The assessment of antimalarial drug efficacy

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Antimalarial drug efficacy in uncomplicated malaria should be assessed parasitologically in large, community-based trials, enrolling the age groups most affected by clinical disease. For rapidly eliminated drugs, a 28-day follow-up is needed, but, for slowly eliminated drugs, up to nine weeks could be required to document all recrudescences, and, when possible, the drug levels should also be measured. The WHO 14-day assessments are neither sensitive nor specific. In tropical *Plasmodium vivax* and *Plasmodium ovale* infections treated with chloroquine, the first relapse is usually suppressed by residual drug levels. A relapse cannot be distinguished confidently from a recrudescence. Host immunity is a major contributor to the therapeutic response, and can make failing drugs appear effective.

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In uncomplicated malaria, the objective of treatment is primarily to provide CURE (see Glossary). Rapid clinical improvement and the prevention of transmission are significant secondary objectives of treatment. In falciparum malaria, preventing the progression to severe disease is important, particularly as the available antimalarial drugs fall to resistance (as most have). In low-transmission settings, cure is defined as eradication (i.e. no reappearance) of the malaria parasites that caused the infection. In high-transmission areas, where infection is frequent, true cure is more difficult to identify because re-infection occurs rapidly.

Because antimalarial drug resistance has worsened steadily over the past 30 years, the available affordable drugs have become increasingly ineffective. Insensitive, simple methods of assessment have been introduced, which can detect only high levels of drug resistance [1]. In high-transmission settings, the important contribution of immunity to resolving the acute infection, independent of drug treatment, is not appreciated sufficiently. Together, this has resulted in a systematic underestimation of the adverse impact of antimalarial drug resistance on morbidity and mortality. In this review, the current methods of assessing therapeutic responses are discussed in relation to the pharmacokinetic and pharmacodynamic properties of the antimalarial drugs, and some approaches to evaluating *Plasmodium vivax* and *Plasmodium ovale* treatment are proposed.

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Parasite clearance

Antimalarial drugs kill malaria parasites, thereby preventing multiplication and leading to their removal from the circulation [2,3]. The spleen has a central role in the clearance of circulating drug-affected parasites, removing damaged or dead parasites, and returning the 'once parasitized' red blood cells to the circulation [4]. Most antimalarial drugs act predominantly on the more-mature trophozoites, which, in *Plasmodium falciparum* infections, cannot be seen by the microscopist because they are sequestered in capillaries and venules [3]. These parasites die *in situ*, and are cleared slowly as the infected cells degenerate. Young ring-stage parasites and mature schizonts are relatively resistant to antimalarial drugs (with the notable exception of the artemisinin derivatives, which kill a broader range of blood stages than do other drugs, and consequently have a significant effect on developing ring-stage parasites). Chloroquine (CQ) also has a greater effect on ring stages than quinine or mefloquine [5,6]. Thus, in falciparum malaria, the immediate changes in parasitaemia that follow antimalarial drug treatment [with drugs such as sulphadoxine–pyrimethamine (SP), mefloquine or quinine] reflect largely the changes that would have occurred anyway, whether or not treatment had started [7]. Parasitaemias can either rise abruptly, reflecting release of merozoites and invasion from hidden sequestered schizonts, plateau as young ring-stages continue to circulate, or decline sharply as the parasites sequester.

The elimination of parasites from the blood of people with malaria appears to be a first-order process [8] (Fig. 1a). Thus, while blood concentrations of the antimalarial drug exceed those required for a MAXIMUM EFFECT (E_{max}), a fixed fraction of the infecting parasites is cleared per asexual cycle [PARASITE REDUCTION RATIO (PRR)] [3]. Concentrations below the MINIMUM INHIBITORY CONCENTRATION (MIC) for the infection are associated with net parasite growth, so to cure the blood-stage infections in a patient with no background immunity, antimalarial blood concentrations must exceed the MIC until the last asexual parasite is cleared from the blood. By contrast, in immune individuals, host defence might clear most or all the parasites independent of drug treatment. As a consequence, if the PRR and the slope of the concentration–effect relationship, and the pharmacokinetic properties of the drug are known (Fig. 1b), then the duration for which antimalarial drugs must be present in the blood to ensure cure can be calculated [3]. Most effective antimalarial drugs have PRR values >100 (Fig. 1a). For rapidly eliminated drugs [e.g. those with terminal elimination half-lifes ($t_{1/2\beta}$) of <1 day such as quinine, proguanil and the artemisinin

