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Review

Perspective: Filling in the gaps of the global research agenda for eliminating malaria

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Despite the scaling-up of existing control measures and the commitment to controlling malaria the global situation has worsened. Concurrently, the drug, which was hailed as the biggest hope for eradicating malaria, Artemisinin by having significant activity against developing gametocytes, is showing signs of resistance at the Thailand-Cambodia border. This article purports to draw on lessons learnt from this epicentre of drug resistance and raise a red flag for sub-Saharan Africa. Firstly, the limitations of the current weapons of the WHO Global Malaria Control Strategy will be highlighted in both low transmission and high transmission settings. Secondly, it will be explored how a malaria-transmission blocking vaccine (TBV) as a community approach would have a high value in combination with drug treatment and other stage vaccines to break the vicious cycle of antimalarial drug resistance. Thirdly, it will be argued that prioritising vaccine development over improving diagnostic capacity may constitute a threat to the control strategy. Finally, it will be argued that if the ambitious aim of achieving malaria elimination is to be pursued, investment in TBV research should be stepped up.

Key words: Artemisinin combination therapy, transmission blocking vaccine, drug resistance, improving diagnostic capacity.

INTRODUCTION

Artemisinin combination therapy (ACT) has been considered the best current treatment for delaying the development of microbial resistance to *Plasmodium falciparum,* Health Organization Guidelines for the treatment of malaria (2006) following resistance to the low-cost mainstay drugs for malaria, choloroquine and sulphadoxine-pyrimethamine (SP). The most common artemisinin derivatives used in ACT are artesunate and artemether. Several drugs can be used in combination. The WHO currently recommends the following combination therapies, artemether/lumefantrine, artesunate plus amodiaquine in areas where the cure rate of amodiaquine monotherapy is greater than 80%, artesunate plus mefloquine and artesunate plus sulfadoxine/pyrimethamine in areas where the cure rate of sulfadoxine/pyrimethamine is greater than 80% (WHO, 2006).

Artemisinins have action against a broader range of parasite developmental stages with quinine (Peter, 2001). ACT reduces the asexual parasite population, the source of new gametocytes, more swiftly (White, 2004). By reducing the infectiousness of treated, symptomatic patients, ACT may be used to reduce overall transmission intensity (Karen et al., 2005; Nguyen et al., 2005) and the spread of parasite strains resistant to the drug combined with artemisinin derivatives (Rachel et al., 2004; Nosten et al., 2000).

A randomized controlled trial (RCT) by Sutherland and colleagues (2005) shows conclusively that a six- dose course of artemether-lumefantrine given to children with *P. falciparum* malaria in Gambia reduces gametocyte prevalence, duration of gametocyte carriage and infectiousness to mosquitoes, compared to dual treatment with choloroquine and SP (CQ/SP)(Sutherland et al., 2005). Even though the public health impact of ACT is significant, in the most severe form of human malaria, *P. falciparum*, adequate treatment of an attack does not necessarily prevent the infected person from transmitting the disease to others. Mature gametocytes present at the time of treatment may be unaffected and parasites already committed to gametogenesis will continue their development. Therefore, infectious gametocytes may be present in the blood for many days after the patient has been treated and feels better. Gametocytes responsible

for such “post-treatment transmission” are more likely to carry and spread drug-resistant alleles (Paul and Patricia, 2005).

In low transmission settings, where the basic reproductive rate is lower than 1 setting, ACT has been successful in preventing the mortality caused by the rise of an uncontrolled parasite biomass and reducing gametocyte carriage. It has reduced the transmission of resistant strains and has had dramatic effects on malaria incidence in low transmission areas such as recently in north-western Thailand, KwaZulu Natal and Zanzibar, where a greater proportion of infected individuals are symptomatic (Karen et al., 2005; Achuyt et al., 2007).

There are now signs that Artemisinin resistance is emerging near the Thailand-Cambodia border where standard ACT is artesunate plus and mefloquine (Richard et al., 2009). It is believed that ACT failures might be caused by high-level mefloquine resistance because mefloquine was used for monotherapy long before the introduction of ACT (Chansuda and Steven, 2008). This resistance may have been compounded by the shorter half-life of artesunate relative to that of mefloquine which would make tolerance to mefloquine more likely when treated patients are reinfected (Hastings IM, Ward SA, 2005). Another reason which could theoretically explain why the Thailand-Cambodia border has been the epicentre of drug resistance is that in low transmission settings a higher proportion of potentially transmissible infections are exposed to ACT and lower immunity increases the individual probability of treatment failure and transmission of resistant parasites.

