob Saralamba provided computing resources as well as advice on statistical programming and methodology. Shanghavie Loganathan assisted with spatial data standardisation and visualisation with programming input from Dr Michael Chipeta of the Big Data Institute.

6 The Cardiovascular Effects of Amodiaquine

6.1 SUMMARY

Background

Amodiaquine is a 4-aminoquinoline antimalarial used extensively for the treatment and prevention of malaria. Data on the cardiovascular effects of amodiaquine are scarce, although transient effects on cardiac electrophysiology (electrocardiographic QT interval prolongation and sinus bradycardia) have been observed. We investigated the cardiovascular effects of amodiaquine to aid development of dose optimisation and risk minimisation measures to improve tolerability of this important antimalarial.

Methods

We conducted a meta-analysis of individual patient data from studies of amodiaquine for malaria identified from a systematic review. Heart rates and QT intervals with study-specific heart rate correction (QTcS) were compared within studies then pooled for multivariable linear mixed effects regression. Concentration-effect analyses were also performed where amodiaquine and desethylamodiaquine concentrations were available for pharmacokinetic modelling.

Findings

The meta-analysis included 2,681 patients from four randomised controlled trials. Amodiaquine prolonged QTcS (mean=16.9ms, 95% CI: 15.0-18.8) less than chloroquine (21.9ms, 18.3-25.6, p=0.0069) and piperaquine (19.2ms, 15.8-20.5, p=0.0495) but more than lumefantrine (5.6ms, 2.9-8.2, p<0.0001) and pyronaridine (-1.2ms, -3.6 to +1.3, p<0.0001). In individuals aged ≥12 years, amodiaquine reduced heart rate (15.2bpm, 95% CI: 13.4-17.0) more than piperaquine (10.5, 7.7-13.3, p=0.0013), lumefantrine (9.3bpm, 6.4-12.2, p=0.0001), pyronaridine (6.6bpm, 4.0-9.3, p<0.0001) and chloroquine (5.9bpm, 3.2-8.5, p<0.0001),

and was associated with a higher risk of potentially symptomatic sinus bradycardia (≤50bpm) than lumefantrine (risk ratio: 6.4, 95% CI: 1.5-27.4, p=0.0031) and chloroquine (risk difference: 8%, 95% CI: 4.0-12.0, p<0.0001). Amodiaquine-related changes were transient and concentration-dependent (QTcS: 1.4ms per 100nmol/L; heart rate: 1.9bpm per 100nmol/L). Adverse event data were insufficient for analysis but no serious complications were documented.

Interpretation

Amodiaquine is safe for the treatment and prevention of malaria. Bradycardia may underlie amodiaquine-associated asthenia. Caution is advised in the use of amodiaquine in adolescent and adult patients with cardiac conduction disorders and concomitant use of heart rate-reducing medications, particularly in the presence of other torsade de pointes risk factors.

6.2 INTRODUCTION

Amodiaquine, a 4-aminoquinoline structurally similar to chloroquine, is an important antimalarial drug which has been deployed extensively in both the treatment and prevention of malaria over the past 60 years. The artemisinin-based combination therapy (ACT) artesunate-amodiaquine (ASAQ) is recommended by the World Health Organization (WHO) for the treatment of uncomplicated *Plasmodium falciparum* and *P. vivax* malaria[30] and is the first-line oral antimalarial in more than 20 African countries[16] where malaria is endemic. The WHO also recommends that amodiaquine with sulfadoxine-pyrimethamine (AQ + SP) is given as seasonal malaria chemoprevention to young children (aged 3-59 months) living in areas of seasonal high-intensity malaria transmission in the Sahel subregion of Africa, with millions of children protected every year[33]. Both ASAQ and AQ + SP are administered daily over three days[30].

Amodiaquine is generally well-tolerated. Serious adverse reactions[96] of neutropaenia[88], hepatitis[90], and extrapyramidal movement disorders[104,309] are rare to very rare[105] following standard treatment doses. In large cohort event monitoring and safety studies, artesunate-amodiaquine has been associated with a higher incidence of asthenia[310,311], gastrointestinal disorders (nausea or vomiting[310], appetite disorders[310]), neurological disorders (dizziness[311], headaches[310]), and pruritus[310] than other ACTs, with asthenia[309-311] having been consistently the most commonly reported reaction. The physiological mechanisms underlying these non-serious but common adverse drug reactions are not well-understood.

The cardiotoxicity of the quinoline antimalarials has received renewed interest[47] following the withdrawal of halofantrine and findings of QT interval prolongation during pre-registration safety evaluations of the ACT dihydroartemisinin-piperaquine[119]. However, the risk of sudden death following dihydroartemisinin-piperaquine has since been found

to be not higher than baseline at standard treatment doses[209]. Conversely, the structurally related antimalarials quinidine and halofantrine have marked effects on ventricular repolarisation and clinically unacceptable risks of life-threatening cardiovascular effects including torsade de pointes (TdP) and sudden death[47]. Data on the cardiovascular effect profile of amodiaquine are scarce[248] despite its widespread use[48], amodiaquine having been introduced in the 1950s, when the risk of drug-induced heart rhythm abnormalities was not appreciated.

In the few studies in which patients have undergone electrocardiographic monitoring[248] after a full treatment course of amodiaquine, sinus bradycardia[190,253,254] and QT interval prolongation[122,189,190,253,254] have been observed. Cardiovascular effects may provide a unifying explanation for the asthenia and asthenia-like reactions commonly reported after amodiaquine. Further characterisation of how amodiaquine affects the cardiovascular system would aid development of dose optimisation and risk minimisation measures to improve tolerability of and adherence to this important antimalarial. I therefore undertook a meta-analysis of all available clinical data for systematic evaluation of the cardiovascular effects of amodiaquine, focusing on the heart rate and the electrocardiographic QT interval.

6.3 METHODS

6.3.1 Search Strategy and Selection Criteria

Studies were identified from a systematic review of the quinoline and structurally related antimalarials for malaria-related indications with ECG monitoring[248], which was last updated on 21 August 2017 as described in Chapter 5 (see also 2.4.2). Amodiaquine was one of the nine antimalarials reviewed.

Prospective randomised-controlled trials or cohort studies published from 1988 onwards in which five or more human participants were given amodiaquine as monotherapy or as part of an ACT in a full 3-day treatment course with systematic ECG monitoring before and after antimalarial administration were eligible for inclusion in this meta-analysis.

I contacted study authors with a request for clinical study reports and protocols as well as anonymised individual patient-level datasets of the following pre-specified variables identified from expert consultation[47]: age, weight, sex, body temperature, parasitaemia, haemoglobin or haematocrit, blood pressure, heart rate or RR interval duration, uncorrected QT interval duration, QRS interval duration, PR interval duration, ECG abnormalities, other cardiovascular adverse events, antimalarial dose received, concomitant medications, and antimalarial pre-treatment received. In addition, amodiaquine and structurally-related quinoline antimalarial drug concentrations were requested for the studies in which these were measured. Data gathering was in line with existing ethical approvals (see 2.4.3.1).

6.3.2 Data Extraction and Standardisation

I screened titles, abstracts, full texts, trial documentation, and anonymised datasets, and agreed study eligibility with another independent reviewer. From study publications,

reports, and protocols I extracted study-level characteristics including location, patient population, antimalarial treatment indication, antimalarial dosing regimen, inclusion and exclusion criteria, temperature measurement method, and ECG measurement methodology into a standardised database (see 2.4.3.2). Where required, trial registry records and study investigators were consulted for further information.

Individual patient-level datasets were standardised and checked according to a pre-specified data dictionary (see 2.4.3.3). Only data from scheduled time points and from the first treatment episode for studies of repeated treatments were extracted. Individual patient records were excluded if both QT and RR interval measurements were unavailable, or if antimalarial treatment arm was missing.

6.3.3 Data Analysis

Measurements from fixed and non-fixed dose ASAQ arms were pooled for all analyses as these dose formulations are known to be bioequivalent for amodiaquine and to not have an effect on the pharmacokinetic parameters of these drugs[191,312]. It is generally accepted that artesunate does not have a significant effect on the QT interval[53].

In view of the inverse relationship between the QT interval and heart rate, measured QT intervals were adjusted for heart rate with the widely-used Bazett[269] ($QTcB = \frac{QT}{\sqrt{RR}}$) and Fridericia[270] ($QTcF = \frac{QT}{\sqrt[3]{RR}}$) correction formulae. Age group-specific correction formulae for each individual study ($QTcS = \frac{QT}{\beta_{age}\sqrt{RR}}$) were also applied with the correction exponent β_{age} derived from log-log linear regression (see 2.4.5.1). Scatterplots of resulting corrected QT and RR interval relationships with linear regression and 95% confidence intervals were produced for each correction method, individual study, and ECG time point.

6.3.3.1 Study-Level Analyses

Within each study, electrocardiographic heart rate and corrected QT interval measurements were compared across treatment arms at each available scheduled time point, namely: baseline/pre-dose (Day 0), post-dose (Day 2 or 3), and, where available, late (Day 28).

Mean heart rates as well as the proportion of participants with heart rates of ≤60 and ≤50 beats/minute or age-equivalent thresholds (see 2.4.5.1) and their risk ratios were evaluated, the higher bradycardia threshold being the lower limit of the normal range, and the lower threshold the rate below which individuals could be symptomatic. Mean corrected QT interval comparisons and categorical analyses of the proportion of participants with corrected QT intervals >450, >480, and >500 milliseconds as well as corrected QT interval prolongation of >30 and >60 milliseconds from baseline at each time point were also performed[136] (see 2.4.5.2). As body temperature is known to affect both heart rate and the QT interval, the median change in body temperature from baseline for each time point was also computed (see 2.4.5.2).

For the studies for which cardiovascular vital signs were available, scatterplots of pulse rate and blood pressure over time with loess regression and 95% confidence intervals were also produced.

6.3.3.2 Individual Patient-Level Data Meta-analyses

Data from baseline/pre-dose (Day 0) and within 6 hours after the final dose of antimalarial treatment (Day 2) from all studies for which these time points were available were pooled for multivariable linear mixed effects analyses. Where there were multiple Day 2 post-dose readings, the recording closest to the time of maximum concentration of the relevant antimalarial was used.

Two sets of models were fitted, with heart rate and the corrected QT interval from ECG measurements as the respective response variables. Study, site, and individual patient were modelled as nested random effects while fixed effect variable selection was based on directed acyclic graphs of proposed causal relationships among available variables (see 2.4.5.3).

In the heart rate models, body temperature and antimalarial drug arm as an interaction term with day of ECG measurement were the fixed effects. The demographic fixed effect in the heart rate model for adolescents and adults (age ≥12 years) was sex, and age in the heart rate model for children (age <12 years) (see also 2.4.5.3).

In the corrected QT interval models, body temperature and antimalarial drug arm as an interaction term with day of ECG measurement were again the fixed effects. In addition, as age and sex are known to interact to have a non-linear effect on the QT interval, sex was modelled as an interaction term with age group, a categorical variable created from grouping age into 5-year bands (see also 2.4.5.3).

6.3.3.3 Concentration-Effect Analyses

For the one study[189] for which antimalarial drug concentrations were available, pharmacokinetic modelling was used to generate predicted amodiaquine and desethylamodiaquine concentrations for the time points at which cardiovascular vital signs (pulse rate and blood pressure) and electrocardiographic intervals (QT, QRS, and PR) were measured (see 2.4.5.4).

Using these predicted drug concentrations, I performed multivariable linear mixed effects modelling with the change from baseline of pulse rate and blood pressure (systolic and diastolic in the supine and erect positions with their postural differences) as well as the corrected QT, QRS, and PR intervals as response variables. Individual patient was the

random effect while fixed effect selection was based on directed acyclic graphs of proposed causal relationships among available variables (see 2.4.5.5).

The drug effect was evaluated using the total predicted concentration of amodiaquine plus its metabolite desethylamodiaquine based on prior evidence that both amodiaquine[313,314] and desethylamodiaquine[253] affect cardiovascular physiology as well as lack of evidence either was more potent from descriptive analyses.

In the ECG interval models, the other fixed effects were body temperature change, age, and sex, with the addition of RR interval change to adjust for residual heart rate changes. For cardiovascular vital signs which had multiple time points after recovery from malaria, the malaria disease effect denoting a binary categorical variable which was present during days 0-2 of treatment was a fixed effect, with the addition of body temperature change and sex for the change in pulse rate model (see 2.4.5.5).

6.4 RESULTS

6.4.1 Studies

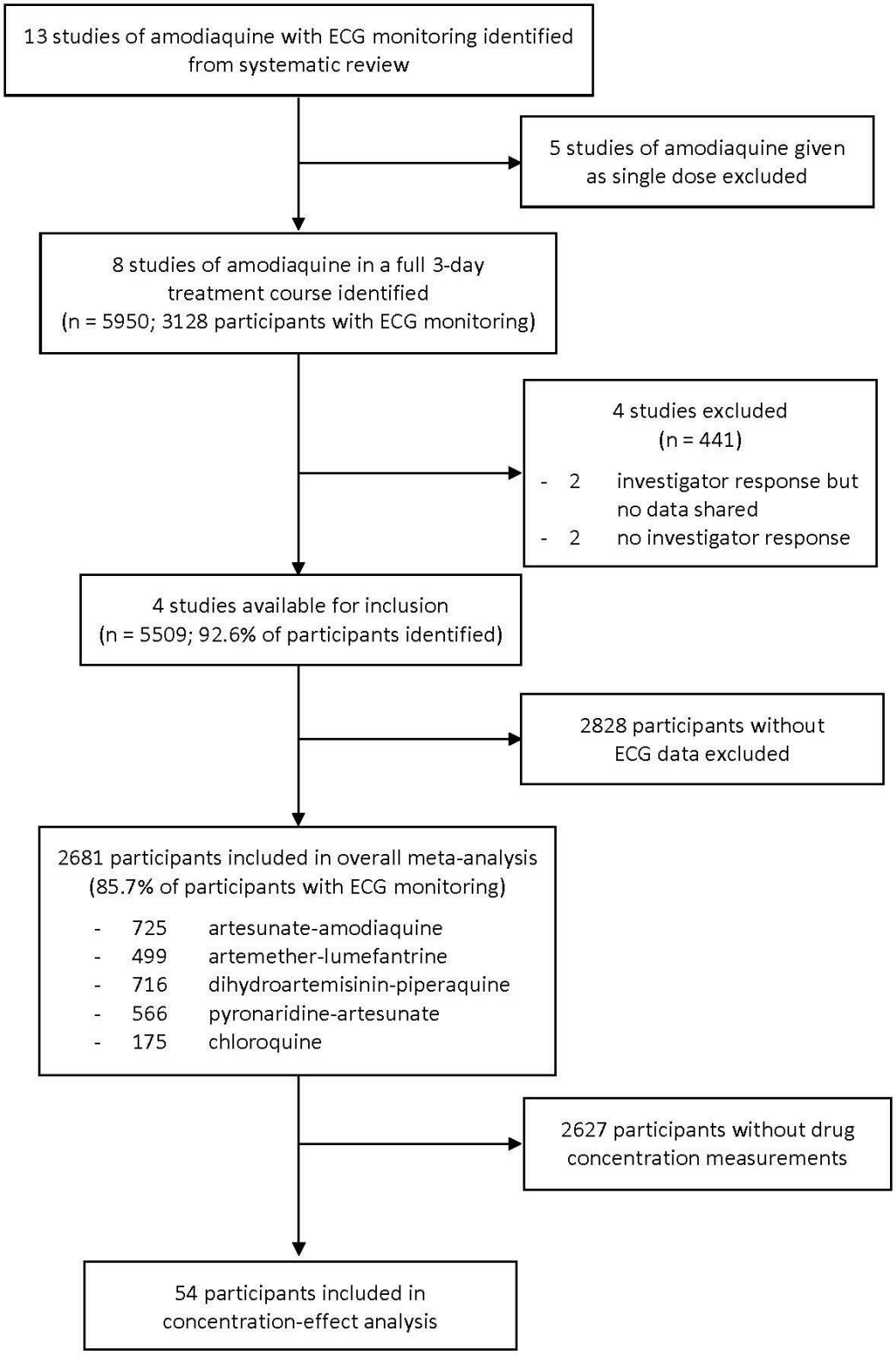
6.4.1.1 Study and Participant Selection

The systematic review, described in detail in Chapter 5, identified 159 studies of the quinoline and structurally related antimalarials with ECG monitoring before and after antimalarial treatment. Of these, thirteen studies were of amodiaquine administered as monotherapy or as an ACT with artesunate: eight[122,188-190,253,254,315,316] were of amodiaquine in a full 3-day once daily treatment course for uncomplicated *P. falciparum* or *P. vivax* malaria while the remaining five[265,285,317-319] were healthy volunteer pharmacokinetic studies in which only a single dose of amodiaquine was administered. These five studies of amodiaquine in a single dose were excluded (Figure 6.1). There were no studies of amodiaquine as preventive therapy such as seasonal malaria chemoprevention.

There were no cases of sudden cardiac death, life-threatening ventricular tachyarrhythmias (ventricular fibrillation or ventricular tachycardia), TdP, or any other serious cardiovascular events reported in any of the 5950 patients in the eight studies from which individual patient-level data were sought.

Data were available from four studies which enrolled 5,509 participants, of which 2,681 individuals had ECG data. These comprised 85.7% of the 3,128 participants identified who had ECG monitoring (Figure 6.1).

Figure 6.1: Selection of Studies of Amodiaquine with Electrocardiographic Monitoring



6.4.1.2 Included Studies – Design, Dosing, and Outcomes

Four studies shared individual patient data and were included. These were randomised-controlled trials[122,188-190] (Table 6.1) which compared the fixed-dose ACT artesunate-amodiaquine with either non-fixed dose amodiaquine with artesunate or another WHO-recommended oral antimalarial – artemether-lumefantrine, chloroquine, dihydroartemisinin-piperaquine, or pyronaridine-artesunate – in 3-day treatment courses dosed according to body weight (Appendix 11.4.1 – Table 6.5).

All four studies performed heart rate and QT interval measurements, while three[122,188,189] measured QRS and PR intervals in addition. While all four studies collected cardiovascular vital sign measurements and individual patient-level adverse event data, only two[122,189] shared these (Table 6.2). Of these, one study of fixed versus non-fixed dose artesunate-amodiaquine (n = 54) measured amodiaquine and desethylamodiaquine drug concentrations over time using liquid chromatography-mass spectrometry/mass spectrometry methods[189].

Table 6.1: Characteristics of Included Studies of Amodiaquine with Electrocardiographic Monitoring

Study Reference	Ndiaye 2011[188]	Ogutu 2014[189]	Siqueira 2017[190]	WANECAM 2018[122]
Country	Senegal	Kenya	Brazil	Burkina Faso, Guinea, Mali
Trial Population (with ECG Monitoring)	Adults & Children (ECG monitoring if aged ≥12 years)	Adults (All)	Adults & Children (ECG monitoring if aged >10 years)	Adults & Children (All)
Treatment Indication	Uncomplicated *P. falciparum*	Uncomplicated *P. falciparum*	Uncomplicated *P. vivax*	Uncomplicated *P. falciparum*
Days of Follow-up	28	28	42	42
Amodiaquine-Containing Drugs (Manufacturer)	ASAQ (Sanofi)	ASAQ (Sanofi) & AQ + AS (Sanofi + Guilin)	ASAQ (Sanofi)	ASAQ (Sanofi)
Non-amodiaquine Drugs (Manufacturer)	AL (Novartis)	None	CQ (Farmanguinhos)	AL (Novartis), DP (Sigma Tau), PA (Shin Poong)
Directly Observed Therapy	All doses	All doses	All doses	All doses
Number of Participants in Amodiaquine Drug Arms (with ECG Monitoring)	184 (77)	54 (54)	189 (179)	1061 (417)
Number of Participants in Non-amodiaquine Drug Arms (with ECG Monitoring)	182 (77)	0	190 (175)	3649 (1708)
TdP Risk Factors Excluded at Screening	Yes	Yes	No	Yes
Pharmacokinetic Sampling (Food Intake)	No	Yes (starved)	No	No
Vital Sign Measurement Time Points, Protocol Days	0 (pre-dose), 1, 2, 3, 7, 14, 28	0 (pre-dose), 1, 2, 3, 7, 14, 21, 28	0 (pre-dose), 1, 2, 3, 7, 14, 28, 42	0 (pre-dose), 1, 2, 3, 7, 14, 28, 35, 42
ECG Measurement Time Points, Protocol Days	0 (pre-dose), 3 (post-dose)	0 (pre-dose, +2h, +4h), 2 (+2h, +4h), 28	0 (pre-dose), 2 (post-dose), 28	0 (pre-dose), 2 (+2-4h)
Pharmacokinetic Sampling Time Points, Protocol Days	None	0 (pre-dose, +0.25-4h), 1 (+0.25-4h), 2 (+0.25-4h), 7, 14, 21, 28	None	None

ECG = electrocardiogram, TdP = torsades de pointes, PK = pharmacokinetic, ASAQ = artesunate-amodiaquine fixed-dose combination, AQ + AS = artesunate + amodiaquine non-fixed dose combination, AL = artemether-lumefantrine fixed-dose combination, CQ = chloroquine, DP = dihydroartemisinin-piperaquine fixed-dose combination, PA = pyronaridine-artesunate fixed-dose combination

Table 6.2: Adverse Events, Pre-Treatment and Concomitant Medications in Included Studies of Amodiaquine

Study Reference	Ndiaye 2011[188]	Ogutu 2014[189]	Siqueira 2017[190]	WANECAM 2018[122]
Adverse Event IPD	Not available	Available	Available	Not available
Antimalarial Pre-Treatment, Subjects (% Total Subjects)	Not available	7 (13%)	55 (14%)	Not available
Antimalarial Pre-Treatment Drug (% Pre-treated Subjects)	Not available	Chloroquine (71%) or Artemether-lumefantrine (29%)	Artesunate-mefloquine (95%) or Chloroquine (4%) or Artemether-lumefantrine (1%) +/- Primaquine (96%)	Not available
Cardiovascular Concomitant Medications, Subjects (% Total Subjects)	Not available	None	18 (4.7%): 3 with more than one drug	Not available
Cardiovascular Concomitant Medication (% Concomitantly Treated Subjects)	Not available	None	ACE inhibitor (39%) Beta blocker (33%) Thiazide or thiazide-like diuretic (22%) A2R blocker (17%) Calcium channel blocker (5.6%)	Not available

IPD = individual participant-level data; ECG = electrocardiogram; ACE = angiotensin-converting enzyme; A2R = angiotensin II receptor

6.4.2 Risk of Bias Assessment

Compared to included studies, studies for which data were unavailable were older (all except one completed enrolment before 2007), used amodiaquine in non-fixed dose combination therapies or monotherapy, had smaller populations from a more restricted demographic, and were less likely to have cardiologists as ECG readers. Other characteristics were otherwise comparable (Table 6.3).

All studies had low risk of bias overall (Appendix 11.4.2 – Table 11.6).

Table 6.3: Comparison of Characteristics of Included and Excluded Studies of Amodiaquine with Electrocardiographic Monitoring

	Included Studies (n = 4)	Excluded Studies (n = 4)
Antimalarial Treatment Indication, studies (%)		
Uncomplicated malaria	4 (100%)	4 (100%)
P. falciparum mono- or mixed infection	3 (75%)	4 (100%)
P. vivax mono-infection	1 (25%)	0
Amodiaquine Formulation, amodiaquine-containing drug arms (%)		
Fixed-dose combination therapy with artesunate	4 (80%)	0
Non-fixed dose combination therapy	1 (20%)	4 (66.7%)
With artesunate	0	3 (50%)
With sulfadoxine-pyrimethamine	0	1 (16.7%)
Monotherapy	0	2 (33.3%)
Patient Population, studies (%)		
Adults & children	3 (75%)	0
Adults only (male and female)	1 (25%)	1 (25%)
Adults only (males only)	0	1 (25%)
Children only	0	1 (25%)
Pregnant women only	0	1 (25%)
Geographical Region, studies (%)		
Africa	3 (75%)	4 (100%)
Americas	1 (25%)	0
Year Enrolment Completed, studies (%)		
2007-2017	4 (100%)	1 (25%)
Pre-2007	0	3 (75%)
Study Design, studies (%)		
Randomised Controlled Trial	4 (100%)	3 (75%)
Cohort	0	1 (25%)
ECG Reader, studies (%)		
Cardiologist	4 (100%)	1 (25%)
Physician or other trained personnel	0	1 (25%)
Machine	0	1 (25%)
Not detailed	0	1 (25%)
Torsade de Pointes Risk Factors Excluded, studies (%)	3 (75%)	2 (50%)
Mean Age in Years, median (IQR)	17.8 (11.4-26.6)	24.0 (17.4-28.6)
Percentage of Females, median (IQR)	49.55 (40.5-53.7)	Not reported*

*1 study of pregnant women, 1 study of adult males only, 2 studies did not report number of females

6.4.3 Populations and Study-Level Analyses

6.4.3.1 Included Population – Baseline Characteristics

Baseline characteristics of the included population in the ECG interval analyses of artesunate-amodiaquine and comparator antimalarials of artemether-lumefantrine, chloroquine, dihydroartemisinin-piperaquine, and pyronaridine-artesunate are summarised in Table 6.4.