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Glossary

Cure: The parasites causing the infection are eradicated from the body.
Maximum effect: The maximum effect produced by a drug; increasing concentrations produce no additional effect (the top of the concentration–effect relationship).
Minimum inhibitory concentration: Plasma or blood concentrations of a drug resulting in a parasite multiplication rate of one.
Minimum parasiticidal concentration: The lowest plasma or blood concentration of a drug that produces the maximum antimalarial effect.
Parasite multiplication rate: Fractional increase in parasite numbers in one complete asexual cycle – the reciprocal of parasite reduction ratio.
Parasite reduction ratio: Fraction of parasites removed in each asexual cycle.
R_1, R_2 and R_3: The different levels of therapeutic failure defined parasitologically (see Fig. 2 in main text).
Recrudescence: The parasite causing the initial infection are not eradicated by the treatment but, after declining below the threshold of microscopic detection, their numbers then re-expand to reach detectable parasitaemias.
Relapse: *Plasmodium vivax* and *Plasmodium ovale* infections produce dormant liver-stage parasites called hypnozoites. Relapses are produced when, weeks or months after the initial infection, these hypnozoites awake and generate a blood-stage infection.

derivatives], then with PRR values of 1000, four asexual cycles must be covered to ensure cure of infections with parasite burdens >10^9 (parasitaemia >200 μl^{-1}). Resistance, or a right shift in the concentration–effect relationship (Fig. 1c), is associated eventually with a decrease in the PRR and increased rates of treatment failure. Background immunity complements drug treatment [9,10]. In areas of moderate or high transmission, spontaneous resolution of parasitaemia is usual, whether or not drug treatment is given. Apparently, good therapeutic responses are commonly seen with ineffective drugs. Even a small drug effect, combined with immunity, could be sufficient to cure the infection in a patient with 'good immunity', whereas in a non-immune young child from the same locality, infected with the same parasites, there could be a high-grade failure (i.e. parasite multiplication continues, despite the treatment) with the attendant risk of a fatal outcome. In a non-immune subject, the initial rate of parasite clearance is determined by the intrinsic activity of the drug, the susceptibility of infecting parasites, and the drug levels achieved [3]. The pattern of treatment failure is also determined by these factors.

Problems with the WHO 14-day test

The original WHO 14-day test [1] was introduced for use in intense-transmission settings because of operational difficulties in conducting longer follow-up in trials, problems distinguishing RECRUDESCENCE from re-infection, and because it was considered that clinical response was the main criterion upon which national malaria treatment policy was based. Unfortunately, the 14-day test has been applied widely, both in high-transmission and low-transmission settings. Recrudescences can now be distinguished from new infections with PCR genotyping. The 14-day test, which defined three levels of response, early treatment failure (ETF), late treatment failure (LTF) and adequate clinical response (ACR), has serious limitations (Box 1). A patient is classified as having an ETF, if the fever is ≥37.5°C (axillary) and parasites are still present on the blood film on Day 3 after drug treatment. This is