In a high transmission setting, where the basic reproductive rate is higher than 1 setting, where the major transmission reservoir is asymptomatic a large proportion of malaria cases in the sub-Saharan region may not be treated. This may lead to older asymptomatic infections also contributing to the presence of gametocytaemia at baseline (Robert et al., 2000), although the relative contribution of asymptomatic and symptomatic infections to transmission is not known. This may reduce ACT impact in high transmission settings or towards the end of a period of seasonal transmission.

Although, the findings of three-year mass drug administration project in Cambodia has illustrated that mass drug administration (MDA) of artemisinin-piperaquine and low doses of primaquine can be an effective, safe and affordable strategy for efficiently eliminating malaria parasites in human carriers and interrupting parasite transmission, (Jianping, 2010) a large scale MDA study conducted in the Gambia 2007, using S/P plus a single dose of artesunate and a dose of primaquine on day 3 (Clinical Trial Gov, 2008) illustrated the limitations in terms of public health impact.

Possible reasons for this failure in the MDA in Gambia included a less than optimal gametocydical impact of the drug regimen, incomplete coverage, the relatively high transmission intensity in the area and the migration of individuals between villages. Also, the parasitological response to treatment of acute malaria among HIV-seropositive individuals and other prevalent immuno-suppressive states has not been evaluated. If it is proven that malnutrition or HIV infection plays a significant role in facilitating the development or intensification of antimalarial drug resistance, the high prevalence of these illnesses in sub-Saharan Africa could pose an additional threat to existing and future antimalarial drugs. But there are successful elsewhere with the widespread use of antimalarials population, as reported recently. Jianping Song et al. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine (Malaria Journal 2010, 9: 57).

Lack of public health infrastructure poses a challenge to delivery and effectiveness of ACT and in this respect the emergence of artesunate-mMefloquine resistance on the Thailand-Cambodia border should raise a red flag for Africa, where ACT is being promoted in a large scale but not with parallel effort to enhance rational therapy (Chansuda and Steven, 2008).

Approximately 30 years of civil war in Cambodia resulted in the breakdown of the country's public health infrastructure. This continues to impede timely treatment seeking for malaria in rural areas, thereby increasing the duration of symptoms and contributing to the spread of resistant strains. In turn, access has increased the risks to inappropriate antimalarial drugs. In particular, if artemisinins are used on their own or in co-formulation with a drug to which resistance already exists, this may result in a greater risk of drug resistance arising to this precious class of drugs (Bloland et al., 2000).

Clinical diagnosis alone or misdiagnosis as a result of poor microscopy technique, or interpretation, which still persist in remote areas, ACT Malaria EB and Partner meeting in LAO PDR (2009) has played an instrumental role in accelerating the onset of resistance since 2002 at the Thai- Cambodian border (Chansuda and Steven, 2008). In addition to suboptimal facilities for diagnosis and ACT treatment, adherence was shown not to have been adequately emphasized, which in turn led to drugs consumed in incomplete dosages.

Compounding to the problem of adherence, were unreliable services and poor diagnostic capabilities at peripheral health facilities, which further discouraged patients from seeking malaria treatment from the public sector and encourage self-purchase of drugs (Chansuda and Steven, 2008). In the face of the threat of widespread ACT drug resistance, minimising reliance on clinical diagnosis and improving diagnosis will be needed to reduce the overuse of antimalarial drugs. This will be achieved by ensuring that treatment is targeted to patients suffering from malaria as opposed to treating all patients in a febrile state.

Recent studies also suggested that RDTs were less sensitive for non-*P. falciparum* than for *P. falciparum* (Pattanasin et al., 2003). The chance of false negative

results and errors in species identification even to differentiate *P. falciparum* from *Plasmodium vivax* increases with decreasing parasite densities. This is accentuated in remote settings, where there are less skilled microscopists and poor equipment. Unless the 15 tests that now meet minimum WHO performance criteria TDR, (2010) improve in sensitivity for *P. vivax* and in ability to measure parasitemia levels, RDT will not be able to expand peripheral coverage of parasite-based diagnosis and minimize clinical diagnosis. Despite obvious need for improving malaria diagnosis, it remains the most neglected area of research, accounting for less than 0.25% of the US$ 323 million investment in research and development in 2004 (Malaria and Alliance, 2005). Community-based surveillance, relying on increased accuracy of Giemsa microscopy, which is used for histopathological diagnosis of malaria and other parasites and expansion of RDT use, along with national and global surveillance would also have a crucial role to play in extending the life of ACT. Coordination needs to be stepped up and artemisin sold only in combination with other long-lasting antimalarials. It is imperative that no pre-existing resistance be encountered to the long-lasting antimalarial, as any remaining parasites resistant to artemisin may not be mopped up.