The multi-site West African WANECAM 2018 study (n = 2125) had the youngest ECG population with a paediatric median age of 8.3 years (IQR: 5.1-12.1, range: 0.5-71.6), followed by the Ndiaye 2011 study from Senegal (n = 148) with a median age of 15.0 years (IQR: 13.0-20.0, range: 11.0-65.0), and the Ogutu 2014 study from Kenya (n = 54) with a median age of 24.0 years (IQR: 19.0-32.0, range: 18.0-60.0). These three uncomplicated *P. falciparum* studies from sub-Saharan Africa had otherwise clinically comparable baseline disease characteristics of temperature and parasitaemia between treatment arms and across studies. The remaining study, Siqueira 2017 from Brazil (n = 354), was of older and mostly male (75.1%, 266/354) adults with *P. vivax* malaria and had a median age of 36.4 years (IQR: 26.6-48.7, range: 10.3-74.9).

Table 6.4: Baseline Characteristics of Included Population in Electrocardiographic Heart Rate and QT Interval Analyses

Study Reference	**Ndiaye 2011[188]**		**Ogutu 2014[189]**	**Siqueira 2017[190]**		**WANECAM 2018[122]**			
Drug Arm	*ASAQ*	*AL*	*ASAQ*	*ASAQ*	*CQ*	*ASAQ*	*AL*	*DP*	*PA*
Number of Individuals	75	73	54	179	175	417	426	716	566
Age (years)									
Median (IQR)	19.7 (13.0-18.0)	16.0 (14.0-20.0)	24.0 (19.0-32.0)	37.4 (25.1-50.9)	35.8 (28.5-47.7)	7.7 (5.0-11.4)	8.6 (5.0-13.2)	8.1 (5.1-11.3)	9.0 (5.5-13.6)
<12	1 (1.3%)	1 (1.4%)	0	2 (1.1%)	1 (0.6%)	335 (80.3%)	298 (70.0%)	567 (79.2%)	375 (66.3%)
0.5-<2	0	0	0	0	0	11 (2.6%)	10 (2.3%)	18 (2.5%)	7 (1.2%)
2-<5	0	0	0	0	0	95 (22.8%)	90 (21.1%)	150 (20.9%)	99 (17.5%)
5-<12	1 (1.3%)	1 (1.4%)	0	2 (1.1%)	1 (0.6%)	229 (54.9%)	198 (46.5%)	399 (55.7%)	269 (47.5%)
≥12	74 (98.7%)	72 (98.6%)	54 (100%)	177 (98.9%)	174 (99.4%)	82 (19.7%)	128 (30.0%)	149 (20.8%)	191 (33.7%)
12-<18	52 (69.3%)	45 (61.6%)	0	18 (10.0%)	14 (8.0%)	71 (17.0%)	97 (22.8%)	116 (16.2%)	122 (21.6%)
18-<35	9 (12.0%)	21 (28.8%)	43 (79.6%)	62 (34.6%)	64 (36.6%)	8 (1.9%)	22 (5.2%)	25 (3.5%)	53 (9.4%)
35-<50	11 (14.7%)	4 (5.5%)	8 (14.8%)	50 (28.2%)	62 (35.4%)	3 (0.7%)	6 (1.4%)	6 (0.8%)	14 (2.5%)
≥50	2 (2.7%)	2 (2.7%)	3 (5.6%)	47 (26.3%)	34 (19.4%)	0	3 (0.7%)	2 (0.3%)	2 (0.4%)
Weight (kg)									
Median (IQR)	40.1 (33.3-52.8)	48.6 (32.4-56.8)	59.0 (53.0-62.0)	71.0 (61.1-80.5)	72.0 (61.7-82.0)	20.7 (15.9-28.2)	22.5 (16.2-34.2)	22.0 (16.1-30.7)	24.3 (17.2-41.6)
Sex									
Female	33 (44.0%)	32 (43.8%)	29 (53.7%)	46 (25.7%)	42 (24.0%)	190 (45.6%)	208 (48.8%)	348 (48.6%)	291 (51.4%)
Male	42 (56.0%)	41 (56.2%)	25 (46.3%)	133 (74.3%)	133 (76.0%)	227 (54.4%)	218 (51.2%)	368 (51.4%)	275 (48.6%)
Temperature (°C)									
Median (IQR)	37.5 (37.0-38.8)	37.7 (36.7-39.0)	37.7 (37.1-38.6)	37.7 (36.7-38.7)	37.4 (36.7-38.5)	38.1 (37.3-38.9)	38.2 (37.4-39.1)	38.2 (37.3-39.0)	38.2 (37.3-39.1)
≥37.5	39 (52.0%)	39 (53.4%)	35 (64.8%)	104 (58.1%)	87 (49.7%)	298 (71.5%)	311 (73.0%)	515 (71.9%)	402 (71.0%)
Parasitaemia (parasites/µL)									
Median (IQR) [*P. falciparum*] [*P. vivax*]	12677 (4835-40263)	15813 (5537-32045)	17181 (5317-25876)	1978 (836-3739)	1764 (819-3373)	12940 (840-37160)	24980 (4380-67530)	15680 (1280-41640)	18170 (1695-51870)
>10,000-50,000	29 (38.7%)	33 (45.2%)	24 (44.4%)	15 (8.4%)	4 (2.3%)	153 (36.7%)	150 (35.2%)	269 (37.6%)	195 (34.5%)
>50,000-100,000	9 (12.0%)	12 (16.4%)	6 (11.1%)	0	0	45 (10.8%)	71 (16.7%)	84 (11.7%)	92 (16.3%)
>100,000-250,000	6 (8.0%)	0	1 (1.9%)	0	0	29 (7.0%))	64 (15.0%)	54 (7.5%)	60 (10.6%)

Haemoglobin (g/dL)									
Median (IQR)	12.5 (11.7-13.7)	12.1 (11.1-13.6)	13.2 (11.9-14.8)	13.5 (12.4-14.8)	13.6 (12.5-14.5)	10.5 (9.6-11.3)	10.7 (9.7-11.6)	10.6 (9.6-11.5)	10.8 (9.8-11.8)
<8	0	1 (1.4%)	0	0	1 (0.6%)	17 (4.1%)	15 (3.5%)	41 (5.7%)	26 (4.6%)
Heart Rate (beats/minute)									
Mean (SD)	94.9 (19.2)	95.8 (21.9)	91.7 (15.7)[a]	90.3 (21.3)	88.4 (19.1)	109.1 (22.0)	108.8 (22.0)	108.9 (21.6)	106.0 (22.8)
≥140	1 (1.3%)	2 (2.7%)	0	2 (1.1%)[b]	1 (0.6%) [b]	37 (8.9%)	36 (8.5%)	57 (8.0%)	45 (8.0%)
120-<140	5 (6.7%)	10 (13.7%)	2 (3.8%)[a]	17 (9.6%) [b]	6 (3.5%)[b]	99 (23.7%)	96 (22.5%)	169 (23.6%)	119 (21.0%)
100-<120	27 (36.0%)	15 (20.5%)	17 (32.1%)[a]	42 (23.7%)[b]	39 (22.3%)[b]	138 (33.1%)	149 (35.0%)	248 (34.6%)	174 (30.7%)
80-<100	23 (30.7%)	26 (35.6%)	21 (39.6%)[a]	56 (31.6%)[b]	69 (39.9%)[b]	110 (26.4%)	105 (24.6%)	177 (24.7%)	154 (27.2%)
60-<80	17 (22.7%)	18 (24.7%)	13 (24.5%)[a]	51 (28.8%)[b]	49 (28.3%)[b]	29 (7.0%)	38 (8.9%)	57 (8.0%)	70 (12.4%)
<60	2 (2.7%)	2 (2.7%)	0	9 (5.1%)[b]	9 (5.2%)[b]	4 (1.0%)	2 (0.5%)	8 (1.1%)	4 (0.7%)

IQR = interquartile range, SD = standard deviation, ASAQ = artesunate-amodiaquine, AL = artemether-lumefantrine, CQ = chloroquine, DP = dihydroartemisinin-piperaquine, PA = pyronaridine-artesunate, [a]1 participant had missing baseline heart rate, [b]2 participants had missing baseline heart rates, [c]3 participants had missing baseline heart rates

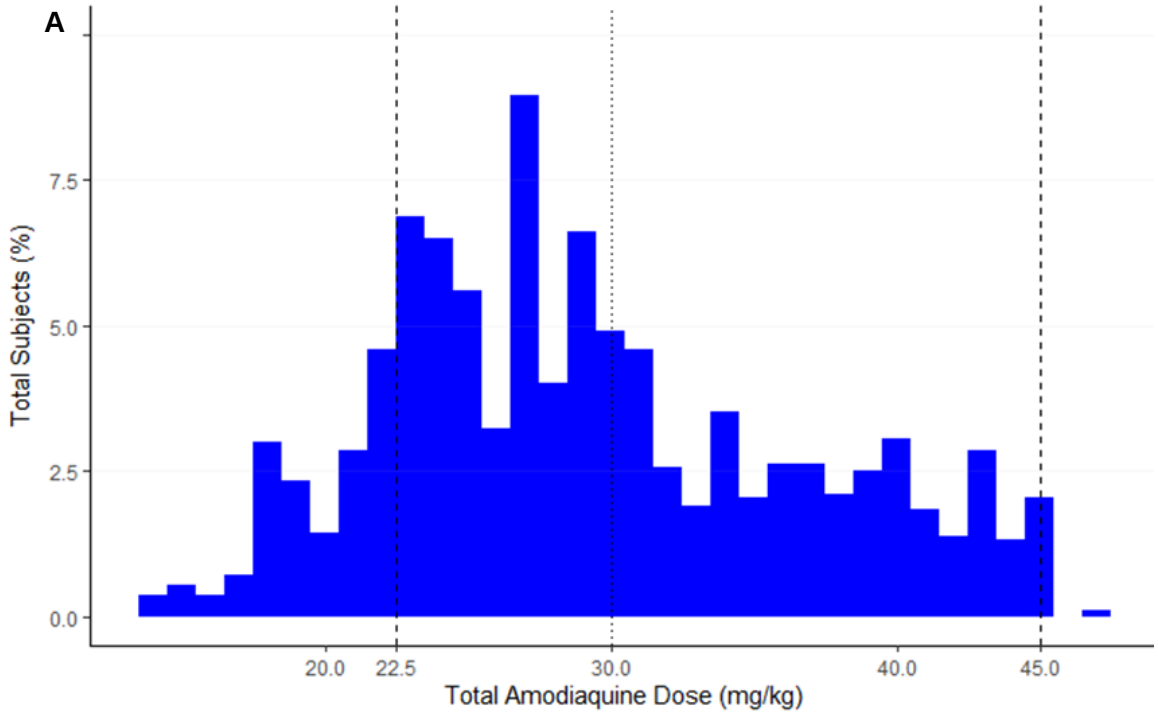
6.4.3.2 Included Population – Quinoline/Quinoline-like Antimalarial Dose Received

Most of the 725 participants treated with artesunate-amodiaquine received total mg/kg doses of amodiaquine over three days within the WHO-recommended range of 22.5-45mg/kg[30] body weight (Figure 6.2, panel A), other than the 86 (11.9%) adult participants weighing more than 72kg who received doses below the recommended range (Figure 6.2, panel B).

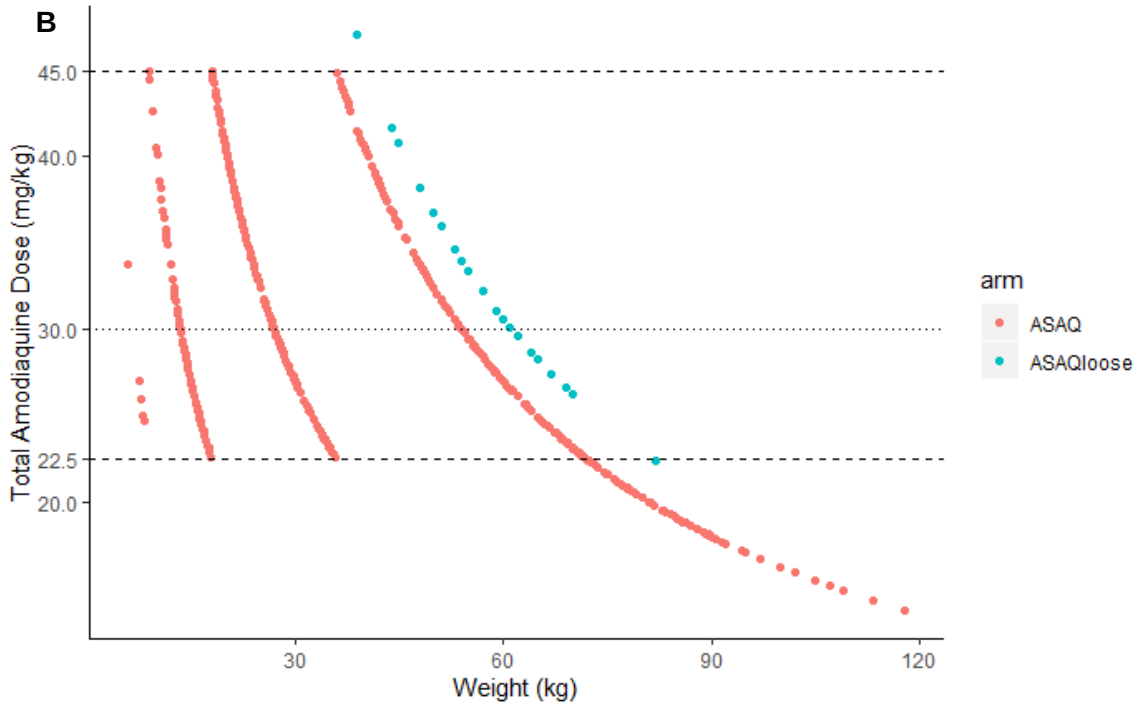
147 (84%) of the 175 participants treated with chloroquine, 139 (94%) of whom were adults weighing more than 60kg, received total chloroquine doses below the WHO-recommended dose of 25mg/kg[30] body weight (Figure 6.3, panel A). Of the 716 participants treated with dihydroartemisinin-piperaquine, 177 (24.7%) received a total dose of piperaquine below the WHO-recommended range of 48-81mg/kg[30], 161 (91%) of whom were children weighing under 25kg (Figure 6.4, panel B).

All participants treated with artemether-lumefantrine and pyronaridine-artesunate received total doses of lumefantrine and pyronaridine within WHO-recommended ranges of 29-144[30] and 22.5-45mg/kg[123] body weight respectively (Figure 6.4, panels C & D).

Figure 6.2: Total Amodiaquine Dose Received by Frequency and Individual Body Weight

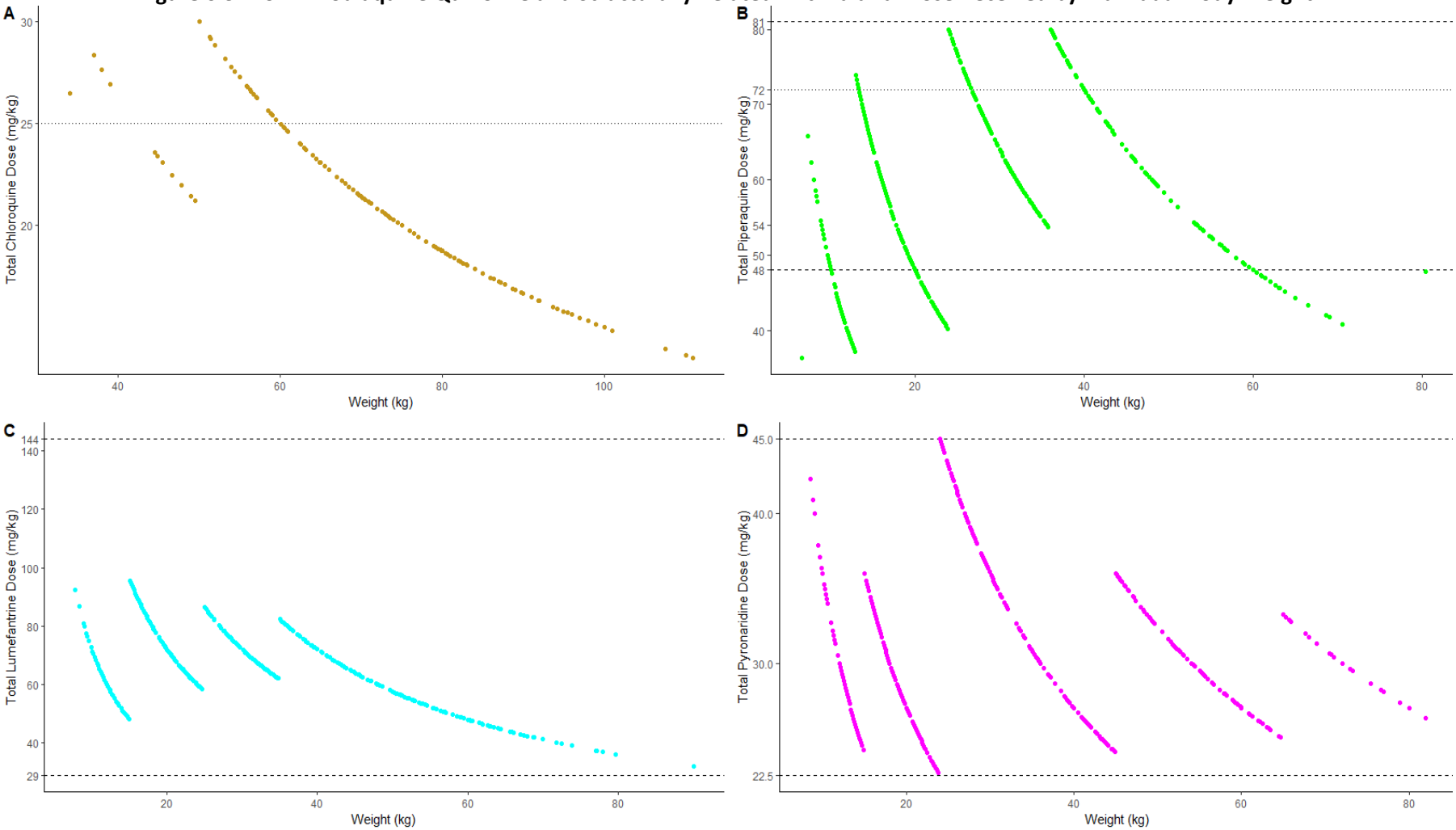


Vertical lines demarcate the WHO-recommended total therapeutic dose (dotted) and range (dashed) for amodiaquine of 30 (22.5-45) mg/kg body weight over 3 days[30]



Horizontal lines demarcate the WHO-recommended total therapeutic dose (dotted) and range (dashed) for amodiaquine of 30 (22.5-45) mg/kg body weight over 3 days[30]

Figure 6.3: Non-Amodiaquine Quinoline and Structurally Related Antimalarial Dose Received by Individual Body Weight



Horizontal lines demarcate the WHO-recommended total therapeutic dose (dotted) and/or range (dashed) over 3 days in units of mg/kg body weight for (A) chloroquine (25), (B) piperaquine (72; 48-81), (C) lumefantrine (29-144), and (D) pyronaridine (22.5-45)

6.4.3.4 Study-Level Analyses – Heart Rate

Study-level analyses of heart rate measurements from ECG interval analysis are presented in Table 6.5.

Day 2 or 3 (Post-Dose)

In three of the studies, mean heart rates after the final dose of antimalarial treatment on day 2 or 3 (post-dose) in the artesunate-amodiaquine arms were just above 60 beats/minute, the lower limit of the normal range for adolescents and adults (Ndiaye 2011: 61.0 beats/minute, 95% CI: 58.9-63.1; Ogutu 2014: 60.4, 95% CI: 58.3-62.6; Siqueira 2017: 61.1, 95% CI: 59.7-62.4). These means were clinically and statistically significantly lower than in the relevant comparator arms of artemether-lumefantrine (mean: 69.9 beats/minute, 95% CI: 67.4-72.4, $p<0.0001$) and chloroquine (mean: 70.3 beats/minute, 95% CI: 68.7-72.0, $p<0.0001$). The proportions of participants with post-dose heart rates ≤60 and ≤50 beats/minute were larger in the artesunate-amodiaquine arms compared to the relevant artemether-lumefantrine (p<0.0001 & p=0.0070) and chloroquine (p<0.0001 & p=0.0004) arms. Patients treated with artesunate-amodiaquine had a higher relative risk of sinus bradycardia than those treated with artemether-lumefantrine (≤60 beats/minute risk ratio: 2.2, 95% CI: 1.4-3.2, p<0.0001; ≤50 beats/minute: 6.4, 95% CI: 1.5-27.4, p=0.0031) and chloroquine (≤60 beats/minute risk ratio: 2.6, 95% CI: 1.9-3.5, p<0.0001; ≤50 beats/minute risk difference: 8%, 95% CI: 4.0-12.0, p<0.0001). All three of these studies had adolescent and adult populations consisting almost entirely of individuals aged 12 years and older (Table 6.4).

Table 6.5: Summary Statistics of Heart Rate Measurements from ECG Interval Analyses of Individual Studies by Drug Arm and Time Point

Study Reference	**Ndiaye 2011[188]**			**Ogutu 2014[189]**	**Siqueira 2017[190]**			**WANECAM 2018[122]**				
Drug Arm	*ASAQ*	*AL*	*p*	*ASAQ*	*ASAQ*	*CQ*	*p*	*ASAQ*	*AL*	*DP*	*PA*	*p*
Total dose (mg/kg)[a]												
Median (IQR)	28.2 (25.0-36.1)	58.8 (50.7-67.3)		29.5 (27.4-31.6)	22.8 (20.1-26.5)	20.8 (18.3-23.4)		31.4 (27.0-38.6)	68.6 (60.5-79.4)	57.8 (48.0-67.6)	30.3 (27.0-34.8)	
Day 0 (Pre-Dose)												
Number of Individuals	75	73		53	177	173		417	426	716	566	
Heart Rate (beats/minute)												
Mean (SD)	94.9 (19.2)	95.8 (21.9)	0.7943[b]	91.7 (15.7)	90.3 (21.3)	88.4 (19.1)	0.3867[b]	109.1 (22.0)	108.8 (22.0)	108.9 (21.6)	106.0 (22.8)	0.0573[f]
≤60 or age-equivalent	2 (2.7%)	3 (4.1%)	0.6789[c]	1 (1.9%)	9 (5.1%)	9 (5.1%)	1[d]	12 (2.9%)	10 (2.3%)	21 (2.9%)	18 (3.2%)	0.8912[d]
≤50 or age-equivalent	1 (1.3%)	1 (1.4%)	1[c]	0	2 (1.1%)	2 (1.1%)	1[c]	2 (0.5%)	3 (0.7%)	4 (0.6%)	3 (0.5%)	1[c]
Day 2 or 3 (Post-Dose)												
Number of Individuals	74	73		51	175	174		417	426	716	566	
Heart Rate (beats/minute)												
Mean (SD)	61.0 (9.2)	69.9 (10.6)	<0.0001[b]	60.4 (7.5)	61.1 (9.0)	70.3 (11.3)	<0.0001[b]	81.5 (16.6)	83.3 (16.6)	85.2 (16.2)	86.8 (18.0)	<0.0001[f]
≤60 or age-equivalent	46 (62.1%)	21 (28.8%)	<0.0001[d]	29 (56.9%)	96 (54.9%)	37 (21.3%)	<0.0001[d]	112 (26.9%)	79 (18.5%)	122 (17.0%)	65 (11.5%)	<0.0001[d]
≤50 or age-equivalent	13 (17.6%)	2 (2.8%)	0.0070[d]	2 (3.9%)	14 (8.0%)	0	0.0004[d]	21 (5.0%)	11 (2.6%)	20 (2.8%)	10 (1.8%)	0.02375[d]
Change in Temperature (°C)												
Median (IQR)	-1.2 (-2.5 to -0.6)	-1.5 (-2.6 to -0.4)	0.9536[e]	-1.2 (-2.2 to -0.6)	-1.6 (-2.7 to -0.6)	-1.3 (-2.5 to -0.4)	0.2653[e]	-1.4 (-2.2 to -0.6)	-1.3 (-2.3 to -0.4)	-1.3 (-2.1 to -0.5)	-1.2 (-2.3 to -0.3)	0.4714[g]
Day 28 (Late)												
Number of Individuals				49	167	164						
Heart Rate (beats/minute)												
Mean (SD)				67.8 (12.9)	66.5 (12.5)	69.5 (11.1)	0.0247[b]					
≤60 or age-equivalent				18 (36.7%)	61 (36.5%)	35 (21.3%)	0.0035[d]					
≤50 or age-equivalent				3 (6.1%)	6 (3.6%)	1 (0.6%)	0.1211[c]					
Change in Temperature (°C)												
Median (IQR)				-1.1 (-2.1 to -0.2)	-1.5 (-2.5 to -0.5)	-1.1 (-2.4 to -0.3)	0.2346[e]					

[a]Quinoline and structurally-related drug only, [b]2-sample t-test with Welch modification for unequal variances, [c]Fisher's exact test, [d]Pearson's Chi-squared test for independence with Yates' continuity correction, [e]Wilcoxon rank sum test with continuity correction, [f]One-way ANOVA test, [g]Kruskal-Wallis test

In the remaining study (n = 2125), which had a median age of under 12 years, the differences among the mean post-dose heart rates in the four arms of artesunate-amodiaquine, artemether-lumefantrine, dihydroartemisinin-piperaquine, and pyronaridine-artesunate were statistically significant (p<0.0001) with the artesunate-amodiaquine arm having the lowest mean heart rate of 81.5 beats/minute (95% CI: 79.9-83.1). However, none of these means were close to or within the bradycardic range for children aged 2-<12 years who were 72.0% (1529/2125) of this study population and any differences among these means were small and of uncertain clinical significance. Similarly, the differences among the proportions of participants with post-dose heart rates below age-specific thresholds in the four arms were statistically significant (p<0.0001 & p=0.02375) with the highest proportions in the artesunate-amodiaquine arm although these were all comparable to the proportions seen in non-amodiaquine comparator arms in the other three studies (Table 6.5).