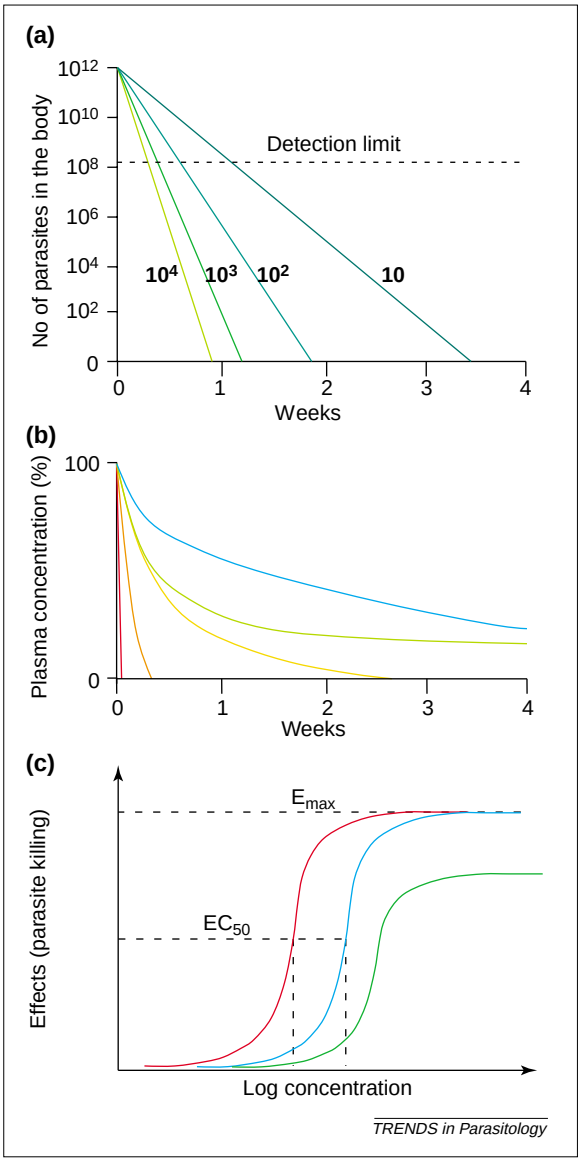

Fig. 1. (a) Pharmacodynamic properties of the antimalarial drugs *in vivo* in an adult patient with 2% parasitaemia. Parasite killing is measured as the fractional reduction in numbers in each asexual cycle, the parasite reduction ratio (PRR) (green colours). The PRR varies from <10 (antibiotics with antimalarial activity or dying antimalarials) (dark green) to 10^4 (artemisinin derivatives) (light green). To prevent recrudescence, antimalarial drugs must be present at levels greater than the minimum inhibitory concentration, until eradication of the infection in non-immune patients. The detection limit (broken black line) is the total number of parasites in the body of an adult (~10^8) below which microscopy cannot detect parasitaemia. (b) Pharmacokinetic properties of the antimalarial drugs are indicated: red, artemisinins; orange, quinine; yellow, pyrimethamine; green, chloroquine; blue, mefloquine. The origin represents the maximum concentration (100%) achieved after a therapeutic dose. (c) Increasing drug resistance leads to a rightward shift in the dose response or concentration–effect relationship (red) (the effect is parasite killing). This shift can be parallel (blue), or the shape of the curve and maximum effect (E_{max}) could change (green). The EC_{50} is the concentration of drug producing 50% of the E_{max}.

not uncommon, particularly in non-immune young children with high initial parasite counts, whose parasite counts are falling satisfactorily, but have not yet cleared (Fig. 1a). It does not necessarily mean that the infection is resistant. However, having persistent parasitaemia, following Day 3 after treatment (and

Box 1. WHO definitions of treatment failure

The widely used definitions [a] designed for assessments in high-transmission settings have been modified recently [b], emphasizing the distinction between methods of assessment in areas of different transmission intensity, and putting more weight on parasitological measures than previously.

Adequate clinical response

An adequate clinical response (ACR) is indicated by one of the following, during the follow-up period from Day 4 to 14 after drug treatment:

- Absence of parasitaemia on Day 14, irrespective of axillary temperature, without previously meeting any of the criteria of early or late treatment failure.
- Axillary temperature <37.5°C, irrespective of the presence of parasitaemia, without previously meeting any of the criteria of early or late treatment failure.

Early treatment failure

An early treatment failure (ETF) is indicated by one or more of the following:

- Development of danger signs or severe malaria on Day 1, Day 2 or Day 3 after drug treatment in the presence of parasitaemia.
- Axillary temperature ≥37.5°C on Day 2 with parasitaemia greater than that of Day 0 count. However, a recent WHO consultation [b] recommends substituting this with: parasite count on Day 2 higher than Day 0, irrespective of temperature.
- Axillary temperature ≥37.5°C on Day 3 in the presence of parasitaemia. WHO consultation [b] recommends that, for areas with low to moderate transmission, there must be a measured increase in axillary temperature on Day 3.
- Parasitaemia on Day 3 ≥25% of count on Day 0.

Late treatment failure

A late treatment failure (LTF) is indicated by one of the following:

- Development of danger signs or severe malaria in the presence of parasitaemia on any day from Day 4 to Day 14 after treatment, without previously meeting any of the criteria for early treatment failure.
- In areas of intense transmission, axillary temperature ≥37.5°C in the presence of parasitaemia on any day from Day 4 to Day 14, without previously meeting any of the criteria of early treatment failure.
- The recent WHO consultation [b] recommends including: in areas of low to moderate transmission, presence of parasitaemia on any day from Day 4 to Day 28, and a measured axillary temperature >37.5°C, without previously meeting any of the criteria of early treatment failure. If a history of fever, rather than measured fever, was accepted as an entry criterion, then parasitaemia with history of fever suffices for late treatment failure.

The WHO consultation [b] has modified the definition of cure to adequate clinical and parasitological response (ACPR). In a high-transmission setting, ACPR is defined as an adequate clinical response, but in low to moderate transmission settings, the assessment is made on Day 28. The consultation has also included the definition late parasitological failure (LPF). In high-transmission settings, LPF is defined as 'presence of parasitaemia on Day 14, and a measured axillary temperature <37.5°C, without previously meeting any of the criteria of early or late treatment failure' [b]. In low to moderate transmission settings, the definition is the same, except that parasitaemia at any time from Day 7 to Day 28 qualifies.

