Surveillance of malaria drug resistance: improvement needed?

"In order to monitor and control the spread of resistances, appropriate surveillance is necessary in a critical number of sites."

Despite recent improvements in case management and mortality, malaria remains one of the biggest health problems affecting many populations, especially in tropical and subtropical countries. It is the growing parasite drug resistance that aggravates the situation in malaria-stricken countries. Whenever drugs are used to control transmissible diseases, it is inevitable that selection of drug-resistant pathogens takes place. The risk is particularly high in malaria due to the high number of treated patients, indiscriminate use of antimalarial drugs and the ability of this relatively complex organism to adapt to new drugs [1,2].

The most fatal malaria parasite, *Plasmodium falciparum*, not only infects large numbers of people, but a single person can also carry an enormous parasite burden: estimations are approximately 10^{11} parasites during an acute phase of the infection [3]. Moreover, the parasites have a short generation time with a large number of offspring and a haploid genome that enables advantageous mutations to penetrate a population very rapidly. Through recombination, drug resistance information can be linked to other gene mutations and rapidly disseminated.

Once the mass use of a drug is tailing off, drug resistance is expected to revert because the price of resistance is often the loss of fitness in the absence of drug pressure. A local reintroduction of chloroquine as a therapeutic agent for the treatment of falciparum malaria has therefore recently been discussed as a future option [4]. However, once selection for resistance has arisen it is virtually impossible to eliminate a resistant subpopulation from the overall parasite pool, and after reintroduction of drug pressure selection may happen very quickly. Particularly with inexpensive drugs, such as chloroquine, it is virtually impossible to eliminate drug pressure due to massive self treatment.

In recent years, malaria control efforts have increased extensively, particularly in Africa, due to growing financial support and governmental involvement in endemic countries. The introduction of new measurements has halved the malaria incidence in many countries. However, the demonstration of significantly longer parasite clearance times after treatment with artemisinin-based combination therapies at the Thai–Cambodian border can be interpreted as a first alert of the diminishing effectiveness of artemisinins [5,6].

If so, the dramatic consequence would be the ineffectiveness of many other endoperoxide drugs under development. In order to monitor and control the spread of resistances, appropriate surveillance is necessary in a critical number of sites. The constant surveillance of drug resistance in endemic countries is essential and should lead to rapid recommendations on an effective treatment by national authorities.

Until recently, several drugs against malaria were in use depending on the recommendations of local health authorities. These drugs include sulfadoxine/pyrimethamine, and chloroquine for uncomplicated malaria. The efficacy of these drugs decreased dramatically or has already been lost in large parts of Africa. The new first-line treatment to date consists of artemisinin-based combination therapy [7], which includes the artemisinin derivatives artesunate, artemether and dihydroartemisinin, in combination with other drugs such as mefloquine or lumefantine.

The gold standard for assessing antimalarial drug efficacy is *in vivo* tests in the frame of clinical trials. However, particularly in high-transmission areas efficacy results are confounded by new infections/re-infections that invariably occur during the follow-up period, and that are inherently difficult to distinguish from recrudescences due to drug resistance [8]. Additionally, *in vivo* tests are logistically difficult to implement, expensive and usually need longer periods of time for recruitment of sufficient patients to fulfil power requirements for equivalence studies.

Jürgen FJ Kun
Author for correspondence:
Institute for Tropical Medicine, University Tübingen, Wilhelmstr. 27, 72074 Tübingen, Germany
Tel.: +49 7071 298 2191
Fax: +49 7071 294 684
juergen.kun@uni-tuebingen.de

Jürgen May
Bernhard Nocht Institute, Hamburg, Germany

Harald Noedl
Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria

future medicine part of fsg

In vitro tests involve evaluating the parasite's intrinsic drug susceptibility in short-term cultures. The read-out of *in vitro* assays is either based on microscopic inspection of parasite growth, fluorescent labeling, the measurement of the amount of incorporated radioactively labeled precursors or secreted parasite products [9–12]. Quality control issues may interfere with results that have sometimes been unsatisfactory [6,13,14]. Moreover, *in vitro* tests take a minimum of 2 days before interpretable results can be observed, and commonly only 60–80% of the cultured parasites progress in a manner that allows assay interpretation, thereby introducing potential for bias in interpretation. Incubators for parasite culture have to be installed, sterile techniques are needed, and the read-out by microscope requires an experienced scientist.

An efficient surveillance system should include up-to-date information on molecular markers for drug resistance. Molecular markers will not cover all possible resistance mechanisms, but for these parasites are relatively comprehensive in comparison to viral or bacterial drug resistances. However, molecular markers can provide rapid results, are informative and can detect slight changes in resistance patterns of parasite populations. Resistance against most drugs is linked to point mutations in distinct genes or to gene amplifications.