Apart from prompt treatment seeking, the WHO global malaria control strategy also highlights the need for scaling up malaria control and prevention through insecticide treated nets (ITN) in high transmission settings and indoor residual spraying (IRS) in low transmission areas. However, resistance to pyrethroid insecticides in the malaria vector anopheles gambia threatens the sustainability of malaria vector control in sub-Saharan Africa and thus the future of Pyrethroid-based IRS may now be uncertain due to a recent discovery of a knock -down resistance (kdr) mechanism, Awolola et al. (2009) which also confers cross-resistance to DDT and a wide range of pyrethroid- based IRS and ITNs. As Kdr confers cross-resistance to DDT as well as a wide range of pyrethroid-based insecticides, (WHO Expert Committee on Malaria, 2009) it is crucial that continuous entomological surveillance take place.

ITNs' plight may be similar, due to the same family of genes that code for enzymes known as cytochrome P450s and its association with resistance to pyrethroids in anopheles funestus and gambiae (Müller et al., 2008). In order not to lose the gains achieved, continuous entomological surveillance, monitoring and research on mechanisms by which mosquitoes have developed a resistance to pyrethroids will remain crucial for the survival of the IRS and ITN - based malaria vector control programme. Given that the future of two of the WHO-recommended three-pronged approach to malaria prevention and control for pregnant women in areas of high transmission, ITN and intermittent preventive treatment (ITP) may now be compromised, there is a sense of urgency to find alternative safe and effective regimens to the one with sulfadoxine primethamine, which is increasingly being undermined in sub-Saharan Africa Florian (2005).

In light of the current gaps left behind and the uncertain future of antimalaria, the deployment of a vaccine appears to be an imperative if malaria control is to be sustained. Vaccine research has focused on preventing clinical malaria, which entails a focus on the antigens expressed in the stages of the parasite's life cycle that reside in the human host, that is, pre-erythrocytic and erythrocytic stage vaccines. Both pre-erythrocytic candidate malaria vaccines RTS,S/AS01$_E$ and RTS,S/AS02$_D$, vaccine candidates which are recombinant proteins that fuse a part of the *P. falciparum* circumsporozoite (CS) protein with the hepatitis B surface antigen, proved to safely induce anti-circumsporozoite responses (Seth, 2009).

They would in their own right become a powerful component of large scale malaria control campaigns under ideal conditions, where no parasite challenge is to be expected. However, a stochastic simulation model predicted that vaccines with efficacy similar to that of RTS, S/ASO2A would have an initial substantial impact on malaria morbidity and mortality but that after 10 years, there would be a net reduction in cumulative numbers of clinical episodes only in low transmission scenarios, with a predicted increase in high transmission area (Nicolas et al., 2006).

This would be due to an increase in severe malaria incidence in children greater than 5 years of age, who would have accrued less immunity to asexual blood stage parasites during their childhood. Also, the accrued risk of severe malaria incidence following a pre-erythrocytic stage vaccine would necessitate an erythrocytic stage vaccine, which controls disease through reduction of parasite load and thereby reduce morbidity and mortality. In the light of these caveats, it could be argued that the transmission blocking vaccine (TBV) may have a high value as a component in a multivalent vaccine.

TBV-induced immunity may reduce malaria transmission by female anopheles mosquitoes, by preventing the fertilization or the subsequent development of malaria parasites in the mosquito midgut. A DNA vaccine containing both Pfs48/45 and Pfs230 gamete surface antigens and post-fertilisation surface antigens Pfs25 and Pfs28 antigens would have the capacity to complete the cycle against gametocytes, which would have escaped gametocyticidal treatment. Contrarily to antigens in the liver and blood stages, gamete surface and post fertilisation surface antigens in this stage have the advantages of showing little polymorphism (Allan, 2007).

Recently, studies have shown the relative ease of expression and induction of potent transmission blocking antibodies in mice and nonhuman primates provide a compelling rationale and basis for development of a CH-rPfs48/45 based malaria transmission blocking vaccine (Debabani et al., 2009). Although the Phase 1 trial of

Pfs25/ISA 51 vaccine proved that it was feasible to induce transmission blocking immunity in humans, these vaccines proved to be unexpectedly reactogenic and systemic adverse effects have been reported (Yimin et al., 2008). Contrarily to gamete surface and post-fertilisation surface antigens which show little polymorphism, antigenic polymorphism in this stage vaccine has hindered the inducement of a protective immune response.