Day 28 (Late)

In the two studies which also had day 28 (late) ECG measurements (Table 6.5), the mean heart rates in the artesunate-amodiaquine arms had returned to levels which were clinically comparable to the chloroquine arm (mean: 69.5 beats/minute, 95% CI: 67.7-71.2, p=0.0247) by day 28 (Ogutu 2014: 67.8 beats/minute, 95% CI: 64.0-71.5; Siqueira 2017: 66.5, 95% CI: 64.6-68.4). The proportions of participants with heart rates ≤60 or ≤50 beats/minute after artesunate-amodiaquine were higher than in the chloroquine arm (p=0.0035 & p=0.1211). The relative risk of sinus bradycardia after treatment with artesunate-amodiaquine remained higher on day 28 compared to chloroquine (≤60 beats/minute risk ratio: 1.7, 95% CI: 1.2-2.4, p=0.0024; ≤50 beats/minute risk ratio: 5.9, 95% CI: 0.7-48.4, p=0.05968).

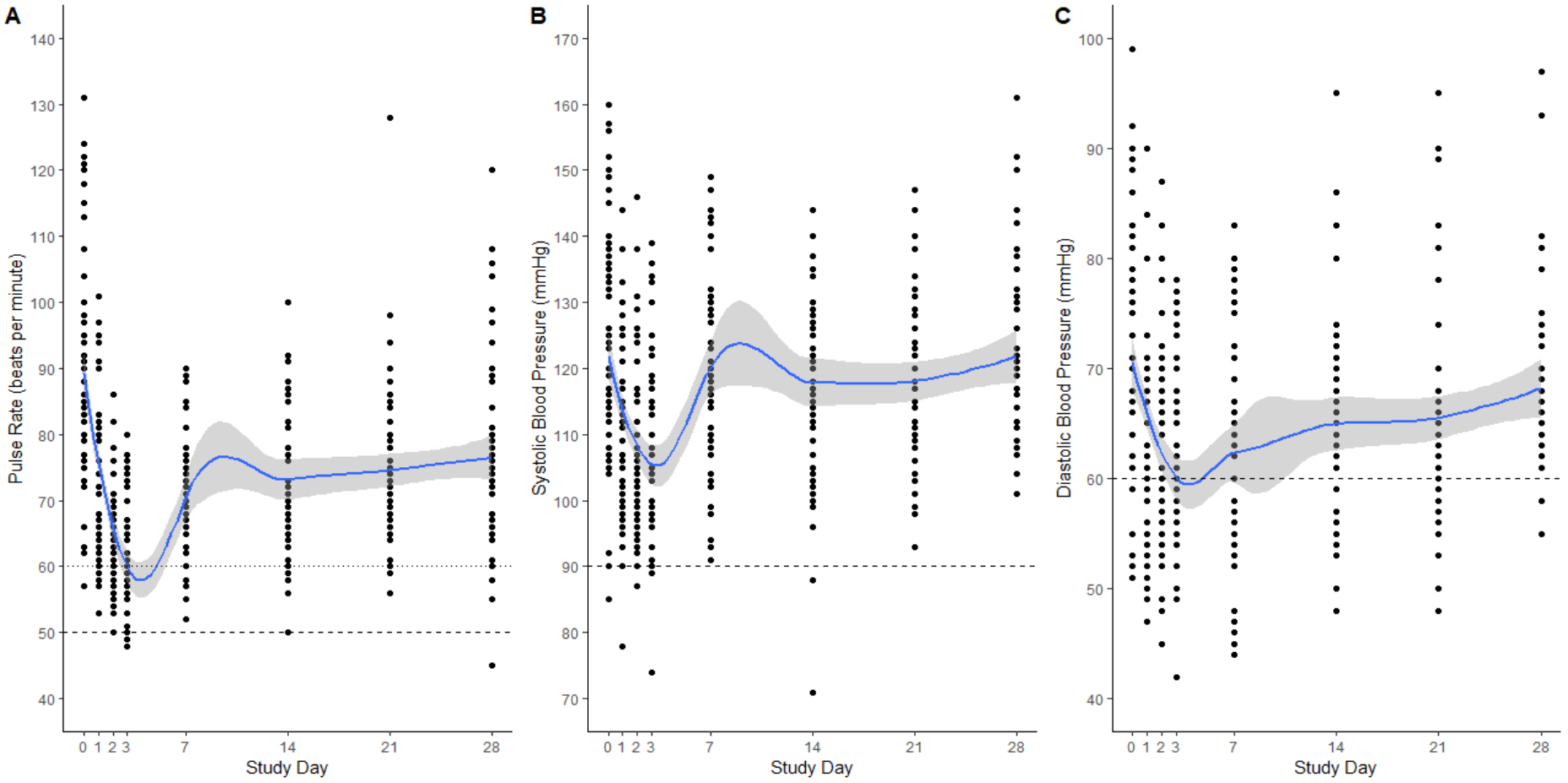
6.4.3.5 Study-Level Analyses – Pulse Rate and Blood Pressure

Cardiovascular vital sign measurements from each study visit were also available from the Ogutu 2014 (Figure 6.4) and Siqueira 2017 (Figure 6.5) studies.

Consistent with heart rates from ECG interval data, in patients above 12 years of age, mean pulse rates fell from day 0 to 3 with lower rates in the bradycardic range (≤60 beats/minute) after artesunate-amodiaquine (Figures 6.4 & 6.5, panels A) compared to chloroquine (Figure 6.5, panel A) before returning to similar levels from day 7 for the remaining 3-5 weeks of observation (Figures 6.4 & 6.5, panels A). Data from children younger than 12 years were few (n = 28) and while mean pulse rates decreased from day 0 to 3, they did not fall into the bradycardic range in either the artesunate-amodiaquine or chloroquine arms (Figure 6.5, panel D).

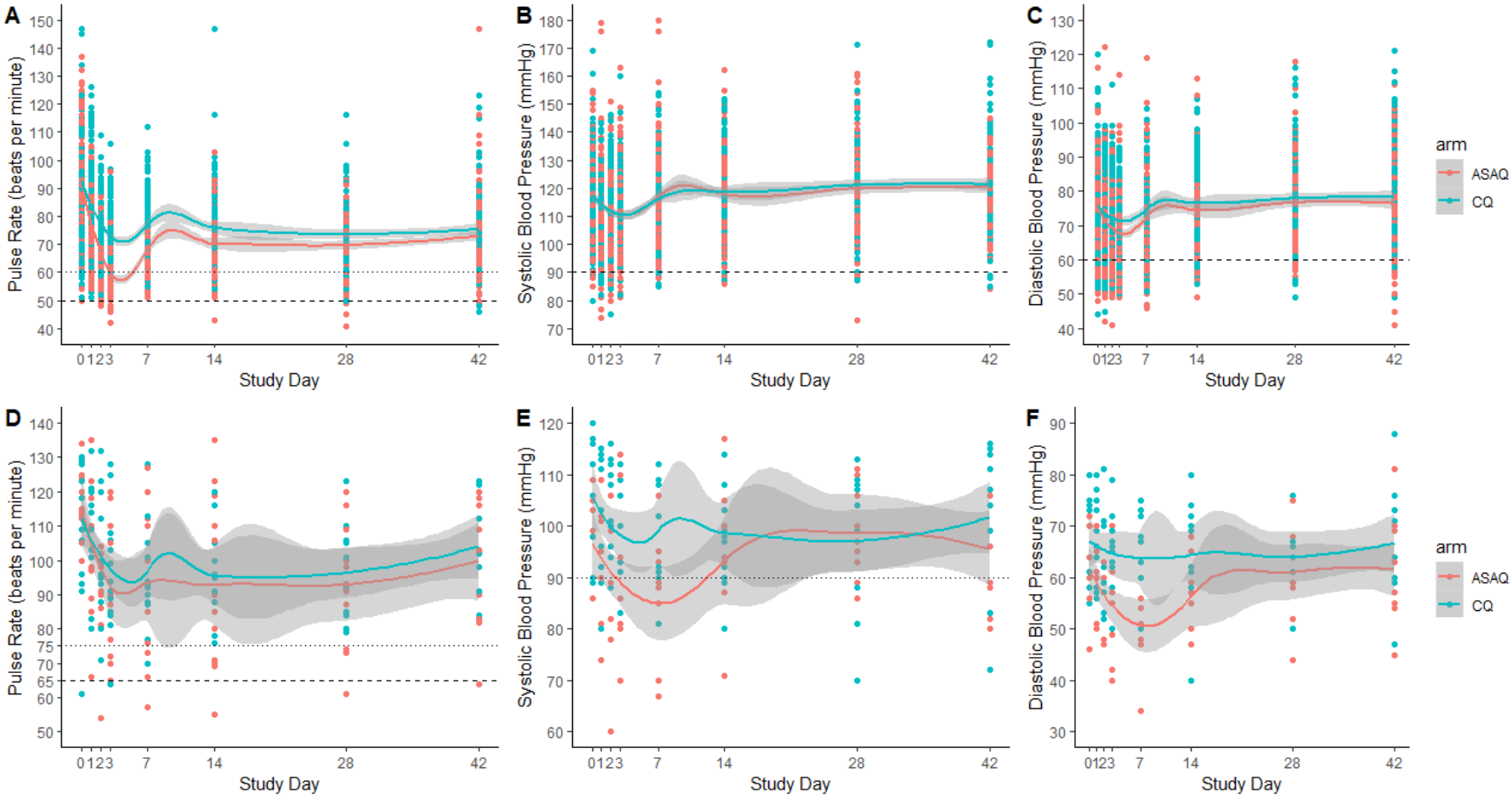
This decrease in heart rate was accompanied by a drop in systolic blood pressure of ~15 mmHg (Figure 6.4 & 6.5, panels B) and diastolic blood pressure of ~10mmHg in both sets of drug arms (Figure 6.4 & 6.5, panels C), with a slightly greater decrease in diastolic blood pressure with artesunate-amodiaquine compared to chloroquine (Figure 6.5, panel C) which had also resolved by day 7 (Figure 6.4 & 6.5, panels B & C). The few blood pressure data from children exhibited similar trends as seen in adolescents and adults (Figure 6.5, panels E & F).

Figure 6.4: Pulse Rate and Blood Pressure Measurements after Artesunate-Amodiaquine for *P. falciparum* Malaria in Kenyan Adults (≥18 Years)



Vital signs from 53 adults with ECG monitoring in a randomised controlled trial of fixed-dose combination versus non-fixed dose combination artesunate-amodiaquine for treatment of *P. falciparum* malaria[189] with means (smooths) and 95% confidence intervals (shaded area) from loess regression. Horizontal lines demarcate lower limit of normal range (dotted) or threshold below which individuals could be symptomatic (dashed).

Figure 6.5: Pulse Rate and Blood Pressure Measurements after Artesunate-Amodiaquine and Chloroquine for *P. vivax* Malaria in Brazilian Adults (≥12 Years) and Children (<12 Years)



Vital signs from 352 adults with ECG monitoring (top panels) and 28 children without ECG monitoring (bottom panels) in a randomised controlled trial of artesunate-amodiaquine (ASAQ) versus chloroquine (CQ) for treatment of *P. vivax* malaria[190] with means (smooths) and 95% confidence intervals (shaded area) by drug from loess regression. Horizontal lines demarcate lower limit of normal range (dotted) or threshold below which individuals could be symptomatic (dashed)

6.4.3.6 Study-Level Analyses – Corrected QT Interval

Study-level analyses of corrected QT interval measurements from ECG interval analysis are presented by individual study in Table 6.6.

Day 2 or 3 (Post-Dose)

The differences in mean day 2 or 3 (post-dose) QTcS duration between treatment arms in the Ndiaye 2011 and Siqueira 2017 studies respectively comparing artesunate-amodiaquine to artemether-lumefantrine and chloroquine were neither clinically nor statistically significant at the sample sizes of the individual studies. Similarly, the differences between treatment arms in proportions of participants with corrected QT interval absolute values and changes from baseline beyond all thresholds recommended in International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines[136] were also neither clinically nor statistically significant regardless of the heart rate correction used (Table 6.6).

In the large WANECAM 2018 study (n = 2125), which investigated artesunate-amodiaquine, artemether-lumefantrine, dihydroartemisinin-piperaquine, and pyronaridine-artesunate, differences in mean post-dose QTcS intervals among the treatment arms were clinically and statistically significant (p<0.0001), with dihydroartemisinin-piperaquine having the highest mean post-dose QTcS (430.6 milliseconds, 95% CI: 428.8-432.4) followed by artesunate-amodiaquine (428.1 milliseconds, 95% CI: 426.0-430.1), then artemether-lumefantrine (415.7 milliseconds, 95% CI: 413.8-417.5) and pyronaridine-artesunate (406.3 milliseconds, 95% CI: 404.7-408.0). However, mean QTcS intervals among the four arms were already significantly different at baseline (p=0.0017) even if the differences were small and of borderline clinical significance. Apart from the QTcF>500 millisecond threshold, differences among treatment arms in proportions of participants with corrected QT intervals above ICH thresholds at the post-dose time point were also clinically and

statistically significant regardless of heart rate correction used, with the highest proportions in the dihydroartemisinin-piperaquine arm followed by the artesunate-amodiaquine arm, then the artemether-lumefantrine and pyronaridine-artesunate arms, in keeping with mean QTcS findings.

Day 28 (Late)

In the two studies which had late ECG measurements, mean QTcS intervals had returned to baseline levels in all treatment arms by day 28. The differences between treatment arms in proportions of participants with corrected QT interval measurements beyond ICH thresholds were once again neither clinically nor statistically significant regardless of the heart rate correction used (Table 6.6).

Table 6.6: Summary Statistics of Corrected QT Intervals from ECG Interval Analyses of Individual Studies by Drug Arm and Time Point

Study Reference	**Ndiaye 2011[188]**			**Ogutu 2014[189]**	**Siqueira 2017[190]**			**WANECAM 2018[122]**				
Drug Arm	*ASAQ*	*AL*	*p*	*ASAQ*	*ASAQ*	*CQ*	*p*	*ASAQ*	*AL*	*DP*	*PA*	*p*
Total dose (mg/kg)[a]												
Median (IQR)	28.2 (25.0-36.1)	58.8 (50.7-67.3)		29.45 (27.4-31.6)	22.8 (20.1-26.5)	20.8 (18.3-23.4)		31.4 (27.0-38.6)	68.57 (60.5-79.41)	57.8 (48.0-67.6)	30.3 (27.0-34.8)	
Day 0 (Pre-Dose)												
Number of Individuals	75	73		51	177	173		417	426	716	566	
QTc Interval (ms)												
Mean QTcS (SD)	391.7 (20.3)	390.1 (25.3)	0.6705[b]	394.9 (19.8)	403.9 (22.2)	403.5 (23.3)	0.8607[b]	408.6 (19.8)	406.6 (21.0)	407.8 (20.5)	403.8 (20.0)	0.0017[e]
QTcS>500	0	0	1[c]	0	1 (0.6%)	0	1[c]	0	1 (0.2%)	0	0	0.3967[c]
QTcF>500	0	0	1[c]	0	0	0	1[c]	0	1 (0.2%)	0	0	0.3967[c]
QTcB>500	0	0	1[c]	0	3 (1.7%)	2 (1.2%)	1[c]	0	1 (0.2%)	0	0	0.3967[c]
480<QTcS≤500	0	0	1[c]	0	0	0	1[c]	0	0	0	0	1[c]
480<QTcF≤500	0	0	1[c]	0	1 (0.6%)	0	1[c]	0	0	0	0	1[c]
480<QTcB≤500	0	1 (1.4%)	0.4932[c]	0	1 (0.6%)	3 (1.7%)	0.3672[c]	2 (0.5%)	1 (0.2%)	2 (0.3%)	3 (0.5%)	0.7553[c]
450<QTcS≤480	0	1 (1.4%)	0.4932[c]	0	4 (2.3%)	7 (4.0%)	0.5149[d]	9 (2.2%)	4 (0.9%)	14 (2.0%)	9 (1.6%)	0.5071[d]
450<QTcF≤480	0	1 (1.4%)	0.4932[c]	0	4 (2.3%)	7 (4.0%)	0.5149[d]	2 (0.5%)	0	0	1 (0.2%)	0.1119[c]
450<QTcB≤480	0	1 (1.4%)	0.4932[c]	0	23 (13.0%)	19 (11.0%)	0.6785[d]	35 (8.4%)	25 (5.9%)	72 (10.1%)	24 (4.2%)	0.0005[d]
Day 2 or 3 (Post-Dose)												
Number of Individuals	74	73		51	175	174		417	426	716	566	
QTc Interval (ms)												
Mean QTcS (SD)	406.7 (25.0)	400.9 (20.5)	0.1245[b]	418.1 (22.4)	428.1 (20.3)	429.6 (20.8)	0.5024[b]	428.1 (21.7)	415.7 (19.3)	430.6 (24.3)	406.3 (19.7)	<0.0001[e]
QTcS>500	0	0	1[c]	0	0	0	1[c]	2 (0.5%)	0	11 (1.5%)	0	0.0006[c]
QTcF>500	0	0	1[c]	0	0	0	1[c]	1 (0.2%)	0	4 (0.6%)	0	0.1487[c]
QTcB>500	0	0	1[c]	0	0	1 (0.6%)	0.4986[c]	2 (0.5%)	0	12 (1.7%)	0	0.0003[c]
480<QTcS≤500	0	0	1[c]	0	1 (0.6%)	0	1[c]	2 (0.5%)	0	14 (2.0%)	0	<0.0001[c]
480<QTcF≤500	0	0	1[c]	0	0	0	1[c]	2 (0.5%)	0	9 (1.3%)	0	0.0030[c]
480<QTcB≤500	0	0	1[c]	0	3 (1.7%)	10 (5.7%)	0.0879[d]	13 (3.1%)	2 (0.5%)	32 (4.5%)	1 (0.2%)	<0.0001[d]
450<QTcS≤480	3 (4.1%)	1 (1.4%)	0.6198[c]	5 (9.8%)	26 (14.9%)	34 (19.5%)	0.3089[d]	56 (13.4%)	18 (4.2%)	106 (14.8%)	7 (1.2%)	<0.0001[d]
450<QTcF≤480	3 (4.1%)	1 (1.4%)	0.6198[c]	3 (5.9%)	24 (13.7%)	31 (17.8%)	0.3657[d]	16 (3.8%)	0	35 (4.9%)	0	<0.0001[d]
450<QTcB≤480	4 (5.4%)	1 (1.4%)	0.3664[c]	6 (11.8%)	32 (18.3%)	45 (25.9%)	0.1147[d]	83 (19.9%)	41 (9.6%)	170 (23.7%)	24 (4.2%)	<0.0001[d]

Change in QTc (ms)												
ΔQTcS >60	2 (2.7%)	3 (4.1%)	0.681[c]	4 (8.3%)[f]	12 (6.9%)[g]	6 (3.5%)[g]	0.231[d]	17 (4.1%)	2 (0.5%)	47 (6.6%)	5 (0.9%)	<0.0001[d]
ΔQTcF >60	10 (13.5%)	5 (6.8%)	0.2882[d]	7 (14.6%)[f]	14 (8.1%)[g]	11 (6.4%)[g]	0.6889[d]	47 (11.3%)	13 (3.1%)	90 (12.6%)	13 (2.3%)	<0.0001[d]
ΔQTcB >60	1 (1.4%)	2 (2.7%)	0.6198[c]	1 (2.1%)[f]	0	1 (0.6%)[g]	0.4986[c]	13 (3.1%)	1 (0.2%)	35 (4.9%)	2 (0.4%)	<0.0001[d]
30<ΔQTcS≤60	16 (21.6%)	12 (16.4%)	0.5551[d]	11 (22.9%)[f]	53 (30.3%)[g]	64 (37.2%)[g]	0.2397[d]	120 (28.8%)	66 (15.5%)	216 (30.2%)	39 (6.9%)	<0.0001[d]
30<ΔQTcF≤60	27 (36.5%)	24 (32.9%)	0.7745[d]	21 (43.8%)[f]	64 (37.0%)[g]	66 (38.4%)[g]	0.8784[d]	155 (37.2%)	104 (24.4%)	271 (37.8%)	72 (12.7%)	<0.0001[d]
30<ΔQTcB≤60	8 (10.8%)	7 (9.6%)	1[d]	6 (12.5%)[f]	16 (9.2%)[g]	27 (15.7%)[g]	0.0989[d]	82 (19.7%)	46 (10.8%)	157 (21.9%)	23 (4.1%)	<0.0001[d]
Day 28 (Late)												
Number of Individuals				49	166	163						
QTc Interval (ms)												
Mean QTcS (SD)				394.1 (22.3)	404.1 (19.3)	407.2 (20.4)	0.157[b]					
QTcS>500				0	0	0	1[c]					
QTcF>500				0	0	0	1[c]					
QTcB>500				0	0	1 (0.6%)	0.4954[c]					
480<QTcS≤500				0	0	1 (0.6%)	0.4954[c]					
480<QTcF≤500				0	0	0	1[c]					
480<QTcB≤500				0	1 (0.6%)	0	1[c]					
450<QTcS≤480				0	4 (2.4%)	3 (1.8%)	1[c]					
450<QTcF≤480				0	3 (1.8%)	4 (2.5%)	0.7215[c]					
450<QTcB≤480				1 (2.0%)	7 (4.2%)	7 (4.3%)	1[d]					
Change in QTc (ms)												
ΔQTcS >60				0	2 (1.2%)[g]	0	0.4985[c]					
ΔQTcF >60				1 (2.2%)[f]	3 (1.8%)[g]	0	0.2477[c]					
ΔQTcB >60				0	1 (0.6%)[g]	0	1[c]					
30<ΔQTcS≤60				3 (6.5%)[f]	9 (5.5%)[g]	10 (6.2%)[g]	0.9669[d]					
30<ΔQTcF≤60				7 (15.2%)[f]	15 (9.0%)[g]	15 (9.3%)[g]	1[d]					
30<ΔQTcB≤60				1 (2.2%)[f]	1 (0.6%)[g]	3 (18.6%)[g]	0.3681[c]					

QTc = Corrected QT interval, QTcS = QT interval with study-specific heart rate correction, QTcF = QT interval with Fridericia heart rate correction, QTcB = QT Interval with Bazett heart rate correction, [a]Quinoline and structurally-related drug only, [b]2-sample t-test with Welch modification, [c]Fisher's exact test, [d]Pearson's Chi-squared test for independence with Yates' continuity correction, [e]Kruskal-Wallis test, [f]3 participants had missing baseline QT intervals, [g]2 participants had missing baseline QT intervals

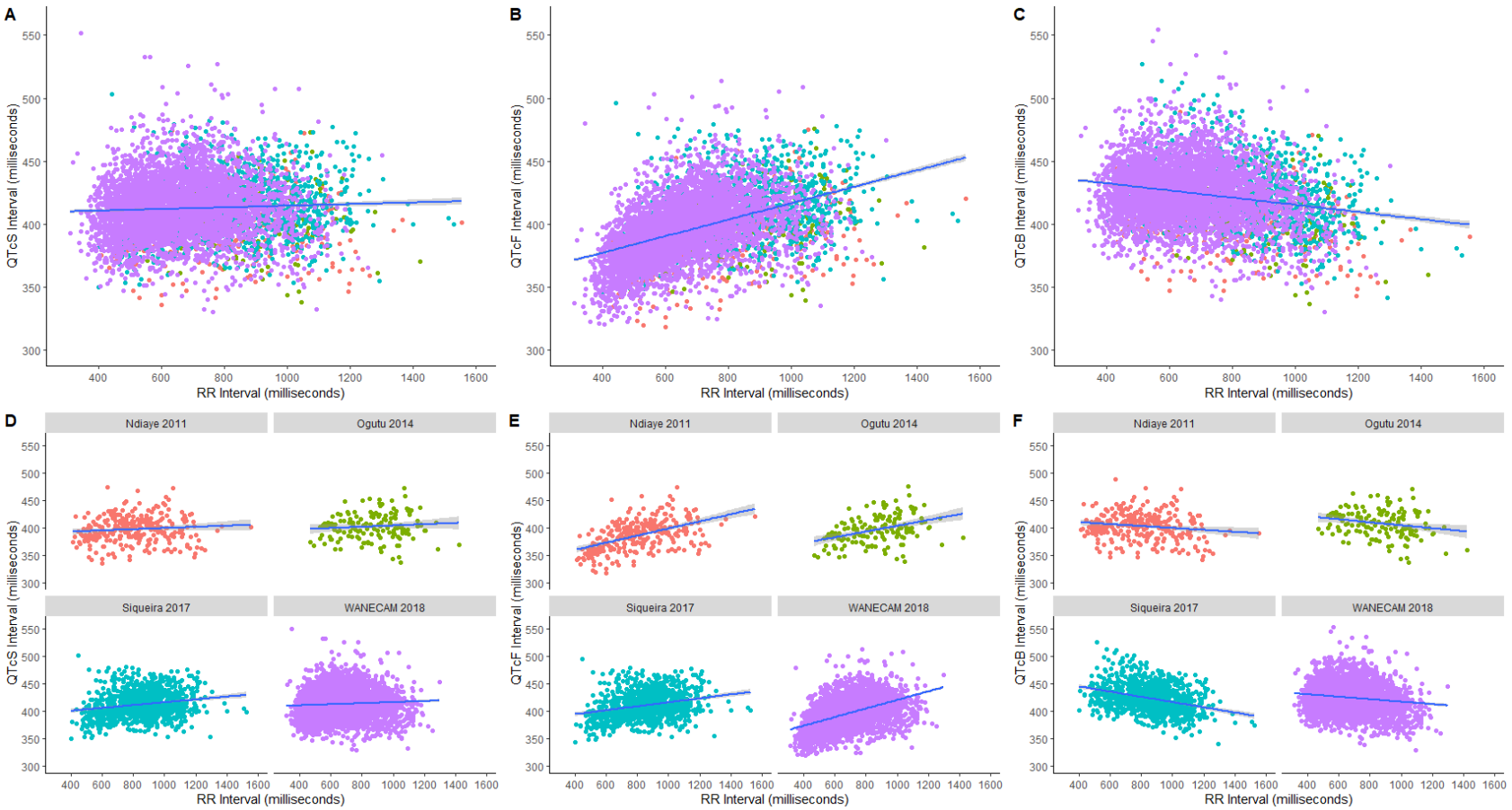
QT Intervals >500 milliseconds

Among all included individuals, four participants had uncorrected QT intervals of >500 milliseconds. All occurred post-dose: three (0.4%) among the 716 individuals treated with dihydroartemisinin-piperaquine and one (0.1%) among the 722 artesunate-amodiaquine treated individuals with post-dose QT interval measurements. None of the patients who had uncorrected or corrected QT intervals >500 milliseconds with any of the three heart rate correction methods experienced any clinical complications.