References

a World Health Organization (1996) Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission. WHO/MAL/96.1077
b World Health Organization (2002) Monitoring antimalarial drug resistance. Report of a WHO consultation, WHO/CDS/CSR/EPH/2002.17

for as long as 14 days) without fever is considered an ACR, yet this is clearly a resistant infection. The host immune response is adequate, but the parasites are resistant, and the next patient might not be so lucky. At lower levels of resistance, the 14-day test misses the majority of treatment failures, which occur after this time. Recently, a WHO consultation has produced a comprehensive review of the methods for monitoring antimalarial resistance [11], which emphasizes the distinction between methods of assessment in areas of high malaria-transmission and those where transmission is low or moderate, and puts more weight on parasitological measures than previously [1] (Box 1). As before, the primary intent of the recommended protocol(s) is the monitoring of drug efficacy over time for strictly programmatic purposes. The objective is to ensure a minimal evidence base from which Ministries of Health can develop informed treatment guidelines and policies. So, does it, and do they?

Patterns of treatment failure

If rapidly eliminated drugs are given for 5–7 days, then at low levels of resistance, recrudescences of falciparum, vivax and ovale malaria appear at approximately three weeks after starting the treatment [3]. This is because concentrations of the drug in the blood decline relatively rapidly, and there is little or no inhibition of parasite multiplication in the asexual cycle two days after stopping treatment [12]. Ten parasites multiplying tenfold per asexual cycle [13] will reach detectable densities in an adult (~50 parasites μl^{-1} or a total of 10^8 parasites) in seven cycles or 14 days. Recrudescences that appear after 28 days (the originally recommended length of follow-up in drug treatment trials) [14] are a minority and result from PARASITE MULTIPLICATION RATES (PMR) of less than six per cycle. However, the 14-day follow-up [1,11] is insensitive because it detects only the high levels of resistance associated with PRR values of <100.

As resistance worsens, parasitaemia no longer clears (R_2 or R_3 responses) (Fig. 2). The R_2 response (still parasitaemic at Day 7 after treatment) is usually associated with PRR values of <10, and the R_3 response with PRR values <4 [3]. Once these responses are encountered, there is an increasing probability that severe anaemia or severe malaria, and death will result. The relationship between these responses in clinical trials, and mortality from malaria is not well characterized (in trials, patients are rescued with another drug – in the community, they are usually not) [15,16].

For slowly eliminated drugs, at low levels of resistance, the situation is more complicated (Fig. 2). As the drug levels decline, they fall below the MINIMUM PARASITICIDAL CONCENTRATION (MPC), and the PRR starts to fall for any residual surviving parasites. Eventually, the drug concentrations fall to MIC levels [3]. Thereafter, the parasite numbers begin to rise again, but multiplication rates are still reduced because of residual drug effects. The net result is that the time to recrudescence is considerably delayed at low levels of resistance. For example, in 1992, in Thailand, a 28-day follow up missed one-third of recrudescences following mefloquine treatment [17]. Earlier in the evolution of

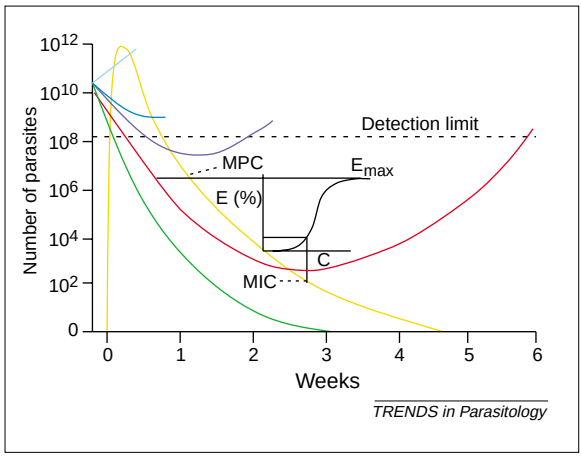

Fig. 2. Therapeutic responses with increasing levels of resistance to a slowly eliminated antimalarial drug. In this case, the drug is mefloquine (yellow). Initially, patients are cured (green), then with increasing resistance, there are late recrudescences (R_1 late) (red) followed by early recrudescences (R_1 early) (purple), failure to clear parasitaemia within one week (R_2) (blue) and, finally, completely unresponsive malaria (R_3) (light blue). The inset shows the pharmacodynamics [the concentration (C) – effect (E) relationship], which occur in the late recrudescent infection. When mefloquine levels fall below the minimum parasiticidal concentration (MPC), the lowest level giving maximum parasite killing (E_{max}), then the rate of decline in parasitaemia (parasite reduction ratio, PRR) starts to fall until the PRR reaches one. A PRR of one results from a minimum inhibitory concentration (MIC) concentration of mefloquine. Thereafter, as drug levels fall further, parasitaemia rises again and recrudescence occurs at six weeks after initial treatment.