A number of methods at various levels of technical sophistication are used to determine these genetic alterations and results are often not directly comparable [15]. Furthermore, molecular technologies are often very costly and difficult to establish in malaria-endemic countries. A further drawback is that if reliable genetic markers for drug resistance are not available (e.g., for artemisinins), resistance can only be detected retrospectively, often too late to adjust treatment policies in times of emerging resistance.

The most accurate method to identify a genetic change is to determine the DNA sequence of the gene in question [16]. The resolution of the assay is down to one base, and the readout is generally unambiguous. The disadvantage of this method is the elaborate machinery necessary. This can either be highly sophisticated automated sequence analysis apparatuses based on fluorochrome chemistry or capillary electrophoresis and the analysis by computer programs. Characterizing microsatellite fragment size polymorphisms requires expensive fragment analyzer systems based on fluorochrome chemistry or using radioactive nucleotides and x-ray technology. The disadvantages of this technology are the same as for the determination of DNA sequences.

Therefore, the standard method currently used does not analyze the complete DNA sequence of defined DNA fragments, but uses restriction length polymorphisms to determine the base sequence of 4- to 8-mer sequences, which represent recognition sites for restriction enzymes [17,18]. In the AT-rich genome of *P. falciparum*, adequate restriction enzymes sites are sometimes hard to find and specific restriction sites may have to be created by the primer design, resulting in distinguishable PCR fragments. Again the equipment necessary consists of agarose gel chambers, power supplies, heating blocks and a large collection of restriction enzymes. Here, the disadvantages, apart from the machinery, lies more in the technology itself: if for whatever reasons the restriction enzymes do not digest a product to completion, misinterpretations are possible: undigested DNA fractions in one sample could be interpreted as more than one parasite strain in this patient.

"Best estimates for the time until availability of an antimalaria vaccine is 5 years; needless to say that this 5-year estimate has been stated since the mid 1980s."

Microarrays to analyze all drug resistance associated point mutations in *P. falciparum* DNA have been developed previously. Using this technology, it is possible to simultaneously analyze hundreds of samples for all SNPs with high accuracy within a few days. This technology has been used and positively validated in Papua New Guinea, the Solomon Islands, Cambodia and in Tanzania [19]. Since these arrays are also associated with a high cost of machinery, the cost can only be covered in a handful of African centers. Service of high-cost machinery is also difficult to obtain. A cheaper technology exists – low cost and density arrays [20]. The necessary equipment consists of very basic wash containers, a centrifuge and the chemicals provided by the producers of the arrays.

Efforts on eradication of malaria parasites have resurged, and the Holy Grail is an effective malaria immunization including RTS,S, which may be the most promising candidate to date. Best estimates for the time until availability of an antimalaria vaccine is 5 years; needless to say that this 5-year estimate has been stated since the mid 1980s. Until then, use of effective drugs will be essential for healthcare in malaria-endemic areas. The best estimate for the vaccine efficacy is a 50–60% decline in malaria cases. This

means that even after the introduction of a vaccine, chemotherapy will be an important tool for the control of malaria. In the near future we will have to cope with drug resistances to antimalaria drugs. Rapid and effective surveillance systems to detect outbreaks of resistance, as well as slow decrease of sensitivity of antimalaria drugs, will remain the key for the control of this disease.

> *"Eradication will probably never be achieved, since there will always be regions inaccessible because of natural or man-made disasters like war or civil unrest."*

To avoid a rapid spread of resistance in the future, a prudent use of antimalarial drugs is crucial – uncontrolled distribution of drugs against a potentially deadly disease should be an event of the past. Treatment should be restricted to 'true' malaria patients. The improvement of diagnostic capacities will therefore be essential. In addition, mass treatment of asymptomatic parasite carriers might be problematic: the detection of these individuals is difficult to achieve and costly.

The development of more affordable drugs, efficacious vaccines, traditional control strategies such as bednets and vector control, an economical surveillance system for resistance and, finally, close communication between countries to introduce novel therapies is needed to control malaria. The recently established WorldWide Antimalarial Resistance Network (WWARN) is a collaborative effort to collect, map and distribute drug resistance data from throughout the malaria-endemic world, with the ultimate goal of making drug-resistance data available to political decision makers [21–23].

Eradication will probably never be achieved, since there will always be regions inaccessible because of natural or man-made disasters like war or civil unrest. Premises for a successful installation of health systems will therefore always include stability, politically and environmentally, and increasing wealth and education in affected countries.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

1 Breman JG: The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am. J. Trop. Med. Hyg.* 64, 1–11 (2001).

2 Breman JG, Egan A, Keusch GT: The intolerable burden of malaria: a new look at the numbers. *Am. J. Trop. Med. Hyg.* 64(1–2 Suppl.), IV–VII (2001).