Heart Rate Correction

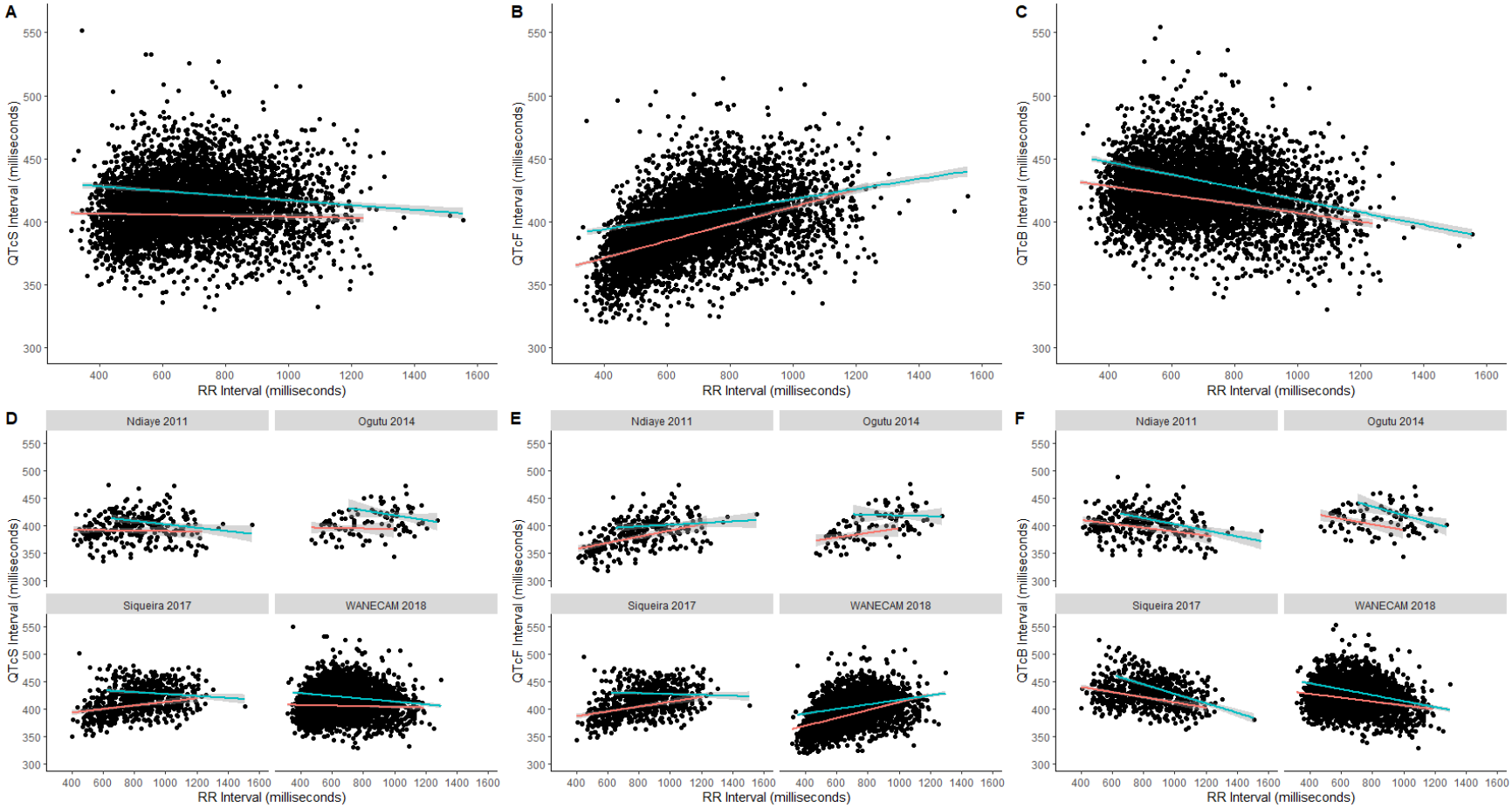
The study-specific correction provided the most effective reduction of heart rate dependence of the QT interval for all individual studies and the pooled dataset (Figures 6.6 & 6.7). In ICH-recommended categorical analyses, use of the Bazett heart rate correction overestimated while use of the Fridericia heart rate correction underestimated the number of individuals with values beyond absolute thresholds of corrected QT interval prolongation (>450, >480, and >500 milliseconds) (Table 6.6). For change from baseline thresholds (>30 and >60 milliseconds), use of the Bazett heart rate correction underestimated while use of the Fridericia heart rate correction overestimated the number of individuals with corrected QT interval prolongation as determined by change from baseline (Table 6.6).

Figure 6.6: Corrected QT Interval and RR Interval Relationships by Correction Method and Individual Study



QT intervals adjusted with study-specific ($QTcS = \frac{QT}{\beta_{age}\sqrt{RR}}$, where β_{age} decreased with increasing age), Fridericia ($QTcF = \frac{QT}{\sqrt[3]{RR}}$), and Bazett heart rate corrections ($QTcB = \frac{QT}{\sqrt{RR}}$), and their relationship with RR intervals in the pooled dataset (top panels) and by individual study (bottom panels) with means (blue line) and 95% confidence intervals (shaded area) from linear regression. A slight positive relationship is expected even with ideal correction because of confounding by body temperature changes, i.e. fever and defervescence, in malaria.

Figure 6.7: Corrected QT Interval and RR Interval Relationships by Correction Method, Treatment Day, and Individual Study



QT intervals adjusted with study-specific ($QTcS = \frac{QT}{\beta_{age}\sqrt{RR}}$, where β_{age} decreased with increasing age), Fridericia ($QTcF = \frac{QT}{\sqrt[3]{RR}}$), and Bazett heart rate corrections ($QTcB = \frac{QT}{\sqrt{RR}}$), and their relationship with RR intervals in the pooled dataset (top panels) and by individual study (bottom panels) with means pre-treatment on day 0 (pink line) and post-treatment on days 2 or 3 (cyan line) with their 95% confidence intervals (shaded area) from linear regression.

6.4.4 Individual Patient Data Meta-analyses

6.4.4.1 Heart Rate

Heart rate data from ECG interval measurements were available from 2680 individuals: 1101 participants aged ≥12 years and 1579 participants aged <12 years. Separate multivariable linear mixed effects models were fitted for these two age groups.

Age ≥12 Years

Results from the model for adolescents and adults (≥12 years) are summarised in Table 6.7. Artesunate-amodiaquine was associated with the greatest mean decrease in heart rate from baseline on day 2 (15.2 beats/minute, 95% CI: 13.4-17.0), followed by dihydroartemisinin-piperaquine (10.5 beats/minute, 95% CI: 7.7-13.3) and artemether-lumefantrine (9.3 beats/minute, 95% CI: 6.4-12.2), then pyronaridine-artesunate (6.6 beats/minute, 95% CI: 4.0-9.3) and chloroquine (5.9 beats/minute, 95% CI: 3.2-8.5) after adjustment for baseline heart rate, body temperature, and sex (Figure 6.8). The greater decrease in heart rate after artesunate-amodiaquine was both clinically and statistically significant versus the comparator antimalarials (artemether-lumefantrine: p=0.0001, chloroquine: p<0.0001, dihydroartemisinin-piperaquine: p=0.0013, pyronaridine-artesunate: p<0.0001).

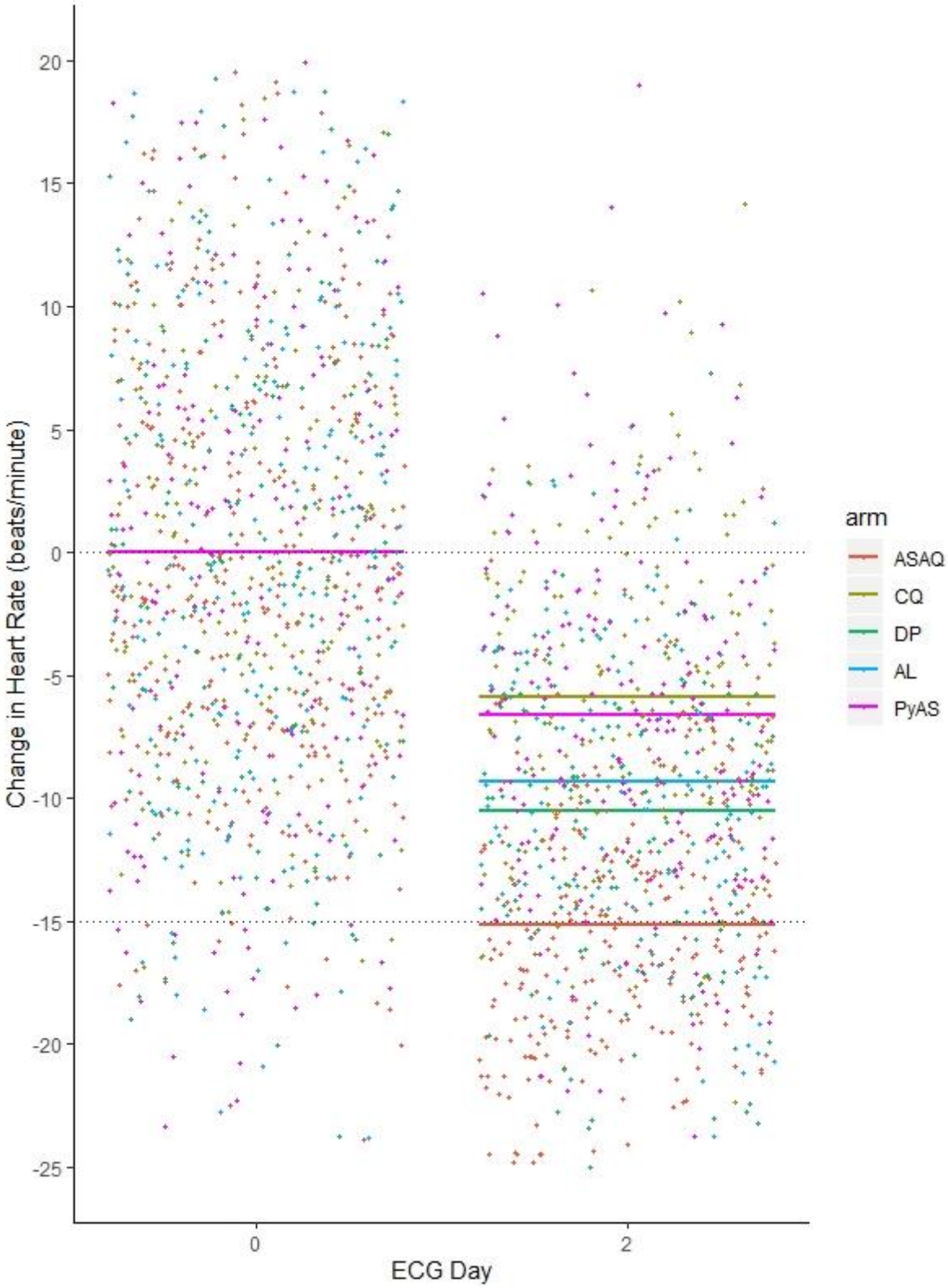
Body temperature was associated with a mean decrease in heart rate of 8.1 beats/minute (95% CI: 7.5-8.7) per 1°C of defervescence with all other variables held constant. Males had heart rates which were lower than females by a mean of 7.8 beats/min (95% CI: 6.4-9.3). The differences in heart rates between artesunate-amodiaquine and comparator antimalarials at baseline were neither clinically or statistically significant after adjustment for body temperature and sex.

Table 6.7: Multivariable Linear Mixed Effects Regression Analysis of Heart Rate after Antimalarial Treatment for Malaria in Individuals Aged ≥12 Years

Variable	Number of Observations	Adjusted Estimate (95% Confidence Interval)	*p* value
Change from baseline after ASAQ	689	-15.17 (-16.98 to -13.35) beats/minute	<0.0001
Change from baseline vs ASAQ, by antimalarial treatment arm	2041		
ASAQ	689	Reference	
CQ	345	9.30 (6.63 to 11.98) beats/minute	<0.0001
DP	298	4.64 (1.82 to 7.45) beats/minute	0.0013
AL	328	5.86 (2.99 to 8.73) beats/minute	0.0001
PA	381	8.54 (5.94 to 11.13)	<0.0001
Baseline measurement vs ASAQ, by antimalarial treatment arm	1096		
ASAQ	384	Reference	
CQ	172	-0.75 (-3.40 to 1.91) beats/minute	0.5816
DP	149	-0.07 (-3.12 to 2.97) beats/minute	0.9620
AL	200	-0.41 (-3.14 to 2.31) beats/minute	0.7672
PA	191	-2.23 (-5.20 to 0.74) beats/minute	0.1408
Body temperature, per 1°C increase	2041	8.14 (7.54 to 8.73) beats/minute	<0.0001
Sex	2041		
Female	829	Reference	
Male	1212	-7.84 (-9.27 to -6.41) beats/minute	<0.0001

ASAQ = artesunate-amodiaquine, CQ = chloroquine, DP = dihydroartemisinin-piperaquine, AL = artemether-lumefantrine, PA = pyronaridine-artesunate

Figure 6.8: Change in Heart Rate after Antimalarial Treatment for Malaria in Individuals Aged ≥12 Years



Change in heart rate from baseline after artesunate-amodiaquine (ASAQ), chloroquine (CQ), dihydroartemisinin-piperaquine (DP), artemether-lumefantrine (AL), and pyronaridine-artesunate (PyAS) in adults aged ≥12 years from multivariable linear mixed effects regression model adjusting for sex, body temperature, and individual study/site/person effects. Lines represent means, and dots are partial residuals, with colour representing antimalarial treatment arm.

Age <12 Years

Results from the model for children (<12 years) are summarised in Table 6.8. Artesunate-amodiaquine was once again associated with the greatest mean decrease in heart rate from baseline on day 2 (21.0 beats/minute, 95% CI: 19.0-23.0) which was similar to that seen with artemether-lumefantrine (20.9 beats/minute, 95% CI: 18.3-23.5), followed by dihydroartemisinin-piperaquine (17.2 beats/minute, 95% CI: 14.9-19.4) then pyronaridine-artesunate (12.7 beats/minute, 95% CI: 10.2-15.1) (Figure 6.9). The decrease in heart rate seen after artesunate-amodiaquine was larger than after dihydroartemisinin-piperaquine (p=0.0007) and pyronaridine-artesunate (p<0.0001), but was neither clinically nor statistically significantly different from that observed after artemether-lumefantrine (p=0.9015).

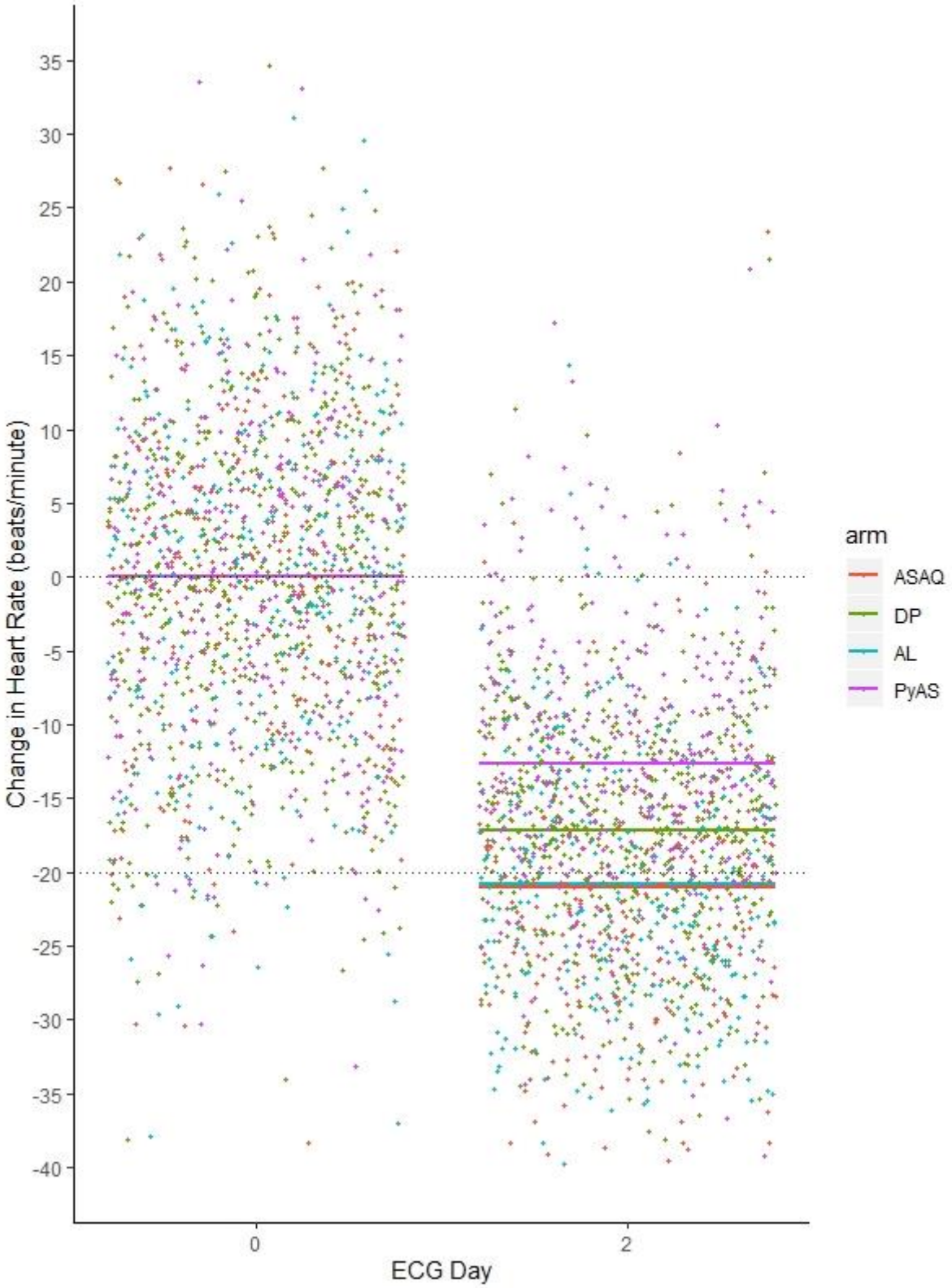
Body temperature was associated with a mean decrease in heart rate of 5.5 beats/minute (95% CI: 4.9-6.1) per 1°C of defervescence and age with a mean decrease of 3.8 beats/minute (95% CI: 3.6-4.0) per year of childhood with all other variables held constant. The differences in heart rates between artesunate-amodiaquine and comparator antimalarials at baseline were neither clinically or statistically significant after adjustment for body temperature and age.

Table 6.8: Multivariable Linear Mixed Effects Regression Analysis of Heart Rate after Antimalarial Treatment for Malaria in Individuals Aged <12 Years

Variable	Number of Observations	Adjusted Estimate (95% Confidence Interval)	*p* value
Change from baseline after ASAQ	674	-21.03 (-23.01 to -19.04) beats/minute	<0.0001
Change from baseline vs ASAQ, by antimalarial treatment arm	3151		
ASAQ	674	Reference	
DP	1131	3.87 (1.63 to 6.12) beats/minute	0.0007
AL	596	0.16 (-2.43 to 2.76) beats/minute	0.9015
PA	750	8.33 (5.88 to 10.77) beats/minute	<0.0001
Baseline measurement vs ASAQ, by antimalarial treatment arm	1579		
ASAQ	338	Reference	
DP	567	-0.09 (-2.11 to 1.93) beats/minute	0.9318
AL	299	0.59 (-1.92 to 3.10) beats/minute	0.6433
PA	375	-0.75 (-2.98 to 1.49) beats/minute	0.5132
Body temperature, per 1°C increase	3151	5.49 (4.91 to 6.07) beats/minute	<0.0001
Age, per 1-year increase	3151	-3.77 (-3.99 to -3.55) beats/minute	<0.0001

ASAQ = artesunate-amodiaquine, DP = dihydroartemisinin-piperaquine, AL = artemether-lumefantrine, PA = pyronaridine-artesunate

Figure 6.9: Change in Heart Rate after Antimalarial Treatment for Malaria in Individuals Aged <12 years



Change in heart rate from baseline after artesunate-amodiaquine (ASAQ), dihydroartemisinin-piperaquine (DP), artemether-lumefantrine (AL), and pyronaridine-artesunate (PyAS) in children aged <12 years from multivariable linear mixed effects regression model adjusting for age, body temperature, and individual study/site/person effects. Lines represent means, and dots are partial residuals, with colour representing antimalarial treatment arm.

6.4.4.2 Corrected QT Interval

QT interval data were available from 2,681 patients. Results from multivariable linear mixed effects modelling for the QT interval with study-specific heart rate correction (QTcS) are summarised in Table 6.9.

Chloroquine was associated with the greatest mean increase in QTcS from baseline on day 2 (21.9 milliseconds, 95% CI: 18.3 to 25.6), followed by dihydroartemisinin-piperaquine (19.2 milliseconds, 95% CI: 15.8 to 20.5), artesunate-amodiaquine (16.9 milliseconds, 95% CI: 15.0 to 18.8), artemether-lumefantrine (5.6 milliseconds, 95% CI: 2.9 to 8.2), and pyronaridine-artesunate (-1.2 milliseconds, 95% CI: -3.6 to +1.3) (Figure 6.10). The QTcS prolongation after artesunate-amodiaquine was less than that after chloroquine (p=0.0069) and dihydroartemisinin-piperaquine (p=0.0495), and more than that after artemether-lumefantrine (p<0.0001) and pyronaridine-artesunate (p<0.0001) (Table 6.9).