mefloquine resistance, this figure could have been even higher [18]. As a consequence, follow-up in clinical trials was lengthened to 63 days. Mefloquine has a $t_{1/2\beta}$ of approximately two weeks in patients with malaria. At low levels of resistance, residual levels six weeks after taking a treatment dose could still suppress parasite multiplication (i.e. if levels were less than MIC) but, as resistance worsened, the MIC rose and these levels were no longer suppressive. Thus, as resistance worsened, the median time to recrudescence shortened, and follow-up for longer than 42 days was no longer necessary (Fig. 3).

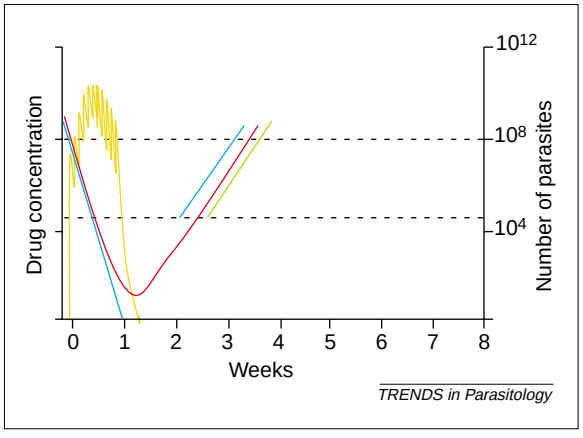

Fig. 3. Treatment failure and re-infection occurring at the same time in malaria caused by frequent-relapse strains of *Plasmodium vivax* and *Plasmodium ovale* following use of a rapidly eliminated drug. In this case, the drug is artesunate or quinine (concentrations in yellow). The blood-stage infection is cured (blue) but the relapse presents three weeks after starting drug treatment. If there is a recrudescence of the blood-stage infection (red), then this occurs at the same time as the genotypically indistinguishable relapse (blue). Re-infection can occur from a mosquito inoculation three days after the seven-day course of drug treatment has stopped (green). This is not a treatment failure and the parasite genotype is usually different to that causing the initial infection. Upper broken line represents level of microscopic detection and symptoms from malaria, and lower broken line represents average number of parasites entering the blood after hepatic schizogony.

The pattern of recrudescence following CQ treatment is less well characterized. CQ exhibits a complex, multi-exponential decline in blood concentrations (Fig. 1b). The terminal elimination phase ($t_{1/2\beta}$ of 1–2 months) occurs at relatively low blood concentrations. Thus, for highly sensitive parasites, CQ behaves as a slowly eliminated drug, but, as resistance rises such that the MIC exceeds the concentrations at the beginning of the terminal elimination phase, CQ effectively becomes a more rapidly eliminated drug. For all drugs, the 14-day follow-up [1] is sufficient only to detect high levels of resistance (i.e. it identifies only those drugs that are dying or dead).

Re-infection or recrudescence?

Patients who are treated in a malaria-endemic area can become re-infected at any time. If the blood-stage parasites emerge from the liver after pre-erythrocytic schizogony and encounter therapeutic blood concentrations of antimalarial drug, then they will be eliminated. Thus, newly acquired infections will be suppressed by residual prophylactic concentrations of a slowly eliminated antimalarial drug following therapeutic administration. Previously, it was difficult to distinguish re-infection from recrudescence unless the patient was studied outside an endemic area. Now, using molecular methods of parasite genotyping, a confident distinction between a recrudescence (with the same genotype as the initial infection) and a re-infection (with a different genotype) can usually be made [19–21]. Sometimes, such a distinction is difficult (e.g. with multiple genotype infections), or an error is made when a minority resistant parasite population is missed in the acute sample and re-infection is incorrectly ascribed to the reappearance of parasites. Nevertheless, the introduction of PCR genotyping based on polymorphic genes such as merozoite surface protein (MSP)1 and MSP2, and glutamate rich protein (GLURP) for *P. falciparum* represents a great advance in the field testing of antimalarial drugs, allowing large community-based trials to be conducted, and also permitting confident description of the temporal pattern of recrudescence. Even in high-transmission areas, where ascribing genotypes is more difficult, genotyping has proved useful in clinical trials. It should also be noted that if the blood concentration profile of the antimalarial drug is known, then the time for which re-infection is suppressed in a high-transmission setting gives an indication of the prevailing level of drug resistance in the parasite population.