A successful TBV would be able to mop up any vaccine escape mutants that would escape control at the liver and blood stage, (WHO, 2000). This would indirectly reduce the number of patients, especially women succumbing to anaemia due to preventing the destruction of a large number of red blood cells and cerebral malaria. With only a minor chance of resistant mutants emerging of its own, the inclusion of a TBV would not only greatly extend the life of ACTs and new generation drugs, but also of vaccines against other stages by preventing the spread of parasites that become resistant to these vaccines**.** If elimination of malaria transmission is to be the objective, a community-based approach such as a TBV will be pivotal. TBV would be based more on the focality of malaria (Woolhouse et al., 1997) rather than on particular groups at risk from the clinical consequences of malaria infection, such as young children or pregnant women. As an alternative use**,** it may prove to be effective in travellers from one endemic area to another and if the vaccine is used as a quarantine vaccine to halt or reduce the spread of drug or vaccine resistant parasites. Even at relatively low coverage, TBV-induced immunity may significantly retard the build- up of a malaria epidemic in a region known to be at risk. Since the vectorial capacity that drives an epidemic is usually time-limited, this could completely abort a potential epidemic or prevent it from reaching a high level, (WHO, 2000). With no other gametocyticidal drug combination in the pipeline as a replacement, a high TBV coverage at the Thailand-Cambodia border may be able to protect the immediate neighbourhood of the vaccinated community from ACT-resistant *P. falciparum* infections.

Low to moderate endemic settings stand to benefit most from a TBV vaccine where incomplete levels of TBV coverage would have less of an impact than in high endemic settings. In a high endemic setting, a community level focus will need to be buttressed by an individual approach, given the high reproductive rate of the parasite and the unrealistic objectives of both attaining a 100% TBV coverage as well as preventing people from an unvaccinated area coming into a vaccinated community. Whilst microscopy remains the gold standard for assessing the outcomes of vaccine and drug and vaccine trials, it is paramount to consider that a high level of false positives may lower the apparent efficacy of antimalarial agents or vaccine. This may result in potentially effective drugs or vaccines to be wrongly discarded (Ohrt et al., 2002). Conversely, a high number of false negatives may result in overly optimistic outcomes of interventions (Chansuda et al., 2007).

The multidrug resistant *P. falciparum* witnessed at the Thai- Cambodian border has made rational therapy the most pressing objective, which can be attained if reliance on clinical diagnosis in areas where laboratory is often out of reach is diminished and diagnosis improved. Whilst the two current diagnostics, Giemsa microscopy and RDTs have instrumental roles in preserving ACT, they are currently both hampered by limitations, which preclude them from attaining this objective. Unfortunately, greatly expanded investment in malaria vaccine research and development in recent years has been to the detriment of investment towards strengthening diagnosis. This approach is dismissive of the possibility that an investment in enhancing the accuracy of diagnostic tools may prove to be cost effective as both potential cases of over diagnosis as well as errors in species identification may be averted. To assess efficacy of future diagnostic methods, including the Cyscope fluorescence micro-scope, which would facilitate detection of the parasites even in low parasitaemia conditions, as well as future vaccines and antimalarials in the pipeline, it is important that an effective system of microscopy quality assurance be put in place so that both sensitivity and specificity be enhanced. In light of the periodic immunity that the pre-erythrocytic candidate malaria vaccine candidate, RTS, S/AS, RTS, S is a vaccine candidate which is a recombinant protein that fuses a part of the *P. falciparum* circumsporozoite (CS) protein with the hepatitis B surface antigen would confer, these limitations in the current diagnostic infrastructure would render it difficult to assess its true efficacy.

Despite TBV's potential value as a tool for reducing the burden of malaria at community level within a multi component vaccine, TBV development, has proceeded slowly, mainly because of lack of a committed industrial partner (Carter, 2000). However, it should be recognised that if the new and ambitious aim of achieving malaria elimination is to be pursued, a different vaccine strategy than one focusing on preventing clinical malaria will be required, since success will depend on killing all parasites in the community in order to stop transmission completely (Target and Greenwood, 2008). There is a need for an immediate overhaul of the current public health infrastructure in all malaria-endemic countries for effective malaria control, which would entail improved surveillance, access to both accurate diagnosis and effective antimalarials, along with the continued prudent use of vector control measures. This should be the foundation of an effective malaria control strategy to be pursued with, in due course the addition and not the substitution of effective vaccines (Müller et al., 2008)

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