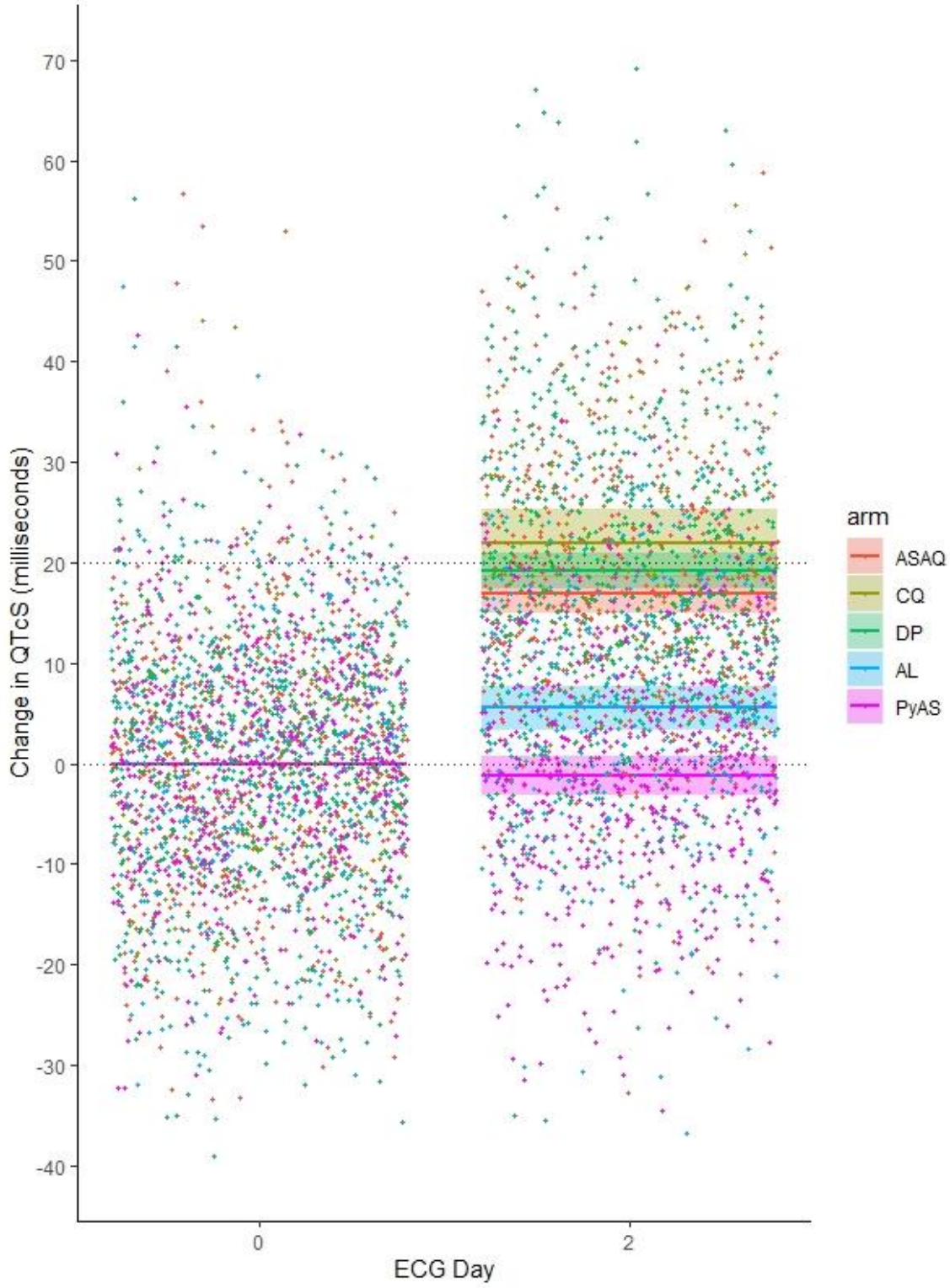
Body temperature was associated with a mean increase in QTcS of 2.7 milliseconds (95% CI: 2.2-3.3) per 1°C of defervescence with all other variables held constant. QTcS became shorter after infancy and early childhood and was comparable in both sexes until puberty when QTcS shortened in males (10.1 milliseconds, 95% CI: 6.0-14.0) but not females (Figure 6.11). The differences in QTcS between artesunate-amodiaquine and comparator antimalarials at baseline were neither clinically or statistically significant after adjustment for body temperature, age group, and sex (Table 6.9).

Table 6.9: Multivariable Linear Mixed Effects Regression Analysis of the QT Interval with Study-Specific Heart Rate Correction

Variable	Number of Observations	Adjusted Estimate (95% Confidence Interval)	*p* value
Change from baseline after ASAQ	1361	16.87 (14.97 to 18.78) milliseconds	<0.0001
Change from baseline vs ASAQ, by antimalarial treatment arm	5192		
ASAQ	1361	Reference	
CQ	347	5.07 (1.39 to 8.75) milliseconds	0.0069
DP	1429	2.34 (0.01 to 4.67) milliseconds	0.0495
AL	924	-11.32 (-13.96 to -8.67) milliseconds	<0.0001
PA	1131	-18.03 (-20.51 to -15.56) milliseconds	<0.0001
Baseline measurement vs ASAQ, by antimalarial treatment arm	2674		
ASAQ	720	Reference	
CQ	173	-1.46 (-5.43 to 2.50) milliseconds	0.4693
DP	716	1.29 (-1.07 to 3.64) milliseconds	0.2833
AL	499	-0.33 (-3.04 to 2.37) milliseconds	0.8091
PA	566	-0.92 (-3.49 to 1.64) milliseconds	0.4808
Body temperature, per 1°C increase	5192	-2.74 (-3.33 to -2.15) milliseconds	<0.0001
Female sex, by age group	1215		
0.5-<5 years	215	Reference	
5-<10 years	419	-8.96 (-11.75 to -6.17) milliseconds	<0.0001
10-<15 years	294	-7.77 (-10.81 to -4.74) milliseconds	<0.0001
≥15 years	287	-8.89 (-12.17 to -5.61) milliseconds	<0.0001
Male vs female sex, by age group	1459		
0.5-<5 years	265	-3.14 (-6.19 to -0.10) milliseconds	0.0431
5-<10 years	413	3.79 (-0.02 to 7.61) milliseconds	0.0514
10-<15 years	329	1.28 (-2.79 to 5.36) milliseconds	0.5381
≥15 years	452	-10.05 (-14.09 to -6.00) milliseconds	<0.0001

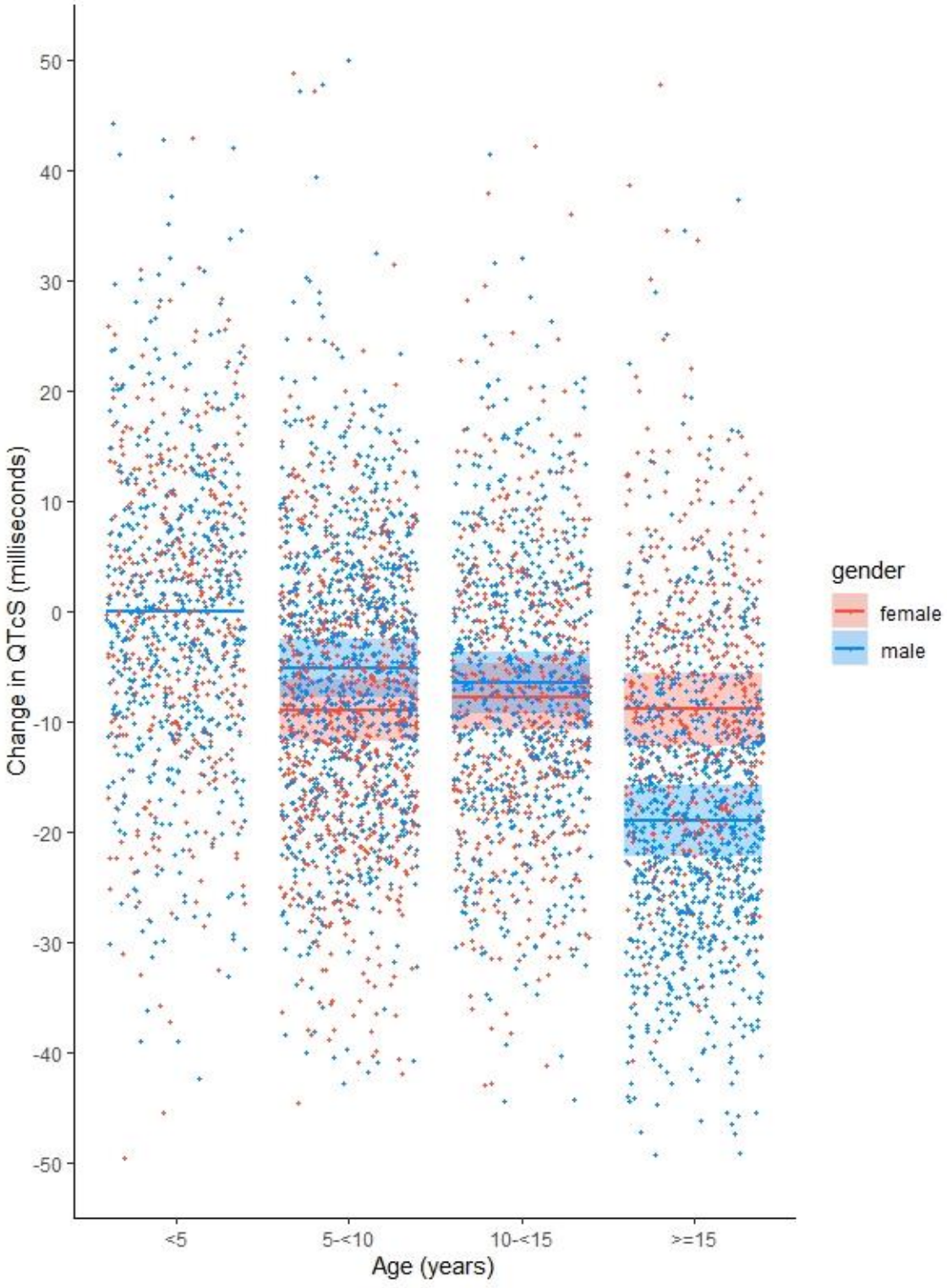
ASAQ = artesunate-amodiaquine; CQ = chloroquine; DP = dihydroartemisinin-piperaquine; AL = artemether-lumefantrine; PA = pyronaridine-artesunate

Figure 6.10 Change in Corrected QT Interval after Antimalarial Treatment for Malaria



Change in QT interval adjusted with study-specific heart rate correction (QTcS) after artesunate-amodiaquine (ASAQ), chloroquine (CQ), dihydroartemisinin-piperaquine (DP), artemether-lumefantrine (AL), and pyronaridine-artesunate (PyAS) from multivariable linear mixed effects regression model adjusting for age group, sex, body temperature, and individual study/site/person effects. Lines represent means, shaded areas 95% confidence intervals, and dots are partial residuals, with colour representing antimalarial treatment arm.

Figure 6.11: Change in Corrected QT Interval with Age by Sex



Change in QT interval adjusted with study-specific heart rate correction (QTcS) compared to early childhood (<5 years) by sex from multivariable linear mixed effects regression model adjusting for treatment day, antimalarial treatment arm, body temperature, and individual study/site/person effects. Lines represent means, shaded areas are 95% confidence intervals, and dots are partial residuals, with colour representing the sex of each participant.

6.4.5 Concentration-Effect Analyses

6.4.5.1 Pharmacokinetic Analyses

In the study from which drug concentration data were available[189], a total of 363 post-dose venous plasma samples from 53 participants were collected, of which 115 (31.7%) observed concentrations for amodiaquine and 352 (97.0%) observed concentrations for desethylamodiaquine were included in the analysis. Only observations below the lower limit of quantification of 1ng/ml for both compounds were excluded from the analysis.

Population pharmacokinetic parameter estimates were reliable, with small relative standard errors (Table 6.10). Secondary pharmacokinetic parameters of maximum concentration, time to maximum concentration, terminal elimination half-life, and total exposure were also computed from the final model (Table 6.10). Goodness-of-fit diagnostics and the prediction-corrected visual predictive checks demonstrated that the model described the observed data adequately.

Table 6.10: Population Pharmacokinetic Parameter Estimates of Amodiaquine and Desethylamodiaquine

Population Pharmacokinetic Parameters	Prior Estimates*	Population Estimates†	95% Confidence Interval‡	%RSE‡
Amodiaquine				
K_a (hour^{-1})	0.418	0.469	0.410–0.508	5.45%
Absorption lag time (hour)	0.314	0.226	0.214–0.233	2.28%
$V_{C(AQ)}$ (litres)	3520	5840	4520–7110	11.1%
$CL_{(AQ)}$ (litres/hour)	2420	2790	2510–3070	4.80%
$V_{P(AQ)}$ (litres)	32500	34200	29600–40100	7.85%
$Q_{(AQ)}$ (litres/hour)	2470	2900	2400–3350	8.09%
σ_{AQ}	N/A	0.108	0.0763–0.145	8.42%
Desethylamodiaquine				
$V_{C(DEAQ)}$ (litres)	198	213	158–272	13.6%
$CL_{(DEAQ)}$ (litres/hour)	34.1	35.2	33.3–37.8	3.25%
$V_{P1(DEAQ)}$ (litres)	2630	2760	2310–3180	7.79%
$Q_{P1(DEAQ)}$ (litres/hour)	163	181	157–206	13.6%
$V_{P2(DEAQ)}$ (litres)	5650	6530	5740–7410	6.24%
$Q_{P2(DEAQ)}$ (litres/hour)	25.4	30	25.6–35.2	7.98%
σ_{DEAQ}	N/A	0.255	0.216–0.304	4.49%
Inter-Individual Variability (%CV)				
Absorption lag time (hour)	0.359 (65.7%)	0.541 (73.6%)	0.342–0.738	9.03%
$V_{C(AQ)}$ (litres)	0.382 (68.2%)	0.772 (87.9%)	0.383–1.34	16.1%
$CL_{(AQ)}$ (litres/hour)	0.0578 (24.4%)	0.0586 (24.2%)	0.0368–0.0890	12.5%
$V_{P(AQ)}$ (litres)	0.0603 (24.9%)	0.0612 (27.4%)	0.0337–0.0814	10.0%
$Q_{(AQ)}$ (litres/hour)	0.0678 (26.5%)	2.94 (171%)	1.95–4.56	11.4%
$V_{C(DEAQ)}$ (litres)	0.196 (46.5%)	0.691 (83.1%)	0.335–1.08	13.4%
$CL_{(DEAQ)}$ (litres/hour)	0.0522 (23.1%)	0.0373 (19.3%)	0.0255–0.0532	9.73%
$V_{P2(DEAQ)}$ (litres)	0.0239 (15.6%)	0.0211 (14.5%)	0.0132–0.0298	9.87%
F	0.0251 (15.9%)	0.0256 (16.0%)	0.0164–0.0385	10.8%
Secondary Parameters				
$C_{max(AQ)}$ (ng/ml)		13.8	7.86-30.6	
$C_{max(AQ)}$ (nmol/litre)		42.1	24.0-93.3	
$T_{max(AQ)}$ (hours)		1.70	0.793-3.56	
$T_{1/2(AQ)}$ (hours)		5.93	0.861-72.1	
AUC_7 (hours x ng/ml)		494	341-681	
$C_{max(DEAQ)}$ (ng/ml)		240	166-429	
$C_{max(DEAQ)}$ (nmol/litre)		731	506-1310	
$T_{max(DEAQ)}$ (hours)		3.61	2.00-6.67	
$T_{1/2(DEAQ)}$ (days)		13.4	11.8-15.0	
AUC_{28} (hours x µg/ml)		31.7	19.8-46.3	

AQ = amodiaquine; DEAQ = desethylamodiaquine; K_a = absorption first-order rate constant; V_C = apparent volume of distribution of central compartment; CL = apparent elimination clearance rate from central compartment; V_P = apparent volume of distribution of peripheral compartment; Q = inter-compartmental clearance rate between central and peripheral compartment(s); σ = residual error variance; F = relative bioavailability; %CV = coefficient of variation for interindividual variability computed as 100 x $\sqrt{(e^{variance} - 1)}$; C_{max} = maximum concentration; T_{max} = time to maximum concentration; $T_{1/2}$ = terminal elimination half-life; AUC = area under curve, i.e. total drug exposure; *Population pharmacokinetic estimates with corresponding parameter uncertainties from model developed from a separate study with rich pharmacokinetic sampling[192]; †Population mean estimates from NONMEM® for a 'typical' 48.0kg adult patient with uncomplicated malaria; ‡From sampling-importance-resampling procedure[193] of the final model.

6.4.5.2 Cardiovascular Vital Signs – Pulse Rate

The population mean maximum total concentration (C_{max}) of amodiaquine and desethylamodiaquine of approximately 750nmol/L (or 250ng/mL) (Table 6.10) was associated with a mean decrease in pulse rate of 14.6 beats/minute (95% CI: 10.9 to 18.2) in addition to the pulse rate reduction following recovery from fever (5.7 beats/minute per 1°C decrease; 95% CI: 3.7 to 7.6) and from acute malaria (3.0 beats/minute; 95% CI: 0.5 to 5.5) (Table 6.11). Male sex did not have statistically significant effects on pulse rate compared to female sex at this sample size (Table 6.11).

Table 6.11: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on the Pulse Rate in Malaria

		Pulse Rate (beats/minute)			
		Univariable Analyses		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/litre increase	362	-19.51 (-23.10 to -15.92)	<0.0001	-14.57 (-18.22 to -10.92)	<0.0001
Body temperature change, per 1°C increase	362	9.72 (8.32 to 11.12)	<0.0001	5.65 (3.74 to 7.56)	<0.0001
Malaria	362				
Yes (days 0-2)	106	-3.21 (-5.54 to -0.90)	0.0066	2.98 (0.50 to 5.46)	0.0186
No (days 3-28)	256	Reference		Reference	
Sex	362				
Female	193	Reference		Reference	
Male	169	3.06 (-4.81 to 10.93)	0.4388	4.12 (-4.17 to 12.41)	0.3232

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

6.4.5.3 Cardiovascular Vital Signs – Blood Pressure

After adjusting for acute malaria, the total concentration of amodiaquine plus desethylamodiaquine at C_{max} was associated with a mean fall of 12.4mmHg (95% CI: 8.9 to 15.9) in supine systolic blood pressure (Table 6.12), 11.0mmHg (95% CI: 7.4 to 14.7) in erect systolic blood pressure (Table 6.12), 4.7mmHg (95% CI: 1.9 to 7.4) in supine diastolic blood pressure (Table 6.13), and 10.3mmHg (95% CI: 7.4 to 13.1) in erect diastolic blood pressure (Table 6.13).

Total drug concentration effects on the postural change between supine and erect blood pressure measurements were small and of unclear clinical significance after adjustment for malaria (Table 6.14).

Table 6.12: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on Systolic Blood Pressure in Malaria

		Systolic Blood Pressure – Supine (mmHg)				*Systolic Blood Pressure – Erect (mmHg)*			
		Univariable Analyses		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/litre increase	362	-18.09 (-20.99 to -15.20)	<0.0001	-12.43 (-15.95 to -8.92)	<0.0001	-12.33 (-15.21 to -9.46)	<0.0001	-11.05 (-14.75 to -7.35)	<0.0001
Body temperature change, per 1°C increase	362	4.46 (3.14 to 5.79)	<0.0001			3.00 (1.76 to 4.24)	<0.0001		
Malaria	362								
Yes (days 0, 1, 2)	106	-9.41 (-11.52 to -7.30)	<0.0001	-4.53 (-6.93 to -2.13)	0.0002	-4.73 (-6.91 to -2.55)	<0.0001	-0.39 (-2.92 to 2.14)	0.7617
No (days 3, 7, 14, 21, 28)	256	Reference		Reference		Reference		Reference	

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

Table 6.13: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on Diastolic Blood Pressure in Malaria

		Diastolic Blood Pressure – Supine (mmHg)				*Diastolic Blood Pressure – Erect (mmHg)*			
		Univariable Analyses		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/litre increase	362	-7.97 (-10.25 to -5.69)	<0.0001	-4.65 (-7.37 to -1.94)	0.0008	-10.64 (-13.03 to -8.25)	<0.0001	-10.26 (-13.11 to -7.42)	<0.0001
Body temperature change, per 1°C increase	362	2.08 (1.11 to 3.05)	<0.0001			2.97 (1.94 to 4.00)	<0.0001		
Malaria	362								
Yes (days 0, 1, 2)	106	-2.17 (-3.71 to -0.63)	0.0058	-0.35 (-2.20 to 1.51)	0.7141	-1.13 (-2.84 to 0.58)	0.1958	2.91 (0.97 to 4.85)	0.0034
No (days 3, 7, 14, 21, 28)	256	Reference		Reference		Reference		Reference	

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

Table 6.14: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on Postural Blood Pressure Change in Malaria

		Systolic Blood Pressure – Postural Change (mmHg)				*Diastolic Blood Pressure – Postural Change (mmHg)*			
		Univariable Analyses		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/litre increase	362	-5.01 (-8.06 to -1.97)	0.0013	-0.38 (-4.32 to 3.55)	0.849	2.31 (-0.10 to 4.71)	0.0602	4.87 (1.90 to 7.84)	0.0014
Body temperature change, per 1°C increase	362	0.52 (-0.43 to 1.47)	0.2787			-0.50 (-1.14 to 0.15)	0.1331		
Malaria	362								
Yes (days 0-2)	106	-4.50 (-6.77 to -2.23)	0.0001	-4.35 (-7.10 to -1.59)	0.0021	-1.00 (-2.75 to 0.75)	0.2621	-2.94 (-5.03 to -0.85)	0.0059
No (days 3-28)	256	Reference		Reference		Reference		Reference	

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

6.4.5.4 Electrocardiographic Intervals – Corrected QT, QRS, and PR Intervals

Once adjusted by change in body temperature, age, sex, and change in RR interval, the mean corrected QT interval prolongation resulting from amodiaquine plus desethylamodiaquine at C_{max} was very similar irrespective of the heart rate correction factor used (QTcS: 10.4 milliseconds, 95% CI: 5.9 to 15.0; QTcF: 10.7 milliseconds, 95% CI: 6.1 to 15.2; QTcB: 10.3 milliseconds, 95% CI: 5.7 to 14.8). Unlike the study-specific corrected QT interval, the Fridericia- and Bazett-corrected QT intervals retained clinically and statistically significant heart rate dependency after adjustment although in opposite directions (QTcF: 11.6 milliseconds per 300-millisecond increase in RR interval, 95% CI: 7.3 to 16.0; QTcB: -11.5 milliseconds; 95% CI: -15.8 to -7.1). Without adjustment, use of the Fridericia heart rate correction overestimated amodiaquine-related QT prolongation from baseline while using the Bazett correction underestimated it (Table 6.15).