Plasmodium vivax and *P. ovale*

The assessment of the acute response to drug treatment of the relapsing parasites *P. vivax* and *P. ovale* is the

same as that for *P. falciparum*. It is the occurrence of the RELAPSE that confounds the overall interpretation of therapeutic response. *Plasmodium vivax* is a diverse species with considerable differences between strains in relapse intervals [22]. These range from the 10–12 months relapse intervals associated with *P. vivax* isolated in temperate areas to three-week intervals in some tropical strains. The *P. vivax* with a long incubation period was the prevalent parasite in northern Europe and Russia, and was considered to form a subspecies *P. vivax hibernans*. Parasites with a similar characteristic long incubation period (*P. vivax multinucleatum*) are found in parts of China, and in North and South Korea. By contrast, the tropical strains of *P. vivax* prevalent in Southeast Asia have short relapse intervals. Long relapse intervals make assessment of radical cure problematic, in that at least one-year follow-up is required. For the tropical strains, confident distinction between recrudescence and relapse is not possible because the parasites causing relapse appear to be genetically identical to those causing the acute infection. The first relapse in the Southeast Asian strains of *P. vivax* tends to occur around three weeks (for Thai strains; mean = 21 days; SD = 4 days) after the start of treatment, which happens to coincide with the time of recrudescence following administration of short acting antimalarial drugs [23] (Fig. 3).

Following CQ treatment, the first relapse (which would normally emerge at around three weeks) of sensitive tropical strains is always suppressed by residual prophylactic concentrations of CQ. The blood concentrations of CQ decline to levels that are generally insufficient to suppress the second relapse. This presents clinically with fever and parasitaemia at approximately six weeks after drug administration. In an individual patient, it is not possible to distinguish recrudescence from relapse [23]. Furthermore, in high-transmission areas, genotyping of *P. vivax* might incorrectly assign a relapse to a new infection, if the hypnozoites derive from a previous inoculation (i.e. one before the inoculation that caused the acute malaria attack), or the parasitaemia of the relapsing genotype was below the level of PCR detection in the acute presentation.

This does not preclude drug assessment, provided that the relapse interval is known. The true relapse incidence in any setting is assessed by providing a highly effective treatment for the blood-stage infection with drugs that are eliminated rapidly. In practice, this means the administration of artesunate or quinine for 5–7 days (Fig. 3). Artesunate is more active than quinine and provides a better assessment [23]. The relapse interval will vary between strains and geographical areas. For example, in Thailand, the majority of relapses will have appeared within one month and, therefore, 28-day follow-up will catch 92% of all the relapses. Elsewhere, longer follow-up periods might be necessary.

Assessing resistance in *Plasmodium vivax*

For rapidly eliminated drugs, the initial therapeutic response (assessed from the rates of parasite and fever clearance) can be compared or monitored every few years. If radical curative treatment with primaquine is not given, and the relapse proportion has been identified previously, then resistance will be reflected by an increasing fraction of patients with recurrent parasitaemia within one month (representing an increased rate of recrudescence).

CQ is still the main treatment for *P. vivax*, worldwide. Significant levels of CQ resistance have now been documented in parts of Oceania, Indonesia and the Americas [24,25]. As CQ suppresses the first relapse in the tropical frequent-relapsing strains of vivax malaria, the first sign of developing resistance is an increasing proportion of patients with recurrent parasitaemia within one month of treatment [26]. This reflects the rising MIC and the resulting failure to suppress the first relapse (Fig. 4). As resistance worsens further, eventually the blood-stage infection is not cured and recrudescences join the relapses. The median time to reappearance of parasites shortens. Although mefloquine resistance in *P. vivax* has not been reported, it would be expected to follow a similar pattern of evolution.

CQ is commonly combined with primaquine for radical cure. Primaquine kills hypnozoites, and thereby reduces the number of subsequent relapses [27], and its asexual-stage activity against *P. vivax* will tend to mask low levels of CQ resistance [28]. Importantly, primaquine also protects against CQ resistance because the mechanisms of resistance in the asexual stage parasites are likely to be different for the two drugs [29]. Short courses of primaquine are widely used and they are probably ineffective in preventing relapses, but they will provide blood-stage activity for three asexual cycles and could reduce the rate of recrudescence if CQ resistance emerges. Thus, CQ resistance in *P. vivax* could be underestimated if combined with primaquine, even in areas where a short course of primaquine (five days) is used.