3 Dondorp AM, Desakorn V, Pongtavornpinyo W *et al.*: Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. *PLoS Med.* 2(8), E204 (2005).

4 Laufer MK, Thesing PC, Eddington ND *et al.*: Return of chloroquine antimalarial efficacy in Malawi. *N. Engl. J. Med.* 355(19), 1959–1966 (2006).

5 Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM: Evidence of artemisinin-resistant malaria in western Cambodia. *N. Engl. J. Med.* 359(24), 2619–2620 (2008).

6 Dondorp AM, Nosten F, Yi P *et al.*: Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 361(5), 455–467 (2009).

7 White NJ: Malaria – time to act. *N. Engl. J. Med.* 355(19), 1956–1957 (2006).

8 Juliano JJ, Ariey F, Sem R *et al.*: Misclassification of drug failure in *Plasmodium falciparum* clinical trials in southeast Asia. *J. Infect. Dis.* 200(4), 624–628 (2009).

9 Elabbadi N, Ancelin ML, Vial HJ: Phospholipid metabolism of serine in Plasmodium-infected erythrocytes involves phosphatidylserine and direct serine decarboxylation. *Biochem. J.* 324(Part 2), 435–445 (1997).

10 Desjardins RE, Canfield CJ, Haynes JD, Chulay JD: Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* 16(6), 710–718 (1979).

11 Noedl H, Wernsdorfer WH, Miller RS, Wongsrichanalai C: Histidine-rich protein II: a novel approach to malaria drug sensitivity testing. *Antimicrob. Agents Chemother.* 46(6), 1658–1664 (2002).

12 Noedl H, Wongsrichanalai C, Wernsdorfer WH: Malaria drug-sensitivity testing: new assays, new perspectives. *Trends Parasitol.* 19(4), 175–181 (2003).

13 Borrmann S, Binder RK, Adegnika AA *et al.*: Reassessment of the resistance of *Plasmodium falciparum* to chloroquine in Gabon: implications for the validity of tests *in vitro* vs. *in vivo*. *Trans. R. Soc. Trop. Med. Hyg.* 96(6), 660–663 (2002).

14 Schwenke A, Brandts C, Philipps J, Winkler S, Wernsdorfer WH, Kremsner PG: Declining chloroquine resistance of *Plasmodium falciparum* in Lambarene, Gabon from 1992 to 1998. *Wien. Klin. Wochenschr.* 113(1–2), 63–64 (2001).

15 Farnert A, Arez AP, Babiker HA *et al.*: Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. *Trans. R. Soc. Trop. Med. Hyg.* 95(2), 225–232 (2001).

16 Sanger F, Coulson AR: A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* 94(3), 441–448 (1975).

17 Duraisingh MT, Curtis J, Warhurst DC: *Plasmodium falciparum*: detection of polymorphisms in the dihydrofolate reductase and dihydropteroate synthetase genes by PCR and restriction digestion. *Exp. Parasitol.* 89(1), 1–8 (1998).

18 Grobusch MP, Adagu IS, Kremsner PG, Warhurst DC: *Plasmodium falciparum*: *in vitro* chloroquine susceptibility and allele-specific PCR detection of *Pfmdr1* Asn86Tyr polymorphism in Lambarene, Gabon. *Parasitology* 116, 211–217 (1998).

19 Crameri A, Marfurt J, Mugittu K *et al.*: Rapid microarray-based method for monitoring of all currently known single-nucleotide polymorphisms associated with parasite resistance to antimalaria drugs. *J. Clin. Microbiol.* 45, 3685–3691 (2007).

20 Aragon LM, Navarro F, Heiser V, Garrigo M, Espanol M, Coll P: Rapid detection of specific gene mutations associated with isoniazid or rifampicin resistance in *Mycobacterium tuberculosis* clinical isolates using non-fluorescent low-density DNA microarrays. *J. Antimicrob. Chemother.* 57(5), 825–831 (2006).

21 Bacon DJ, Jambou R, Fandeur T *et al.*: World Antimalarial Resistance Network (WARN) II: *in vitro* antimalarial drug susceptibility. *Malar. J.* 6, 120 (2007).

22 Plowe CV, Roper C, Barnwell JW *et al.*: World Antimalarial Resistance Network (WARN) III: molecular markers for drug resistant malaria. *Malar. J.* 6, 121 (2007).

23 Sibley CH, Barnes KI, Plowe CV: The rationale and plan for creating a World Antimalarial Resistance Network (WARN). *Malar. J.* 6, 118 (2007).