The mean effect of amodiaquine plus desethylamodiaquine at C_{max} on the QRS and PR intervals was neither clinically nor statistically significant once adjusted by change in body temperature, sex, age, and change in RR interval (QRS: -0.47 milliseconds, 95% CI: -2.51 to 1.57; PR: 2.01 milliseconds; 95% CI: -1.29 to 5.31) (Table 6.16)

Table 6.15: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on the Corrected QT Interval in Malaria

		QTcS – Study-specific Correction (milliseconds)				*QTcF – Fridericia Correction (milliseconds)*				*QTcB – Bazett Correction (milliseconds)*			
		Univariable Analyses		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**	**Crude Estimate (95% CI)**	***p* value**	**Adjusted Estimate (95% CI)**	***p* value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/litre increase	356	12.98 (8.73 to 17.16)	<0.0001	10.43 (5.92 to 15.00)	<0.0001	20.35 (15.56 to 25.15)	<0.0001	10.67 (6.13 to 15.21)	<0.0001	6.10 (1.80 to 10.38)	0.0055	10.26 (5.71 to 14.81)	<0.0001
Body temperature change, per 1°C increase	356	-5.30 (-7.12 to -3.47)	<0.0001	-3.97 (-6.49 to -1.46)	0.0021	-10.59 (-12.52 to -8.65)	<0.0001	-4.04 (-6.57 to -1.52)	0.0018	-0.374 (-2.25 to 1.50)	0.6948	-3.90 (-6.43 to -1.37)	0.0026
Sex	356												
Female	187	Reference		Reference		Reference		Reference		Reference		Reference	
Male	169	-16.24 (-25.48 to -7.00)	0.0009	-17.35 (-26.62 to -8.08)	0.0004	-12.72 (-22.11 to -3.34)	0.0088	-13.30 (-22.94 to -3.66)	0.0078	-19.54 (-29.00 to -10.08)	0.0001	-21.05 (-30.57 to -11.52)	<0.0001
Age, per 10-year increase	356	2.33 (-0.25 to 0.71)	0.3339	4.16 (-0.17 to 8.49)	0.0595	2.87 (-1.78 to 7.52)	0.2209	4.47 (-0.30 to 8.98)	0.0515	1.78 (-3.32 to 6.88)	0.4859	3.87 (-0.57 to 8.32)	0.0864
RR interval change, per 300-millisecond increase	356	7.50 (4.46 to 10.54)	<0.0001	-0.42 (-4.80 to 3.95)	0.8475	19.69 (16.65 to 22.74)	<0.0001	11.63 (7.25 to 16.01)	<0.0001	-3.69 (-6.75 to -0.65)	0.0177	-11.45 (-15.84 to -7.06)	<0.0001

$QTcS = \frac{QT}{RR^{0.42}}$ & $QTcF = \frac{QT}{\sqrt[3]{RR}}$ & $QTcB = \frac{QT}{\sqrt{RR}}$, where RR is in units of seconds

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

†Mean change in RR interval from baseline (rounded) after last dose of amodiaquine treatment in this study

Table 6.16: Multivariable Linear Mixed Effects Regression Analysis of Effect of Total Amodiaquine and Desethylamodiaquine Concentration on the QRS and PR Intervals in Malaria

		QRS Interval (milliseconds)				*PR Interval (milliseconds)*			
		Univariable Analyses		**Multivariable Analyses**		**Univariable Analyses**		**Multivariable Analyses**	
	Number of Observations	**Crude Estimate (95% CI)**	**p value**	**Adjusted Estimate (95% CI)**	**p value**	**Crude Estimate (95% CI)**	**p value**	**Adjusted Estimate (95% CI)**	**p value**
Total concentration of amodiaquine and desethylamodiaquine, per 750* nmol/l increase	356	2.34 (0.38 to 4.29)	0.0194	-0.47 (-2.51 to 1.57)	0.6525	6.68 (3.51 to 9.84)	<0.0001	2.01 (-1.29 to 5.31)	0.2313
Body temperature change, per 1°C increase	356	-2.08 (-2.86 to -1.30)	<0.0001	-0.30 (-1.42 to 0.82)	0.5953	-3.86 (-5.15 to -2.57)	<0.0001	-0.54 (-2.38 to 1.30)	0.5647
Sex	356								
Female	187	Reference		Reference		Reference		Reference	
Male	169	5.44 (0.68 to 10.2)	0.0259	5.09 (0.27 to 9.91)	0.0388	1.60 (-11.84 to 15.04)	0.8124	-0.06 (-13.72 to 13.62)	0.9935
Age, per 10-year increase	356	1.53 (-0.76 to 3.82)	0.186	1.08 (-1.17 to 3.34)	0.3386	4.03 (-2.17 to 10.22)	0.1976	4.11 (-2.29 to 10.51)	0.2027
RR interval change, per 300 millisecond increase	356	4.43 (3.15 to 5.71)	<0.0001	4.20 (2.22 to 6.18)	<0.0001	8.18 (6.10 to 10.26)	<0.0001	6.91 (3.68 to 10.14)	<0.0001

*Mean maximum total drug concentration (rounded) after a 3-day course of amodiaquine from pharmacokinetic analysis of same study

†Mean change in RR interval from baseline (rounded) after last dose of amodiaquine treatment in this study

6.5 DISCUSSION

To my knowledge, this study is the largest analysis to date of the cardiovascular effects of amodiaquine, with data from 5509 participants in trials with amodiaquine treatment arms including 2681 individuals who had electrocardiographic monitoring. These trials compared artesunate-amodiaquine to the most widely-used front-line oral antimalarials for uncomplicated *P. falciparum* (artemether-lumefantrine and dihydroartemisinin-piperaquine) and *P. vivax* (chloroquine) malaria as well as the latest ACT indicated for use in treatment of both *P. falciparum* and *P. vivax* malaria (pyronaridine-artesunate). This is also the first study to formally investigate the concentration-effect relationships between amodiaquine and the electrocardiographic QT interval as well as cardiovascular vital signs of heart rate and blood pressure.

6.5.1 Heart Rate

In adolescents and adults (12 years and older), artesunate-amodiaquine was associated with a significantly higher incidence of sinus bradycardia and a greater mean reduction in heart rate compared to front-line antimalarials even after adjusting for body temperature and sex. More than 50% of adults developed sinus bradycardia, 15% of these to a degree they could be symptomatic. This heart rate reduction was concentration-dependent, reaching its nadir around the time of maximum amodiaquine and desethylamodiaquine concentrations before returning to baseline levels by 3-5 days later. In children (younger than 12 years), as in adults, artesunate-amodiaquine was associated with the greatest mean heart rate reduction of the antimalarials under investigation although unlike in adults this was similar to that after artemether-lumefantrine and did not result in a clinically significant increase in the incidence of sinus bradycardia versus comparator antimalarials. These decreases in heart rate were accompanied by a small concentration-dependent drop

in blood pressure similar to chloroquine which also returned to baseline levels on the same timescale. This overriding of baroreceptor reflex activity provides further evidence of a pharmacological effect in addition to malaria recovery-related changes.

In keeping with my findings, 16 (64%) of 20 patients had sinus bradycardia after amodiaquine monotherapy at total doses of 30-35mg/kg over three days in the only cardiac safety study of amodiaquine in adults with uncomplicated malaria[254], which was conducted in Cameroon. In the few studies[253,254] of amodiaquine with electrocardiographic monitoring not included in this meta-analysis and in case reports[320,321], sinus bradycardia after amodiaquine has been recorded almost entirely (24/25) in patients 12 years and older around the time of expected maximum drug concentration of amodiaquine and desethylamodiaquine. This bradycardia can be symptomatic with easy fatiguability[320] and dizziness when accompanied by hypotension[321]. Higher day 3 desethylamodiaquine concentrations have previously been associated with higher rates of bradycardia[253] while higher day 7 concentrations have been less consistently associated with fatigue[322], and vomiting[322].

Could these drug-related changes in cardiovascular vital signs explain the higher incidence of asthenia and asthenia-like reactions after amodiaquine compared to other antimalarials? The symptoms of bradycardia and hypotension include dizziness, weakness, and fatiguability. Amodiaquine-associated asthenia has been more frequently observed in adolescents and adults 12 years and older than in children[323], and is typically self-limiting, lasting for about 3 days after the end of amodiaquine treatment[323]. Pregnant women with malaria appear to be more susceptible[321,324] possibly because of gestational-related changes in heart rate and blood pressure[325]. As asthenia is also a common feature of clinical malaria, discriminating between adverse events which are symptoms of the acute illness and treatment-emergent signs and symptoms, such as through active elicitation of carefully

defined adverse events of special interest at pre-specified timepoints, would be especially important to improve detection of this possible adverse drug reaction[326]. My analysis was limited by the unavailability of individual patient-level data on adverse events for the majority of included participants and heterogeneity in data collection methodology of the two studies for which individual patient-level adverse event data were shared.

Possible molecular mechanisms are both neurological and cardiovascular. Amodiaquine exhibits potent reversible acetylcholinesterase inhibition[327] (more than chloroquine and much more than mefloquine)[328,329], which could potentiate the effects of parasympathetic activation and account for vagal presentations like bradycardia and dizziness as well as vomiting and diarrhoea. It also reduces the pacemaker "funny" current at the sino-atrial node[330], which directly causes bradycardia.

Further clinical characterisation of the possible relationship between asthenia and bradycardia through systematic cardiovascular vital sign monitoring and exercise testing after amodiaquine would help us better understand the contribution of the cardiovascular system to this common presentation after amodiaquine. Prospective validation of these cardiovascular findings in adult healthy volunteers (NCT04080895) is ongoing.

6.5.2 Corrected QT Interval

Artesunate-amodiaquine was also found to transiently prolong the corrected QT interval in a concentration-dependent manner regardless of correction factor used. This corrected QT interval prolongation after artesunate-amodiaquine was more than that after pyronaridine-artesunate and artemether-lumefantrine but less than after dihydroartemisinin-piperaquine and chloroquine including after adjusting for body temperature, sex, and age. However, these differences were small, and unlike heart rate did not reach statistical significance when analysed at the level of the individual single-site trial[253].

The clinical significance of QT interval prolongation is as an imperfect surrogate marker for risk of development of TdP, a polymorphic ventricular tachycardia that can degenerate in some cases into ventricular fibrillation and lead to sudden cardiac death. Iatrogenic QT prolongation remains a source of discussion and debate. In contrast to how it is often used, the relationship between QT prolongation and proarrhythmia is inconsistent and qualitative rather than quantitative: drugs which prolong the QT interval range from having potent torsadogenic activity (e.g. halofantrine) to no proarrhythmic action and can even be anti-arrhythmic (e.g. amiodarone); TdP can occur without QT prolongation and most QT prolonging drugs do not cause TdP[331]. The lack of consensus about thresholds and definitions of drug-induced QT prolongation reflects these limitations. ICH-recommended categorical analyses using multiple thresholds (absolute value: >500, >480 and >450 milliseconds; change from baseline: >60 and >30 milliseconds) are proposed to be a reasonable approach to address this uncertainty[136] and provide useful information about the QT prolonging profile of a drug. However, QT values beyond these thresholds have been frequently misinterpreted as being clinical complications necessitating intervention in their own right even though only QT values of >500 milliseconds – corrected or uncorrected for heart rate – are considered definitely abnormal[136,138]. Similarly, thresholds for mean prolongations in ICH guidelines do not identify drugs as proarrhythmic but that they could have an increased likelihood of being proarrhythmic and could have clinical arrhythmic events detected during drug development[136]. All of this information is meant to contribute towards risk-benefit evaluations which ultimately determine whether the drug receives regulatory approval and how it is used.

In the case of the quinoline and structurally-related antimalarials, QT prolongation is a well-known feature of this drug class. Indeed, QT prolongation with TdP was once termed the "quinidine effect"[129] and quinidine's main use for the past 100 years has been as an anti-arrhythmic. As recommended in ICH guidance[136], comparisons within the drug class are

valuable to further define any proarrhythmic risk. Moreover, evaluation of the cardiotoxicity of antimalarials is aided by decades of clinical experience with the widespread deployment of these drugs throughout the malaria-endemic world. Of the oral quinoline and quinoline-like antimalarials, only halofantrine is considered to have an unacceptable risk of arrhythmia when used for malaria indications and has never been recommended for use by the WHO[47]. This risk was first identified – after less extensive use than current front-line antimalarials – following a sudden death[147], which was soon followed by reports of further deaths as well as ventricular fibrillation, TdP, and extreme mean QT interval prolongation[69] (>60 milliseconds)[47].

All current front-line antimalarials, including artesunate-amodiaquine, have mean corrected QT prolongations from baseline of approximately 20 milliseconds or less at standard doses and when administered according to manufacturer's recommendations, such as administering dihydroartemisinin-piperaquine when fasted. The data on proarrhythmic risk of drugs which prolong the mean QT interval prolongation of 20 milliseconds or less is considered inconclusive while those associated with prolongation of more than 20 milliseconds could have clinical arrhythmias captured during drug development[136]. In this analysis, chloroquine had the greatest mean corrected QT interval prolongation and was the only drug with a mean prolongation of just above 20 milliseconds. It is one of the most widely-used drugs in humans, with hundreds of metric tonnes dispensed annually since the 1950s and a terminal elimination half-life of over a month, and could well be the drug to which humans have been most exposed. Chloroquine remains a first-line treatment for *P. vivax* malaria. While it has been associated with sudden deaths and TdP, this has been with its use in overdose or for chronic indications other than treatment of malaria[47]. Dihydroartemisinin-piperaquine had the next longest mean QT prolongation of just under 20 milliseconds in this analysis of malaria patients and this was clinically indistinguishable from the prolongation seen in

healthy volunteers receiving a single[119] or repeated[332] courses of the drug. Dihydroartemisinin-piperaquine has now been used extensively with millions of doses distributed for treatment and prevention of malaria including as part of large mass drug administration programmes. The risk of sudden unexplained death after dihydroartemisinin-piperaquine is no higher than baseline[209] and despite having undergone the most extensive cardiac safety evaluation of any antimalarial in history[209,248,333], dihydroartemisinin-piperaquine has never been associated with ventricular fibrillation or TdP[47]. Artemether-lumefantrine was the first fixed-dose ACT to be pre-qualified by the WHO and has been studied intensively[248]. Like with dihydroartemisinin-piperaquine, the QT prolongation after artemether-lumefantrine in my analysis was similar to that observed in healthy volunteers[119] and has not been associated with arrhythmic adverse events[47,248]. Further safety results from the large phase IV study of pyronaridine-artesunate (NCT03201770) are awaited.

Slight QRS prolongation of unclear clinical significance has been previously reported after amodiaquine[334,335], although these analyses did not adjust for heart rate changes. Following adjustment for heart rate and body temperature changes as well as age and sex in this analysis, there was no discernible concentration-dependent effect of amodiaquine and desethylamodiaquine on the QRS or PR intervals.

Artesunate-amodiaquine is associated with QT prolongation intermediate to that after dihydroartemisinin-piperaquine and artemether-lumefantrine as well as clinically significant sinus bradycardia in adults but not children; bradycardia is an important risk factor for drug-induced TdP[138]. Both effects have concentration-dependent relationships with amodiaquine and its metabolite desethylamodiaquine. Despite its effects on cardiac electrophysiology, amodiaquine has not been associated with sudden deaths, life-threatening tachyarrhythmias, or TdP[47] even with extensive use, particularly in seasonal

malaria chemoprevention (at least 85 million and 102.7 million treatments were distributed in 2018 and 2019 respectively). Until further information is available, caution is advised in the use of amodiaquine in adolescent and adult patients with conduction disorders, bradycardia, and concomitant use of other medications which slow the heart rate such as ivabradine, beta-blockers, and calcium-channel blockers, particularly in the presence of other risk factors for drug-induced TdP.

6.5.3 Heart Rate Correction and Non-Drug Factors Affecting QT Interval

In this analysis, an age group-specific correction for each individual study provided the most effective heart rate adjustment for the QT interval. The commonly used Bazett and Fridericia corrections both provided suboptimal heart rate correction in malaria with significant residual heart rate dependency of the QT interval although in opposite directions: the Bazett correction overestimated absolute QT interval values and underestimated QT interval changes while the Fridericia correction underestimated absolute QT values and overestimated QT interval changes. The greater decrease in heart rate seen after amodiaquine further augments the effects of any heart rate dependency on the corrected QT interval, confounding comparison of corrected QT interval changes between amodiaquine-containing antimalarials and those which are associated with smaller changes in heart rate especially in the treatment of malaria.

Although an age group-specific correction for each study resulted in satisfactory heart rate correction of the QT interval in our analysis at the population level, it may be possible to further optimise reduction of heart rate dependence by deriving corrections specific to other factors which affect both the QT interval and heart rate, such as sex and degree of malaria illness in addition to age group. In this analysis, the QT-RR relationship changed with day of treatment with the correction exponent being larger at peak disease and lower during recovery. I previously showed how the correction exponent (i.e. heart rate

sensitivity) increases with malaria severity in Chapter 5. However, population-specific correction factors require specialist input to derive and are of unclear generalisability to individual patients at the point of care as the QT-RR relationship is highly individual[336]. Individual heart rate corrections are not practicable in malaria trials or clinical practice because of resource limitations on ECG monitoring and are complicated by confounding from disease recovery.

For comparison of QT values to assess the relative difference between treatment arms in randomised controlled trials where factors known to affect the QT interval (age, sex, body temperature changes, malaria recovery) are balanced between arms, the use of the Bazett and Fridericia corrections could provide interpretable information. If absolute values of corrected QT interval prolongation are of interest, then optimising heart rate correction with appropriate adjustment for the other factors which affect the QT interval would be necessary to obtain the true drug-related effect.

6.5.4 Conclusion

Amodiaquine is a 4-aminoquinoline antimalarial which, like other quinoline and quinoline-like drugs, has transient effects on cardiac electrophysiology. In particular, it causes the greatest reduction of heart rate of the front-line antimalarials, which results in sinus bradycardia more frequently in adults than children. It also prolongs the QT interval to a smaller extent than chloroquine and piperaquine but more than lumefantrine and pyronaridine. Despite these concentration-dependent effects, there have been no sudden deaths or clinically significant arrhythmias reported throughout its extensive use in both the treatment and prevention of malaria. Amodiaquine is, however, associated with a higher incidence of non-severe and non-specific adverse events like asthenia and dizziness compared to other front-line antimalarials, particularly in adults, to which its cardiovascular effects may contribute. Further clinical and molecular definition of the

effects of amodiaquine on the cardiovascular system is warranted to develop measures to improve the tolerability of this important antimalarial. This would support patients, healthcare providers, policy-makers, and drug development programmes in optimising selection of antimalarial treatment(s) according to the needs of the populations they serve.

6.6 AUTHORSHIP STATEMENT

I designed this study with Professor Nicholas White, conducted the systematic review, carried out the study-level data extraction and individual patient-level data gathering from study investigators, compiled the data dictionary, mapped each received study to the data dictionary, formatted received files for data standardisation, wrote the Python scripts for data standardisation, prepared the standardised data for statistical analysis, performed the statistical analysis, made all the figures and tables, and wrote all the text. Dr Ilsa Haeusler and Dr Yan Naung Win were the additional independent reviewers in the systematic review and data extraction. Dr Yan Naung Win assisted with mapping of individual studies. James Pike and Maryam Hanafiah extended the bespoke Python Application Programming Interface used for data standardisation and provided intensive database programming support. Dr Palang Chotsiri implemented the pharmacokinetic analyses using NONMEM®.

7 Conclusion and Future Directions

7.1 SUMMARY OF OUTPUT

7.1.1 Antimalarial Repolarisation-Related Cardiotoxicity

7.1.1.1 Sudden Cardiac Death or Sudden Unexplained Death

None of the WHO-recommended first-line drugs for the treatment or prevention of malaria[30] is associated with an increased risk of sudden cardiac death or sudden unexplained death when used for these malaria-related indications. This is based on extensive systematic literature reviews as well as searches of WHO and pharmaceutical company drug safety databases[47,209,248].

Despite concerns early in drug development[119], I found the risk of sudden unexplained death after dihydroartemisinin-piperaquine (DHA-PPQ) to be not higher than baseline[209]. DHA-PPQ has since been added to the WHO Essential Medicines List and Essential Medicines List for children, and continues to be used safely in large volumes for the treatment and prevention of malaria.

There have been no sudden unexplained deaths after artemether-lumefantrine (AL) or amodiaquine (AQ) despite vast use[47,248]: >840 million doses of AL were distributed from 1998 to 2016[47], and >100 million doses of co-formulated AQ + SP for seasonal malaria chemoprevention were deployed in 2019 alone. Chloroquine is the most widely used antimalarial drug in history with an annual consumption of hundreds of tonnes over the last six decades[48]. The handful of case reports of sudden death after chloroquine have been for its use for rheumatological indications or in overdose[47].

Halofantrine has been associated with 36 sudden cardiac deaths[47,69] after less extensive use – >23.2 million doses sold between 1988 and 2016 – and has never been recommended for use by the WHO[47]. GlaxoSmithKline have since withdrawn halofantrine from market[47].

7.1.1.2 Torsade de Pointes and Other Arrhythmias

No cases of torsade de pointes (TdP) or other clinically significant arrhythmias have been detected after WHO-recommended antimalarials in the systematic reviews of malaria clinical trials[248] I conducted nor in drug safety databases[47]. This is in contrast to investigations of other widely used QT interval-prolonging anti-infective agents: the macrolide[337] and fluoroquinolone[337,338] antibiotics have both been associated with development and increased risk of clinically significant arrhythmias, including TdP, in a systematic review and meta-analysis of prospective clinical studies[338] as well as a search of the WHO drug safety database[337].

Alongside findings of no excess risk of sudden unexplained death after the same antimalarials, these reassuring results provide further evidence of the extreme rarity of serious arrhythmias after front-line antimalarial therapy. Quinidine, the only intravenous antimalarial approved by the US FDA, was associated with a high risk of TdP in the treatment of severe malaria[339] and has now been discontinued. The first-line drug for severe malaria in the US CDC malaria treatment guidelines is now intravenous artesunate[130] in line with WHO recommendations[30].

It is worth noting that the study of arrhythmia after antimalarial treatment is limited by the scarcity of ECG monitoring and the outpatient basis of antimalarial therapy in the parts of the world where malaria is endemic. These considerations underscore why mandatory ECG monitoring for any antimalarial would severely curtail its use, unnecessarily limiting access to essential life-saving medicines.

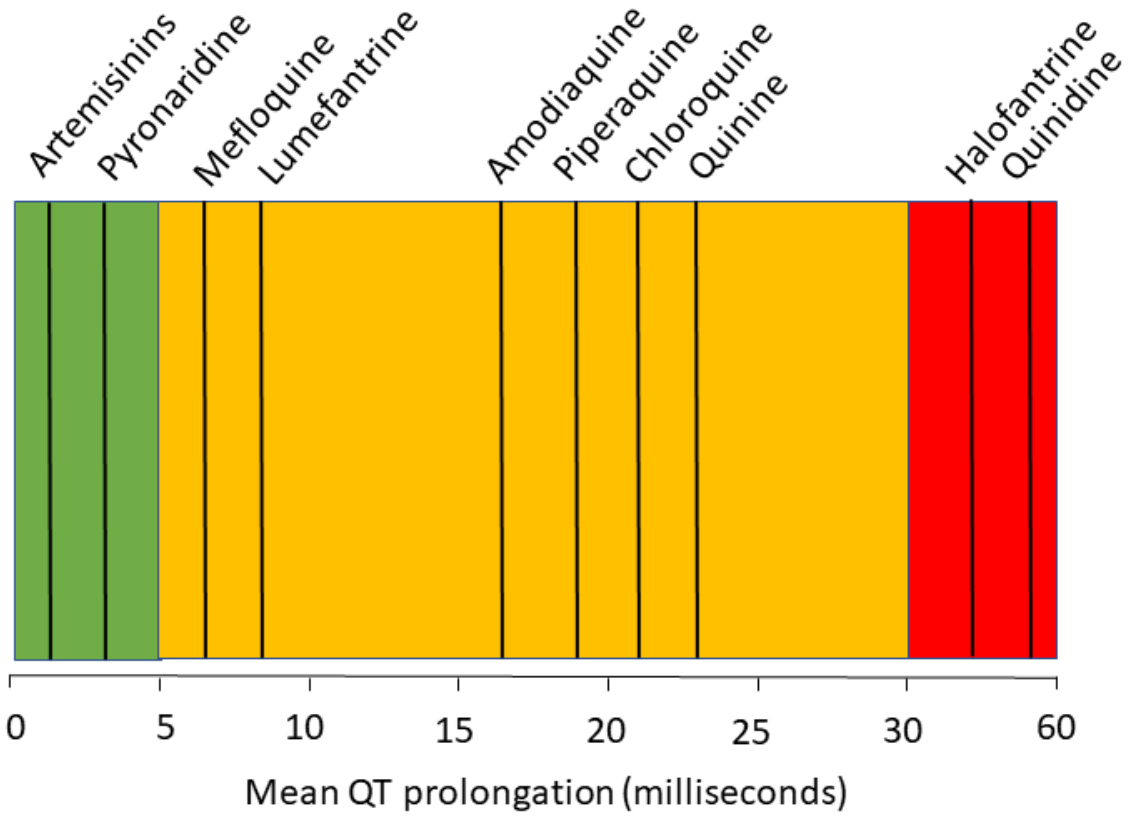
Given the established safety record of front-line antimalarials, it is difficult to see why routine ECG monitoring for these drugs in WHO-recommended doses and combinations would be necessary.

7.1.1.3 QT Interval Prolongation

Investigation of the QT interval in malaria is bedevilled by the considerable heterogeneity in how ECGs are recorded, analysed, and reported across studies[248]. In patients with clinical malaria, there is additional confounding from fever and severity of malaria illness on top of the effects of demographic factors of age and sex. Population-derived heart rate correction formulae[136] and multivariable methods may provide suitable adjustments but this remains an area of active research in cardiology and regulatory science.

In healthy volunteers as well as after adjustments for disease and demographic factors in malaria patients, all front-line antimalarials in current use are associated with drug-related mean QT interval prolongation of approximately 20 milliseconds and under after WHO-recommended treatment regimens. Where present, this QT prolongation is associated with a very low risk of cardiotoxicity which does not limit the continued and extensive global use of these drugs in the treatment and prevention of malaria (Figure 7.1).

Figure 7.1: QT Traffic Lights – Risk of Repolarisation-related Cardiotoxicity after Antimalarials



Schematic summary of risk of cardiac repolarisation-related toxicity of antimalarial drugs adapted from the report of a WHO evidence review[47] with additional data from this thesis.

Colours represent the level of risk minimisation measures implemented: red – withdrawn from market[47] and/or treatment guidelines[130]; amber – QT-prolonging potential without clinically significant risk in recommended treatment regimens, caution is advised when used in individuals with known torsade de pointes risk factors and with drugs that also prolong the QT interval or increase drug levels; green – minimal QT-prolonging potential.