Plasmodium malariae

There are few data on therapeutic responses in *P. malariae* infection. The quartan parasite was considered uniformly CQ sensitive, until a recent report suggested reduced susceptibility to CQ of *P. malariae* from south Sumatra [30]. Parasitaemias are generally low and there are no persistent hypnozoites to cause relapse. In general, the same approach for assessing *P. falciparum* should be taken with the following caveat: *P. malariae* has a three-day asexual life cycle, so if PRR values are ≤ 1000, then, in theory, nine days treatment with a rapidly eliminated drug would be needed to ensure cure. This has never been tested.

When to measure antimalarial blood concentrations

In the assessment of a high level (R_2 and R_3) treatment failure, measurement of antimalarial drug concentrations is informative in that it differentiates high levels of parasite resistance from host pharmacokinetics as the cause of therapeutic failure.

Occasional patients will have low drug concentrations, despite observed treatment. Others will have vomited or failed to absorb the drug, or not taken it as prescribed, or been given an incorrect dose. Substandard or fake drugs also need to be considered. Measurement of the artemisinin derivatives in blood is the exception to this, in that these compounds have elimination half-lifes of approximately one hour. Artesunate or dihydroartemisinin (the metabolite) levels at the time of an early failure will probably not reflect exposure over the previous days. If recrudescence occurs after the treatment course, the levels are not measurable. For patients returning with recurrent parasitaemias following administration of slowly eliminated drugs, blood-level measurements at the time of recurrent parasitaemia are informative, in that they indicate the infecting parasite population could expand to reach levels of detection, despite the presence of the treatment drug. Thus, the drug concentrations measured at the time of a recrudescence must be below the infecting parasites' MIC [3]. The only caveat to be considered is that acute malaria could alter the volume of distribution of the drug, and the levels during recrudescence might be slightly different to those in the preceding days. The same general reasoning applies to recurrent parasitaemias of *P. vivax* following CQ treatment [26]. Whether it is a relapse or recrudescence, the parasite population has expanded in the presence of drug, and the measured level is therefore useful because it must be below the MIC of the infecting parasites.

The relationship between clinical and parasitological responses

In a non-immune subject with an intact spleen, there is a good correlation between clearance of parasites from the blood and clinical recovery from the infection. But in high-transmission settings with ineffective treatments, ACRs are still seen, despite persistent parasitaemia. This is because of host immunity and because malaria can resolve spontaneously. Vigorous host defence means that little or no antimalarial activity is required to reduce PMR values to <1. There is rapid induction of both non-specific and specific host-defence mechanisms, and premunition results in tolerance of parasitaemias, which would be symptomatic in non-immunes. In high-transmission settings, it is not rare to encounter high rates (>5%) of parasitological failures at the R_2 and R_3 level with ACR, and a high proportion of cures in the Day 14 assessments. This reflects good immunity and dangerously poor antimalarial efficacy.

Unfortunately, the ACR is often used to justify continuing to deploy failing or ineffective antimalarial drugs in high-transmission areas. In endemic areas, confirmation of malaria by blood-smear exam or dipstick is commonly unavailable. As a consequence, the majority of antimalarial drug use is for fevers that are not caused by malaria and that resolve spontaneously irrespective of drug treatment, and this supports a popular impression that the drug (usually CQ) still works. There is still extensive use of CQ by immune adults in endemic areas, where falciparum malaria is highly CQ resistant. In these areas of CQ resistance (i.e. most of the tropical world), the relatively high concentrations of CQ that follow oral administrations over three days for symptomatic malaria could still be sufficient to suppress parasite multiplication for two asexual cycles and, in a semi-immune person, that might be all that is

