

Review

Artemisinin resistance, some facts and opinions

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Abstract

Resistance to artemisinin derivatives (ARTs) in malaria disease is currently defined as a delayed parasite clearance following artemisinin combined therapy (ACT). Although ACT is still widely effective, the first evidence of artemisinin resistance was described in 2009 in Southeast Asia. Since then, resistance to ARTs / ACT has been monitored showing an increasing trend. The demonstrated resistance to all drugs that are currently associated to ART, the ambiguous finding that ART resistance is observed only in presence of resistance to the partner drug, the lack of a mechanistic rationale to choose the partner drugs and the lack of markers with known specificity and sensitivity to monitor ART resistance, represent the most worrisome issues.

Key words: artemisinin; delayed clearance of parasites; artemisinin combined therapy.

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Introduction

Worldwide introduction of artemisinin derivatives (ART) improved the effectiveness of this therapy both in uncomplicated and in severe malaria saving a large number of lives and leading to a net reduction of prevalence in many countries. Natural products containing ART have been used in China and in malaria endemic European regions such as Sardinia since centuries; although high rates of recrudescence after ARTs treatment (from 4% to 30%) have been repeatedly reported [1-5], the evidence of ARTs resistance has only recently been outlined. As a matter of fact, ART resistance, defined as a delayed clearance of parasites (DPC) after therapy, has been observed on the Thailand/Cambodia border in 2009 and correlated to higher recrudescence rates in a small cohort of patients [6]. Currently, evidences of artemisinin resistance have been identified in many countries of Southeast Asia such as at the border between Myanmar and India. Despite a demonstrable increase of DPC prevalence in this region, patients generally respond to artemisinin combinatory therapies (ACT). Notably, in these areas the resistance to combinatory drugs is often present and the risk of developing multi-drug resistance is therefore substantial [7].

It is our opinion that the management of ART resistance is particularly troublesome because of two major issues: (i) lack of markers for epidemiological and diagnostic uses; (ii) lack of drugs with proven synergism to ART or at least with no negative interactions.

Markers of artemisinin resistance

It is very difficult to isolate artemisinin resistant strains from patients with a demonstrable therapeutic failure; since no laboratory markers with known sensitivity and specificity are available, it is important to underline that the assessment of ART resistance is more complex and more prone to errors than in the case of any other antimalarial drugs.

Among the available markers of ART resistance the measurement of ART IC₅₀ or estimates of the time required by ART to kill parasites in short term *ex vivo* cultures can provide information; however, these assays need a stringent inter-laboratory standardization and are easily affected by the inconsistent adaptation of parasite to culture conditions [7].

An additional limit is posed by the difficulty to discriminate between recrudescence due to:

(i) therapeutic failure to ARTs, (ii) therapeutic failure to the partner drug; (iii) re-infection; (iv)

inadequate drug uptake/absorption. To discriminate between therapeutic failure and re-infection is clearly more difficult in areas with high prevalence and low seasonality of malaria.

The finding and the validation of new markers to diagnose ART resistance is therefore mandatory, but the fact that the mechanism of action of ART is nearly unknown [8] and ART-resistant strains isolated from patients are generally not available represent a clear disadvantage. As the search of new markers cannot be based on a definite rationale, DPC (*in vivo* and *in vitro*) still represents the golden standard, although its sensitivity and specificity is still undetermined.

More recently, a mutation on the K13-Propeller locus has been observed to be associated to DPC in Cambodia [9]; subsequently, additional mutations on the same gene have been observed in other regions in South Asia [10-16]. This promising marker is currently used in association with DPC to identify ART resistance but both still require a substantial clinical validation. Wide clinical trials to correlate DPC and K13 mutations with confirmed ART resistance are therefore needed to assess the isolated and cumulative performances of the markers [16]. Unfortunately the intrinsic complexity of those markers do not favor the sustainability and the implementation of the large clinical trials that should be required to validate and to assign precise performances to available makers. Table 1 outlines the current state of the technical tools available to estimate the presence of ART resistance.

Drugs for combinatory therapy

Following the alarming evidence on the possible spreading of ART resistant strains, the use of combinatory ART therapy (ACT) has been strongly advised by health authorities and it is now widely used. Lack of information on the mechanism of action of ART, however, limits again a rational choice of the partner drug; and possible negative interferences between drugs cannot be excluded [16]. This issue is even more relevant as most of the available anti-malarial drugs already displayed manifest resistance [17] and as ART resistance is observed only in presence of a demonstrated resistance to the partner drug [7]. The observation that ART activity is functionally related to the hemoglobin metabolism of the parasite poses further concern on the choice of the partner drug as 4-aminoquinolinic compounds interfere with this pathway and have showed negative interaction *in vitro* [18]. Table 2 displays the ACT therapies currently in use.

In conclusion, the emergence of a clinically relevant ART resistance may easily harm the recent advances obtained in the cure and control of malaria. Differently to the resistance observed for other anti-malarial drugs, ART resistance appears subtle and particularly difficult to assess due to the lack of clear-cut clinical counterparts and specific diagnostic tools. Currently, observational studies to monitor the rate of recrudescence taking in account the rates of spontaneous infection and the frequency of asymptomatic carriers are urgently needed to ensure the effectiveness of different therapeutic regimens and clinical validation of available markers.

Table 1. Technical tools to estimate ART resistance

Markers	Sensitivity/Specificity	Notes
Parasitemia at day 28	High sensitivity	Specificity may be affected by false positive results due to re-infection
DPC at 3, 5 days <i>in vivo</i>	Not available	No clinical studies are available to determine their diagnostic performances
K13 mutations	Not available	

Table 2. ACT types currently used in South East Asia

Country	ACT type (2015)		
	Artemether - Lumefantrine	Artesunate-Mefloquine	DHA-Piperaquine
Cambodia	-----	√	√
Laos	√	-----	-----
Myanmar	√	√	√
Thailand	√	√	-----
Vietnam	-----	-----	√

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