7.1.2 Other Cardiovascular Effects of Antimalarials

7.1.2.1 Bradycardia

Bradycardia is a common cardiovascular side effect after antimalarial treatment with amodiaquine and mefloquine[248]. With mefloquine, bradycardia is observed in adolescents and adults more than in children[255], and this is also the case with amodiaquine. I found that in individuals 12 years and older, amodiaquine causes a concentration-dependent reduction in heart rate greater than AL, chloroquine, DHA-PPQ, and pyronaridine-artesunate, which results in a higher risk of potentially symptomatic sinus bradycardia.

Both amodiaquine and mefloquine are associated with reversible inhibition of human acetylcholinesterase (AChE), with amodiaquine having a much higher potency than mefloquine[328,329]. This high potency has led to amodiaquine being investigated for use as a reactivator of inhibited AChE in the treatment of organophosphate poisoning[327]. Amodiaquine, like hydroxychloroquine, may also block the pacemaker current at the sino-atrial node[330], and has been found to have antiarrhythmic activity in pre-clinical studies[313,314].

Cholinergic activity and sinus bradycardia (whether primary or secondary) may provide a unifying explanation for the asthenia and asthenia-like reactions after amodiaquine which are also more common in adults[323]. Further clinical characterisation with pharmacokinetic sampling and detailed treatment-emergent symptom evaluation is recommended.

In addition, bradycardia is a causal risk factor for TdP[139]. Caution is advised in the use of amodiaquine in adolescent and adult patients with cardiac conduction disorders and concomitant use of heart rate-reducing medications, particularly in the presence of other risk factors for TdP such as pre-existing conditions and medications which prolong the QT interval or increase drug concentrations.

7.1.2.2 Hypotension

The dangers of rapid intravenous injection of quinine[61] and chloroquine[84] gained attention after a number of sudden deaths, and led to changes in dosing recommendations[61,84]. Hypotension from peripheral vasodilatation and negative inotropy was the probable cause of these sudden deaths. Pharmacokinetic-pharmacodynamic assessments found that toxicity resulted from transient high plasma concentrations following bolus administration which could be circumvented by switching to controlled continuous infusion[84].

Amodiaquine-related bradycardia is associated with a reduction in blood pressure and borderline diastolic hypotension greater than that after chloroquine which may contribute towards development of drug-related symptoms, including asthenia. This overriding of the baroreceptor reflex provides further evidence of a pharmacological effect in addition to malaria recovery-related change, and is consistent with the possible cholinergic effects of amodiaquine. Further validation of these effects in healthy volunteer studies of a full treatment course of amodiaquine is needed.

7.2 FUTURE DIRECTIONS

7.2.1 Moving from Population-based to Personalised Medicine

Having established the extreme rarity of repolarisation-related cardiotoxicity after antimalarial medicines in front-line use in the general population, risk minimisation efforts are better focused on subgroups and individuals at higher risk of clinical complications. Without a specific biomarker or simple screening test, and with pharmacogenomic risk stratification still in the future[145], the time-honoured use of clinical assessment and judgement[340], where available, to identify risk factors and detect serious reactions is paramount to protect patient safety. Clinician education is therefore key.

At the same time, with antimalarial usage in the malaria-endemic world taking place predominantly in an outpatient setting, continued monitoring for adverse drug effects with rigorous investigation and rapid multidisciplinary expert review of any serious reactions in individuals is a pragmatic approach. It is important therefore to continue to strengthen pharmacovigilance in these less well-resourced settings, including through health and demographic surveillance system record linkage, cohort event monitoring, targeted spontaneous reporting, and regular training for healthcare providers[170,244].

7.2.2 Alternative Assessments for Drug-Induced Torsade de Pointes Risk

More specific alternatives to evaluation of QT interval prolongation for determination of drug TdP risk are being developed, including through the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, a multi-stakeholder global effort among regulators, industry, and academia[341]. CiPA proposes a mechanistic-based, four-component approach coupling *in vitro* assessment of drug effects on multiple ion currents with an *in silico* computational model of the human ventricular cardiomyocyte for predicting

proarrhythmic risk. These assessments would be followed by confirmatory *in vitro* studies on human stem cell-derived cardiomyocytes and *in vivo* phase I ECG safety evaluation. A validation programme is ongoing, and if successful and adopted by regulators, a CiPA evaluation demonstrating low arrhythmogenic risk could potentially obviate the need for intensive ECG monitoring in late phase clinical trials of QT interval-prolonging drugs[341].

7.2.3 New Antimalarial Drugs and Combinations

The antimalarial drug development pipeline may be healthier than ever, although no new drugs are as yet ready for deployment where existing treatments are failing[29]. Many of the novel compounds in development are either quinoline and structurally related compounds themselves (e.g. ferroquine[316] and AQ-13[342]) or are being combined with a quinoline or quinoline-like partner drug (e.g. ganaplicide-lumefantrine and artefenomel-piperaquine[278,295])[29].

The safety of older antimalarials cannot be assumed in new combinations. Careful early phase interaction studies and appropriate cardiac monitoring as recommended by current international guidelines[136] for later phase evaluations should continue. Drug development programmes, usually of randomised controlled trials[171,172,340], are generally not designed to detect harms, particularly rare ones. While Bayesian methods incorporating prior clinical experience (such as described in this thesis) from other drugs with similar properties or the same drug for a different indication can be used to reduce the number of patients needed to draw statistically robust safety conclusions[167], these do not replace continued accumulation of real-world experience with the specific drug and indication of interest.

Evaluation of drug safety is an ongoing process over the lifetime of a drug[168]. High quality post-marketing surveillance and pharmacovigilance are essential, and begin with the individual healthcare provider.

7.3 FINAL THOUGHTS

To my knowledge, this thesis is the largest and most detailed study of the cardiovascular toxicity of the quinoline and structurally related antimalarials when used for malaria-related indications, synthesising over half a century of international experience. It quantifies the risks of sudden death and significant arrhythmias after these drugs as well as the degree of drug-attributable QT prolongation once malaria disease effects (also identified through this work) have been adjusted for. In addition, it contributes important data about the cardiac safety of amodiaquine and chloroquine, extensively used antimalarials for which data were especially scarce.

I conclude that the quinoline and structurally related antimalarials in current use, particularly chloroquine, piperaquine, amodiaquine, and lumefantrine, remain safe at WHO-recommended doses and combinations for the treatment, prevention, and global eradication of malaria.

8 Publications Arising

8.1 THESIS-RELATED

8.1.1 Original Articles

1) **Chan XHS**, Win YN, Mawer LJ, Tan JY, Brugada J & White NJ. Risk of Sudden Unexplained Death after Dihydroartemisinin-Piperaquine: A Systematic Review and Bayesian Meta-analysis. *Lancet Infectious Diseases*. 2018 Aug;18(8):913-923.

2) Haeusler IL†, **Chan XHS**†, Guérin PJ & White NJ. The Arrhythmogenic Cardiotoxicity of the Quinoline and Structurally Related Antimalarial Drugs: A Systematic Review. *BMC Medicine*. 2018 Nov 7;16(1):200.

3) **Chan XHS**, Win YN, Haeusler IL, Tan JY, Loganathan S, Saralamba S, Chan SKS, Ashley EA, Barnes KI, Baiden R, Bassi PU, Djimde A, Dorsey G, Duparc S, Hanboonkunupakarn B, ter Kuile FO, Lacerda MVG, Nasa A, Nosten FH, Onyeji CO, Pukrittayakamee S, Siqueira AM, Tarning J, Taylor WRJ, Valentini G, van Vugt M, Wesche D, Day NPJ, Huang CL-H, Brugada J, Price RN & White NJ. Factors Affecting the Electrocardiographic QT Interval in Malaria: A Systematic Review and Meta-analysis of Individual Patient Data. *PLOS Medicine*. (In Press)

4) A manuscript based on the work in Chapter 6 is being submitted

8.1.2 Technical Reports

5) World Health Organization. Report of the WHO Evidence Review Group on the Cardiotoxicity of Antimalarial Medicines. Geneva, Switzerland. 2017 Mar 22. (as WHO Technical Resource Person & Rapporteur)

8.2 OTHER ASSOCIATED WORK

8.2.1 Original Articles

1) Imwong M, Dhorda M, Tun KM, Thu AM, Phyo AP, Proux S, Suwannasin K, Kunasol C, Srisutham S, Duanguppama J, Vongpromek R, Promnarate C, Saejeng A, Khantikul N, Sugaram R, Thanapongpichat S, Sawangjaroen N, Sutawong K, Han KT, Htut Y, Linn K, Win AA, Hlaing TM, van der Pluijm RW, Mayxay M, Pongvongsa T, Phommasone K, Tripura R, Peto TJ, von Seidlein L, Nguon C, Dysoley L, **Chan XHS**, Rekol H, Leang R, Huch C, Kwiatkowski DP, Miotto O, Ashley EA, Kyaw MP, Pukrittayakamee S, Day NPJ, Dondorp AM, Smithuis FM, Nosten FH & White NJ. Molecular Epidemiology of Resistance of Current Antimalarial Drugs in the Greater Mekong Subregion. (Submitted)

2) Hamilton WL, Amato R, van der Pluijm RW, Jacob CG, Quang HH, Thuy-Nhien NT, Hien TT, Hongvanthong B, Chindavongsa K, Mayxay M, Huy R, Leang R, Huch C, Dysoley L, Amarantunga C, Suon S, Fairhurst RM, Tripura R, Peto TJ, Sovann Y, Jittamala P, Hanboonkunupakarn B, Pukrittayakamee S, Chau NH, Imwong M, Dhorda M, Vongpromek R, **Chan XHS**, Maude RJ, Pearson RD, Nguyen T, Rockett K, Drury E, Gonçalves S, White NJ, Day NP, Kwiatkowski DP, Dondorp AM, Miotto O. Evolution and Expansion of Multidrug-Resistant Malaria in Southeast Asia: A Genomic Epidemiology Study. *Lancet Infectious Diseases*. 2019 Sep;19(9):943-951.

3) von Seidlein L, Peto TJ, Landier J, Thuy-Nhien NT, Tripura R, Phommasone K, Pongvongsa T, Lwin KM, Keereecharoen L, Kajeechiwa L, Thwin MM, Parker DM, Wiladphaingern J, Nosten S, Proux S, Corbel V, Tuong-Vy N, Phuc-Nhi TL, Son DH, Huong-Thu PN, Tuyen NTK, Tien NT, Dong LT, Hue DV, Quang HH, Nguon C, Davoeung C, Rekol H, Adhikari B, Henriques G, Phongmany P, Suangkanarat P,

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4) Tun STT, Lubell Y, Dondorp AM, Fieldman T, Tun KM, Celhay O, **Chan XHS**, Saralamba S & White LJ. Identifying Artemisinin Resistance from Parasite Clearance Half-life Data with a Simple Shiny Web Application. *PLOS One*. 2017 May 22;12(5):e0177840.

8.2.2 Correspondence

5) **Chan XHS**, White NJ & Hien TT. A Temporizing Solution to "Artemisinin Resistance". *New England Journal of Medicine*. 2019 Sep 5;381(10):989-990.

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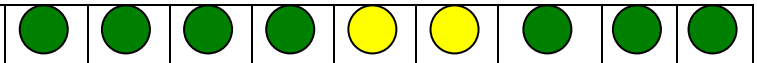
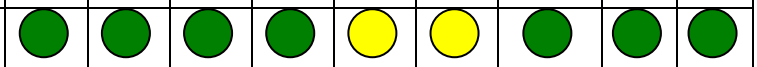
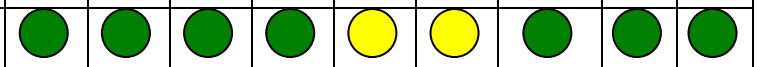
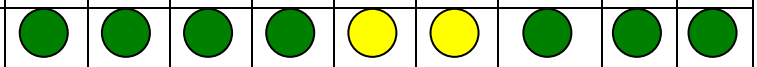
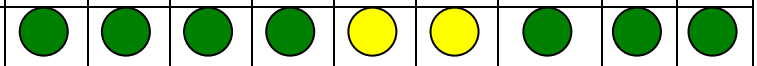
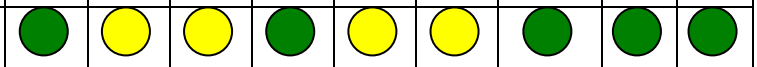
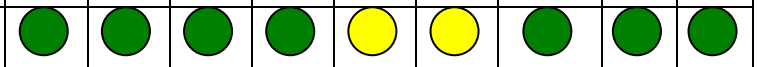
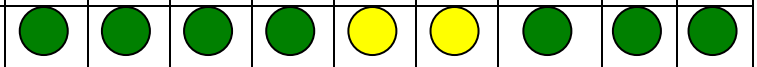
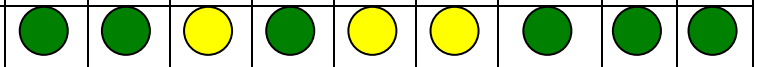
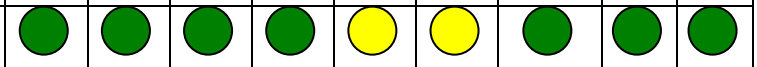
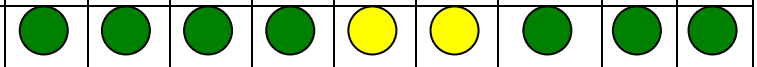
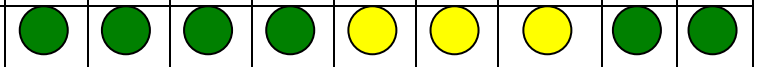
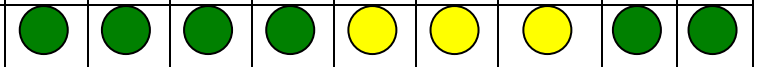
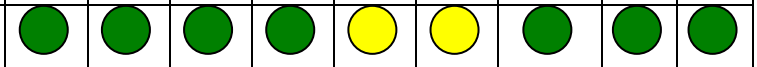
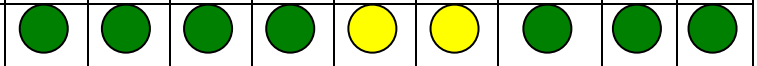
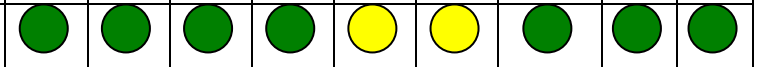
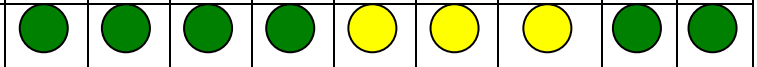
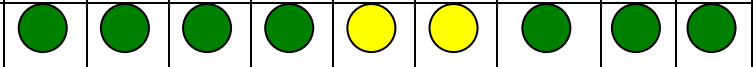
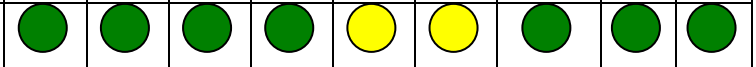
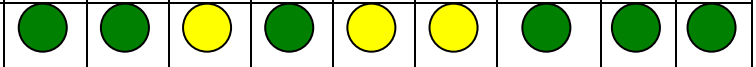
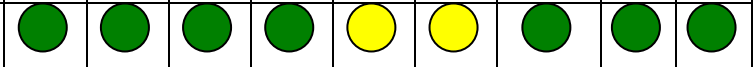
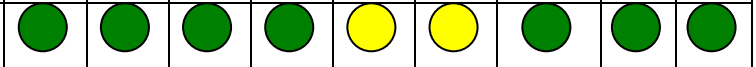
11 Appendices

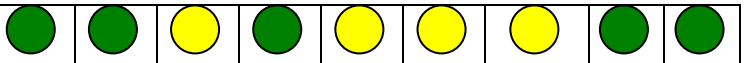
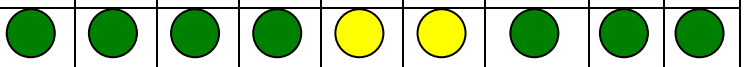
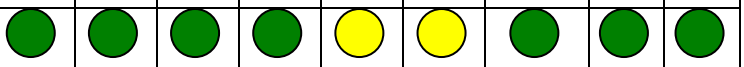
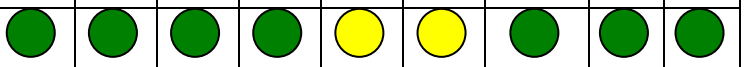
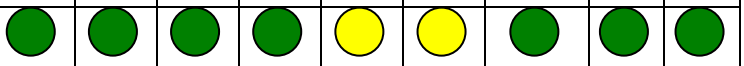
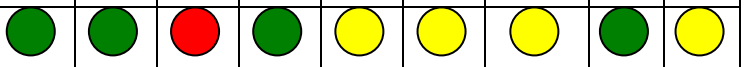
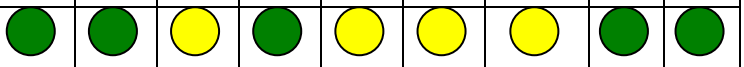
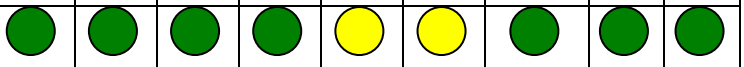
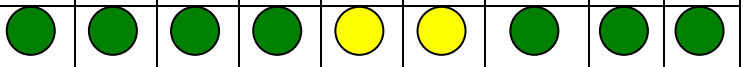
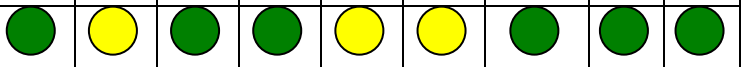
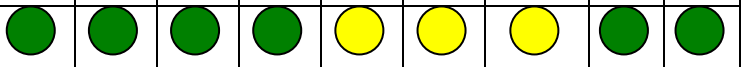
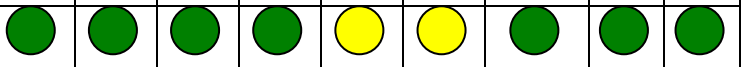
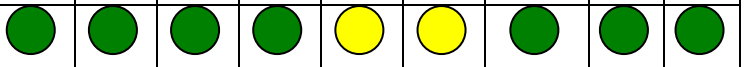
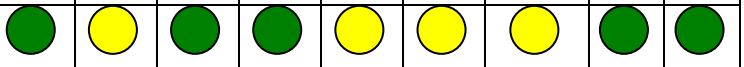
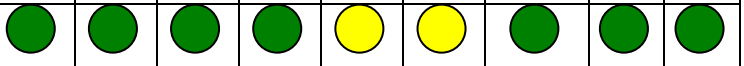
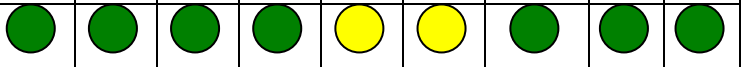
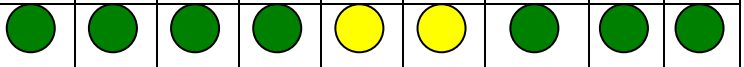
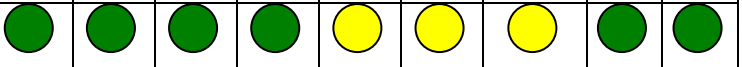
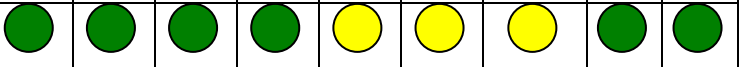
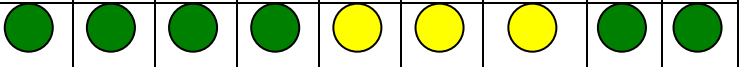
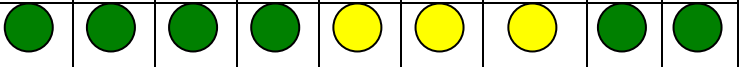
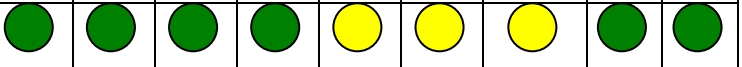
11.1 CHAPTER 3 APPENDIX

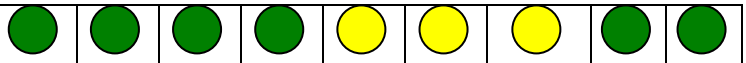
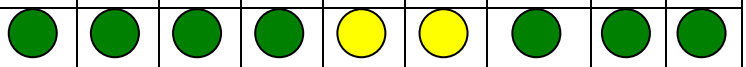
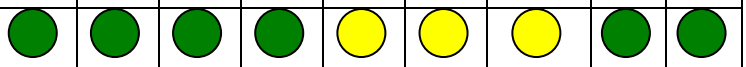
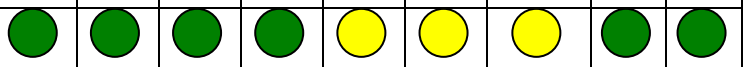
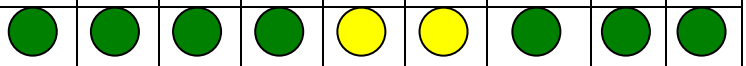
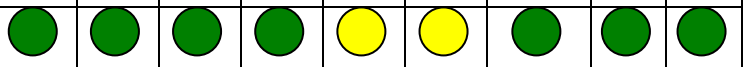
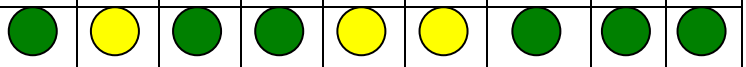
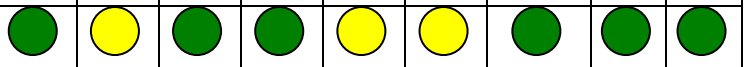
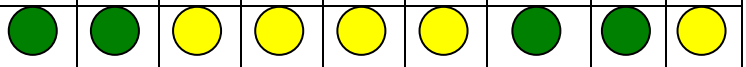
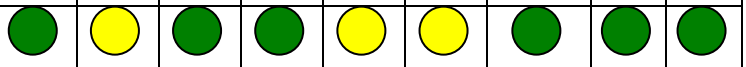
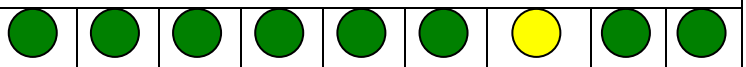
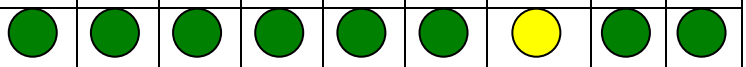
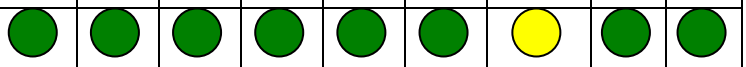
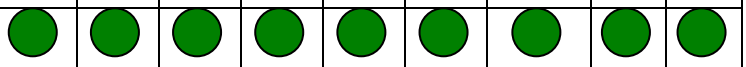
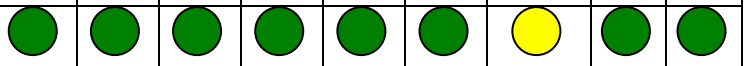
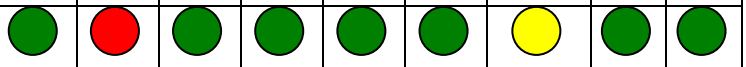
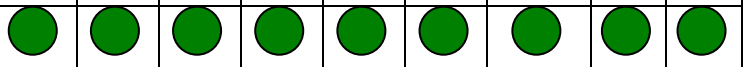
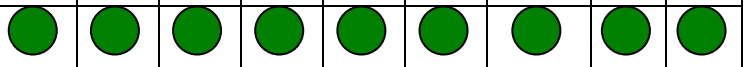
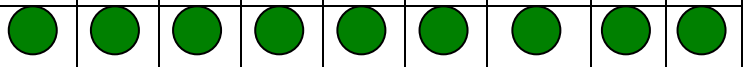
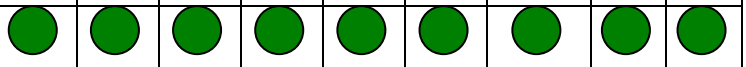
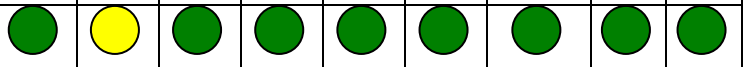
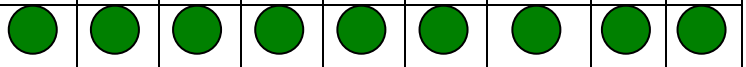
11.1.1 Risk of Bias Assessment

Table 11.1: Risk of Bias Assessment of Studies of Dihydroartemisinin-Piperaquine

	Study design and objectives	Bias in selection of subjects and constitution of study	Bias due to withdrawal or loss to follow up (attrition)	Information bias regarding the drug safety outcome	Other information bias	Statistical methods to control confounding	Statistical methods excluding methods to control confounding	Conflict of interest	SUMMARY RISK OF BIAS
Randomised Controlled Trials									
4ABC 2011[343]									
Adam 2010[344]									
Agarwal 2013[345]									
Annerberg 2011[117]									
Ashley 2004[346]									
Ashley 2005[347]									
Ashley 2014[23]									
Awab 2010[348]									
Awab 2016[349]									
Bassat 2009[286]									
Borrmann 2011[218]									
Dicko 2016[219]									
Grande 2007[350]									
Hasugian 2007[351]									

Hien 2012[352]									
Janssens 2007[353]									
Kamya 2007[354]									
Karema 2006[355]									
Karunajeewa 2008[356]									
Krudsood 2007[357]									
Lon 2014[221]									
Mayxay 2006[358]									
Mens 2008[359]									
Moore 2014[116]									
Nelwan 2015[222]									
Nji 2015[360]									
Ogutu 2014[361]									
Okebe 2016[228]									
Onyamboko 2014[362]									
Pasaribu 2013[235]									
Phyo 2011[363]									
PregACT 2016[214]									
Ratcliff 2007[364]									
Sawa 2013[365]									
Smithuis 2006[366]									
Smithuis 2010[229]									

Sowumni 2016[367]									
Spring 2015[223]									
Sutanto 2013[230]									
Sutanto 2013a[224]									
Sylla 2013[368]									
Tangpukdee 2005[369]									
Thanh 2009[370]									
Thuan 2016[371]									
Tijtra 2012[372]									
Tran 2004[112]									
Ursing 2016[373]									
Valecha 2010[374]									
Wang 2008[375]									
Whegang 2010[376]									
Yavo 2011[377]									
Yeka 2008[378]									
Zongo 2007[379]									
Bigira 2014[225]									
Bojang 2010[380]									
Cisse 2009[381]									
Desai 2015[210]									
Kakuru 2016[211]									

Kamya 2014[226]									
Lwin 2012[227]									
Nankabirwa 2014[382]									
Natureeba 2017[203]									
Wanzira 2014[383]									
Zongo 2015[384]									
START-IPT[204]									
STOPMIP-ID[202]									
MACEPA[208]									
TME[205]									
Cohort									
Adam 2012[212]									
Amaratunga 2016[385]									
Baiden 2015[217]									
Chu 2017[233]									
Irawati 2007[386]									
Kakar 2016[387]									
Kheng 2015[220]									
Leang 2013[388]									
Leang 2015[389]									
Lidia 2015[234]									
Liu 2015[390]									
Mohamed 2017[391]									

Myint 2017[392]									
Plucinski 2015[393]									
Plucinski 2017[394]									
Rijken 2008[215]									
Rijken 2011[216]									
Sow 2016[395]									
Thanh 2017[396]									
Thriemer 2014[397]									
Tun 2016[231]									
Wang 2015[398]									
Zongo 2014[399]									
Poespoprodjo 2014[213]									
MALTEM									
BMEP									
Systematic Reviews & Meta-analyses									
Gutman 2017[201]									
Zani 2014[200]									

Legend

	Low		Unclear		High

11.2 CHAPTER 4 APPENDIX

11.2.1Risk of Bias Assessment

Figure 11.1: Risk of Bias Assessment of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring

11.3 CHAPTER 5 APPENDIX

11.3.1 Individual Patient Data Availability

77 (48.4%) of the 159 of studies for which individual patient data were sought, and 28 (65.1%) of the 43 included studies, were published or conducted between 2007 and 2017 (Table 11.2 & 11.3). 6,852 (65.6%) of the 10,452 included participants were enrolled between 2007 and 2017 inclusive.