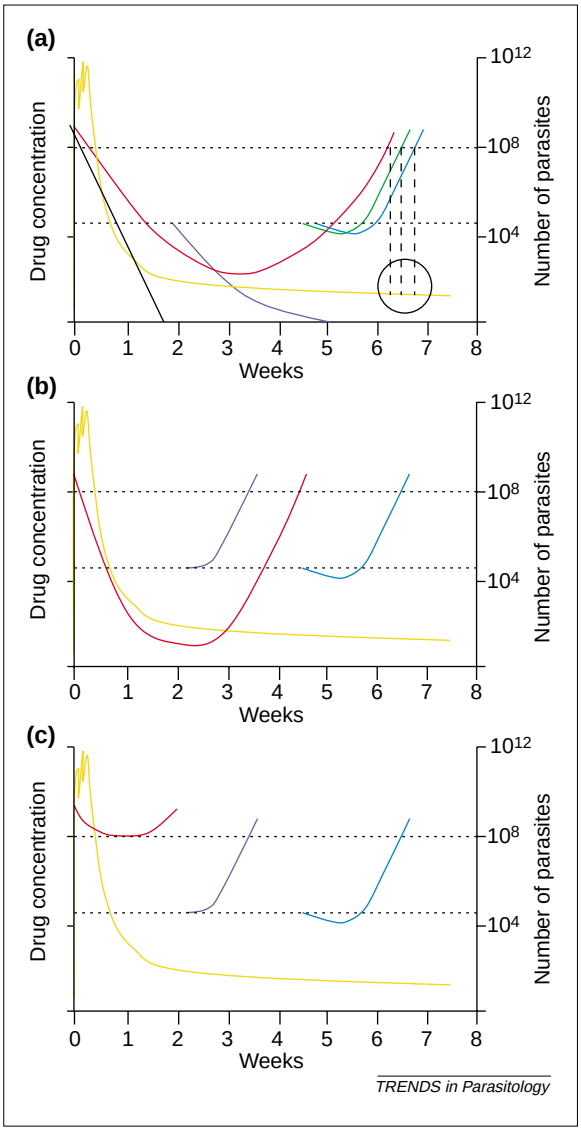

Fig. 4. Patterns of therapeutic response to chloroquine in *Plasmodium vivax* and *Plasmodium ovale* malaria. In (a–c), blood levels of chloroquine (CQ) (yellow) decline multi-exponentially with a long (termination half-life of 1–2 months) terminal phase. This ensures cure of sensitive infections (solid black line), and also suppression of the first relapse (purple) of tropical strains, but levels are often insufficient to suppress the second relapse (blue), or late re-infections (green) (a). With development of CQ resistance, either the first relapse is not suppressed or late recrudescences of the blood-stage infection could occur. In each case, measurement of the blood concentration at the time of recurrent illness is informative (circled) because parasites have been able to multiply at the measured concentration, which must be below the minimum inhibitory concentration of the infecting parasites. (b) As CQ resistance worsens, the first relapse (purple) is not suppressed, and time to reappearance of recrudescent infections shortens (red). (c) At high-level resistance, the initial parasitaemia does not clear (red). Upper horizontal dotted line represents level of microscopic detection and symptoms from malaria, and lower horizontal dotted line represents average number of parasites entering the blood after hepatic schizogony.

needed to prevent progression to a potentially lethal infection, and to allow host defence to become activated and deal with the infection. Alternatively, at even higher levels of resistance, where the drug has no effect, host defence alone could control the infection. This immunity is hard won in endemic areas as a result of repeated infection. Young children are particularly vulnerable because they have lost passive maternal protection, and not yet acquired sufficient immunity to protect against lethal infections. In these less immune children, the same drug-resistant infections are usually not cured, indeed they can persist for weeks or perhaps months, causing anaemia, or they could expand rapidly to fulminant disease. These are a minority of treated cases, and, rightly, they of particular concern to policy makers, but they are not well characterized by small *in vivo* studies of uncomplicated malaria. In the majority, whose infections resolve, the clinical response is deemed adequate. But is it the host defence that is adequate, not the drug treatment? The crucial question is: how often would the infection proceed unabated to kill the patient? Although this occurs in only a small minority of the infections, because malaria is acquired so frequently, the risk is cumulatively significant – and contributes to over a million deaths each year. This risk is not predicted adequately by conventional clinical trials [15].

Conventional drug trials exclude the sicker children, and are sometimes conducted in age groups older than those with the greatest mortality. Assuming that <2% of untreated infections are fatal, and these fatalities occur in young children, large trials would be needed to characterize the true mortality. For example, to detect a fivefold increase in mortality of symptomatic malaria from 0.2% to 1% would require enrolment of 3404 patients in a randomized controlled trial. Furthermore, children developing severe malaria are rescued in well conducted clinical trials. As immunity develops relatively rapidly in high-transmission settings, even young children will resolve infections through host defence mechanisms. Thus, an ACR of 75% in clinical trials can hide high-level drug resistance and potentially lethal treatment failures, when ineffective drugs are used unsupervised in the rural tropics. The rising childhood mortality in Africa is an urgent reminder that we can no longer rely on failing drugs [16,31,32].

Fortunately, drugs are available that will ensure rapid clinical and parasitological responses against all parasites in all settings, and are remarkably effective in preventing the progression to severe disease. In combination with other, more slowly eliminated antimalarials, three-day courses of these drugs reliably provide >95% cure rates. These are the artemisinin derivatives [33].

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