Table 11.2: Availability of Published Datasets by Study Year of Publication

	Studies for which Data Available		*Studies for which Data Not Available*			
Study Year of Publication	**Included in Meta-analysis**	**Insufficient for Inclusion**	**No Data Shared**	**No Response**	**No Contact Details**	***Total Studies for which IPD Sought***
2012-2017	13	1	16	7	1	***38***
2007-2011	7	2	14	3	2	***28***
2002-2006	1	0	7	3	4	***15***
1997-2001	3	0	10	7	1	***21***
1992-1997	3	0	5	15	1	***24***
1988-1992	1	0	5	3	2	***11***
All Years	*28*	*3*	*57*	*38*	*11*	***137***

Table 11.3: Availability of Unpublished Datasets by Study Year of Enrolment

	Studies for which Data Available		
Study Year of Last Enrolment	**Included in Meta-analysis**	**Insufficient for Inclusion**	***Total Studies for which IPD Sought***
2012-2017	5	1	***6***
2007-2011	3	2	***5***
2002-2006	1	2	***3***
1997-2001	0	0	***0***
1992-1997	6	2	***8***
1988-1992	0	0	***0***
All Years	*15*	*7*	***22***

Availability of datasets by study location is presented in Figures 11.2-4.

Figure 11.2: Availability of Datasets by Study Locations – Asia Pacific

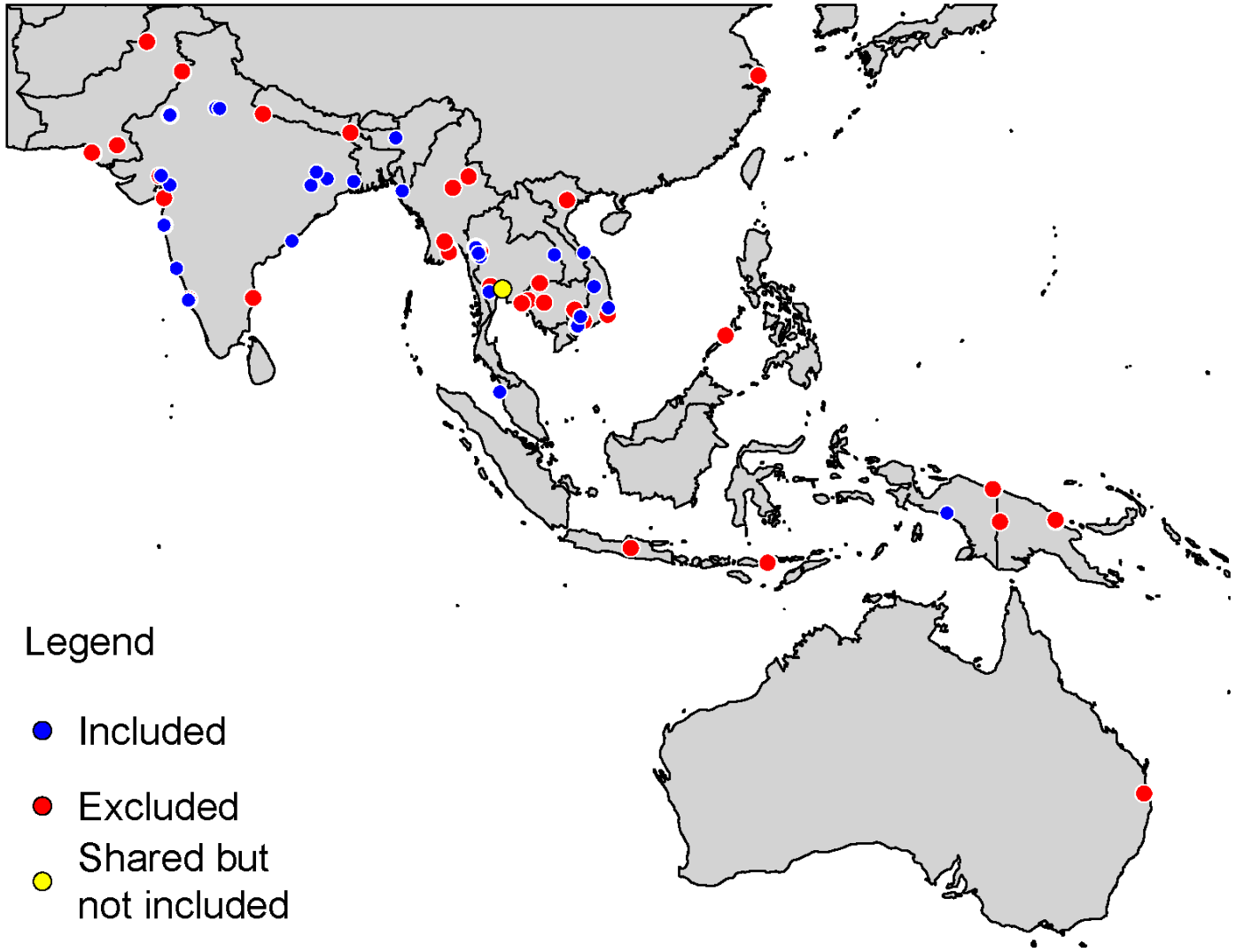


Figure 11.3: Availability of Datasets by Study Locations – Africa & Europe

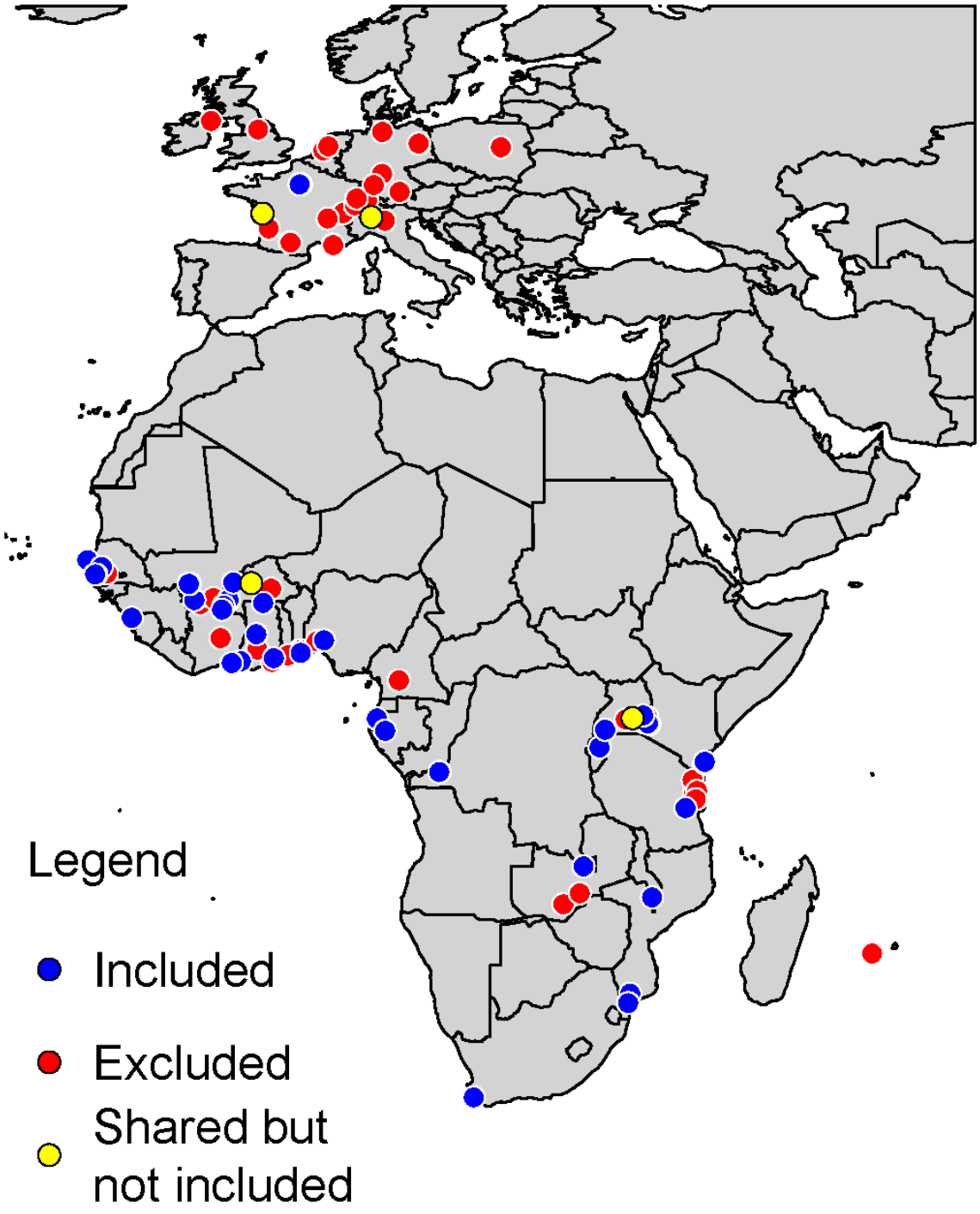
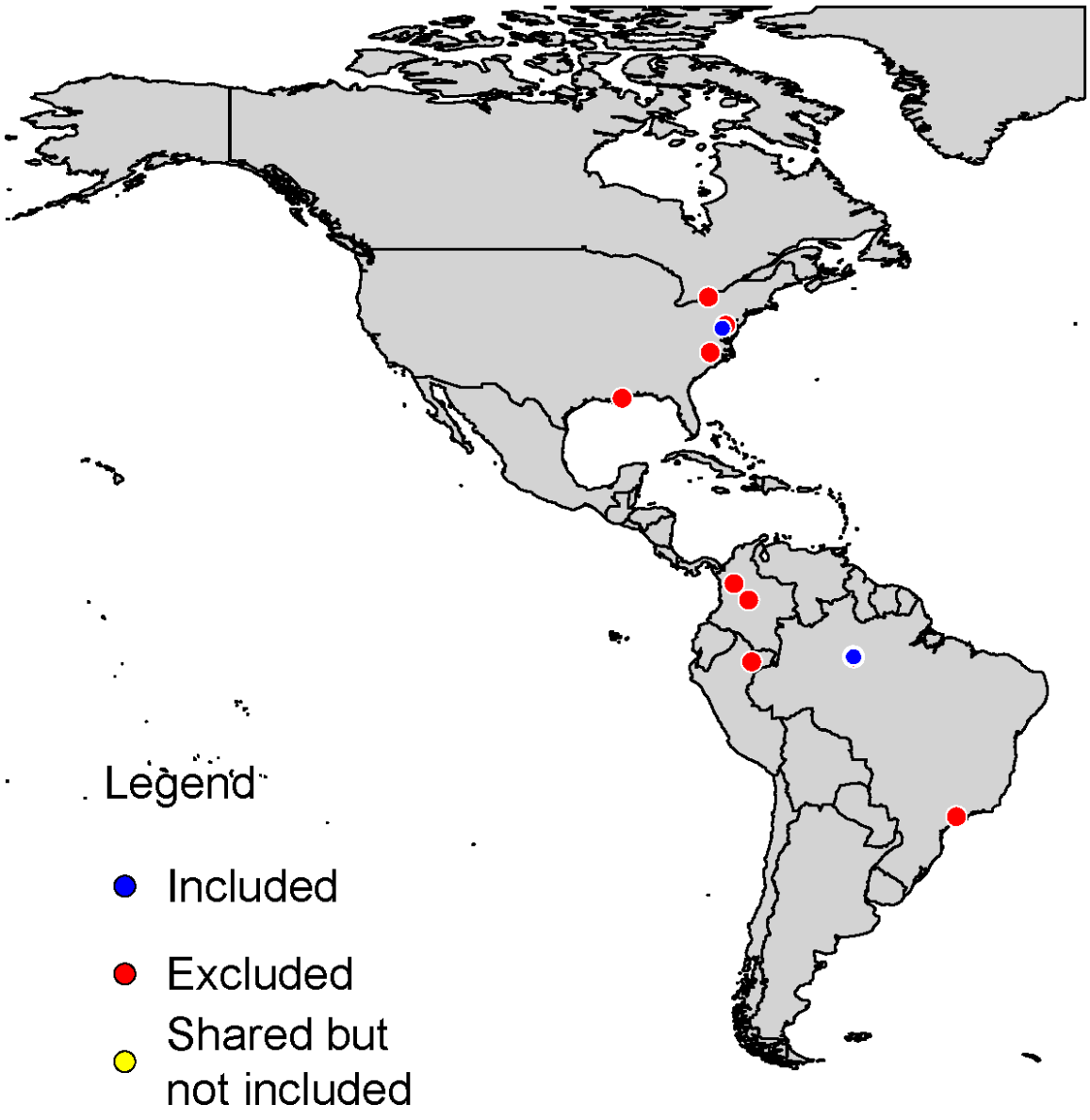


Figure 11.4: Availability of Datasets by Study Locations – Americas



11.3.2 Risk of Bias Assessment

Table 11.4: Risk of Bias Assessment of Studies of the Quinoline and Structurally Related Antimalarials with Electrocardiographic Monitoring for IPD Meta-analysis

	Study design and objectives	Bias in selection of participants and constitution of study groups	Bias due to withdrawal or loss to follow up (attrition)	Information bias regarding the drug safety outcome	Other information bias	Conflict of interest	SUMMARY RISK OF BIAS
Randomised Controlled Trials - Included							
Abernethy 2001[289]							
Bassat 2009[286]							
Bassi 2004[288]							
Darpo 2015[278]							
Funck-Brentano 2019[119] (subsequently published)							
Hanboonkunupakarn 2014[280]							
Hanboonkunupakarn 2019[294] (subsequently published)							
Krudsood 2010[284]							
Macintyre 2017[295] (subsequently published)							
Mytton 2007[400]							
Navaratnam 2009[285]							
Ndiaye 2011[188]							
Ogutu 2014[189]							
Price 1995[293]							
Price 1998b[401]							

PROMOTEi[211]							
Pukrittayakamee 2014a[402]							
Pukrittayakamee 2014b (unpublished)							
Siqueira 2017[403]							
Tandon 2007 (unpublished)							
Toure 2015[404]							
Toure 2016[405]							
Tran 1996[292]							
Valecha 2010[283]							
Valecha 2012[281]							
Valecha 2016[406]							
van Vugt 1999[407]							
van Vugt 2000[290]							
WANECAM 2018[122,408]							
White 1988[84]							
Randomised Controlled Trials - Excluded							
Abdulla 2008[409]							
Abdulla 2010[410]							
Alecrim 2006[411]							
Assimadi 2002[412]							
Benjamin 2015[413]							
Bigira 2014[225]							
Bindschedler 2000[74]							

Bindschedler 2002[414]							
Bouchaud 2000[415]							
Bouyou-Akotet 2010[416]							
Bunnag 1989[417]							
Cao 1997[418]							
D'Alessandro 2006 (unpublished)							
Haroon 2005[419]							
Hien 2011[420]							
Jittamala 2015[421]							
Kakuda 2013[422]							
Karbwang 1991[258]							
Karbwang 1992a[423]							
Karbwang 1992b[424]							
Karbwang 1993b[425]							
Karbwang 1995a[426]							
Karbwang 1995b[427]							
Karbwang 1995c[428]							
Karbwang 1997[429]							
Kayentao 2012[430]							
Kervella 2006 (unpublished)							
Khan 2011[431]							
Kinde-Gazard 2012[432]							

Kshirsagar 2000[151]							
Laman 2014[433]							
Lefevre 2001[434]							
Lefevre 2002a[73]							
Lefevre 2002b[435]							
Lefevre 2013[436]							
Liu 2014[265]							
Llanos-Cuentas 2014[249]							
Lon 2014[437]							
Manning 2014[438]							
Massougbodji 2002[439]							
McGready 2008[440]							
Miller 2013[441]							
Moore 2014[442]							
Morris 2012[443]							
Murphy 1996[444]							
Mutabingwa 2009[315]							
Mzayek 2007[342]							
Na-Bangchang 2000[445]							
Na-Bangchang 2005[446]							
Nasveld 2010[447]							
Nelwan 2015[232]							

Newton 2001[448]							
Ngouesse 2001[254]							
Nosten 1990[449]							
Nosten 1994[450]							
Olliaro 2010[451]							
Omoruyi 2007[318]							
Orrell 2008[319]							
Piola 2010[452]							
Poravuth 2011[250]							
Pyar 2007[453]							
Pyar 2009[454]							
Restrepo 1996[455]							
Rueangweerayut 2012[456]							
Sabchareon 1988[264]							
SB 1993 (unpublished)							
Song 2011[457]							
Sowunmi 1990[458]							
Staedke 2018[204] (subsequently published)							
Supan 2017[316]							
Taylor 1998[459]							
Thapa 2007[460]							
Thuma 2000[461]							

Tjitra 2012[462]							
Touze 2002[463]							
Trung 2009[464]							
Tshefu 2010[465]							
van Agtmael 1999[150]							
van Hensbroek 1996[466]							
Walker 1993[267]							
Cohorts - Included							
Ahmed 2019[202] (subsequently published)							
Baiden 2015[217]							
Jittamala 2011 (unpublished)							
Kredo 2011[282]							
Kredo 2016[276]							
Looareesuwan 2005 (unpublished)							
Nosten 1993i[147]							
Nosten 1993ii[147]							
Nosten 1993iii[147]							
Price 1997i[467]							
Price 1997ii[467]							
Price 1998a[468]							
PROMOTEii[203] (subsequently published)							
Cohorts - Excluded							
Adjei 2012[253]							

Auprayoon 1995[469]							
Bhatt 2006[470]							
Byakika-Kibwika 2011[471]							
Claessen 1998[472]							
Davis 1988[473]							
Davis 1990[474]							
Edwards 1988[475]							
Falade 2005[476]							
Haider 2013[477]							
Hatz 2008[478]							
Hombhanje 1998[479]							
Jaspers 1996[480]							
Karbwang 1993a[481]							
Karunajeewa 2004[482]							
Khan 2006[483]							
Krishna 1993[484]							
Lavallee 2001[262]							
Mansor 1990[485]							
Matson 1996[486]							
Minodier 2005[487]							
Monlun 1995[488]							
Mra 1991[489]							

Na-Bangchang 1994[251]							
Nyunt 2012[490]							
Ogunkunle 2011[491]							
Roggelin 2014[60]							
Rusca 2007 (unpublished)							
SB 1994 (unpublished)							
Sowunmi 1998[261]							
Stein 2015[492]							
Sukontason 1996[493]							
Supanaranond 1997[494]							
Touze 1996[495]							
von Seidlein 1997[271]							
Win 1992[256]							

Legend

	Low		Unclear		High

As this systematic review was conducted to identify studies for an individual patient data meta-analysis, risk of bias assessment of statistical methods of individual studies was considered not relevant.

11.4 CHAPTER 6 APPENDIX

11.4.1 Dosing Characteristics

Table 11.5: Weight-based Drug Dosing Tables of Amodiaquine and Comparator Antimalarials

Drug	Weight Band (kg)	Dose per day (mg)	Dose per 3-day course (mg)	Studies
Artesunate-Amodiaquine FDC	5 to <9	25:67.5	75:202.5	Ndiaye 2011[188], Ogutu 2014[189], Siqueira 2017[190], WANECAM 2018[122]
	9 to <18	50:135	150:405	
	18 to <36	100:270	300:810	
	≥36	200:540	600:1620	
Artesunate + Amodiaquine Non-FDC	≥36	200 + 612	600 + 1836	Ogutu 2014[189]
Artemether-Lumefantrine FDC	5 to <15	20:120	60:360	Ndiaye 2011[188], WANECAM 2018[122]
	15 to <25	40:240	120:720	
	25 to <35	60:360	180:1080	
	≥35	80:480	240:1440	
Chloroquine	5 to <10	Day 1: 75	150	Siqueira 2017[190]
		Days 2 & 3: 37.5		
	10 to <15	Day 1: 150	300	
		Days 2 & 3: 75		
	15 to <25	Day 1: 150	450	
		Days 2 & 3: 150		
	25 to <35	Day 1: 300	900	
		Days 2 & 3: 300		
	35 to <50	Day 1: 450	1050	
		Days 2 & 3: 300		
	≥50	Day 1: 600	1500	
		Days 2 & 3: 450		
Dihydroartemisinin-Piperaquine FDC	5 to <7	10:80	30:240	WANECAM 2018[122]
	7 to <13	20:160	60:480	
	13 to <24	40:320	120:960	
	24 to <36	80:640	240:1920	
	36 to <75	120:960	360:2880	
	≥75	160:1280	480:3840	
Pyronaridine-Artesunate FDC	5 to <8	60:20	180:60	WANECAM 2018[122]
	8 to <15	120:40	360:120	
	15 to <24	180:60	540:180	
	24 to <45	360:120	1080:360	
	45 to <65	540:180	1620:540	
	>65	720:240	2160:720	

FDC = fixed-dose combination

11.4.2 Risk of Bias Assessment

Table 11.6: Risk of Bias Assessment of Studies of Amodiaquine with Electrocardiographic Monitoring

	Study design and objectives	Bias in selection of subjects and constitution of study groups	Bias due to withdrawal or loss to follow up (attrition)	Information bias regarding the drug safety outcome	Other information bias	Conflict of interest	SUMMARY RISK OF BIAS
Randomised Controlled Trials - Included							
Ndiaye 2011[188]							
Ogutu 2014[189]							
Siqueira 2017[403]							
WANECAM 2018[122]							
Randomised Controlled Trials - Excluded							
Mutabingwa 2009[315]							
Ngouesse 2001[254]							
Supan 2017[316]							
Cohorts - Excluded							
Adjei 2012[253]							

Legend

	Low		Unclear		High

As this systematic review was conducted to identify studies for an individual patient data meta-analysis, risk of bias assessment of statistical methods of individual studies was considered not relevant.