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Review

Impact of seasonal malaria chemoprevention of sulphadoxine–pyrimethamine plus amodiaquine on molecular markers resistance of *Plasmodium falciparum* malaria: A review in West Africa

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The study discuss the potential impact of seasonal malaria chemoprevention (SMC) on the cases of malaria, anaemia and molecular markers resistance *Plasmodium falciparum* malaria; and also review the mechanism of action and the effect on immunity of SMC. SMC using an efficacious drug is likely to substantially reduce cases of clinical malaria in high transmission settings. However, an increase of molecular markers could hamper rolling out SMC as a national policy.

Key words: Seasonal malaria chemoprevention impact, malaria molecular markers, review.

INTRODUCTION

Across the Sahel sub-region of Africa, most childhood mortality and morbidity from malaria occurs during the rainy season, which is generally short. Giving effective antimalarial medicines at full treatment doses at appropriate intervals during this period has been shown to prevent illness and death from malaria in children. The interventions currently recommended by the World Health Organization (WHO) (WHO, 2011) for the control of malaria are use of long-lasting insecticidal mosquito nets and/or indoor residual spraying for vector control, prompt access to diagnostic testing of suspected cases and treatment of confirmed cases with effective artemisinin-based combination therapy. In addition to these, other interventions recommended for specific high-risk groups in areas of high transmission include intermittent preventive treatment in pregnancy and infancy. Intermittent Preventive Treatment of malaria (IPT) is a form of chemoprevention achieved by giving therapeutic doses of antimalarials at pre-defined time points (Greenwood, 2007).

IPT during pregnancy (IPTp) is now widely adopted in Africa to reduce low birth weight and maternal anaemia, the main consequences of malaria in pregnancy. Randomized placebo controlled trials of IPT in infants (IPTi) (Grobusch et al., Kobbe et al., 2007; Mockenhaupt et al., 2007), in <5-year-old children (IPTc) (Cairns et al., 2008; Dicko et al., 2011) and in primary school children (IPTsc) (Clarke et al., 2008; Barger et al., 2009) have

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have shown that IPT can reduce the incidence of malaria among these target groups. Both pregnant women and older children are mostly semi-immune and when infected are more likely to develop asymptomatic parasitaemia than to develop acute clinical malaria in moderate and high transmission areas. In these groups, where asymptomatic parasitaemia is common, clearing existing parasitaemia and preventing new infections would be important. By contrast, the prevalence of asymptomatic parasitaemia in infants is very low in areas of low and moderate transmission (Gosling et al., 2009) and varies remarkably between dry and rainy seasons in areas of highly seasonal transmission (Chandramohan et al., 2005). Thus the effects of intermittent preventive treatment of infants (IPTi) will depend on the endemicity of malaria. IPTi with sulphadoxine-pyrimethamine (SP) is recommended as an additional malaria control intervention in high transmission areas of sub-Saharan Africa, provided its protective efficacy is not compromised by SP resistance.

Recently, WHO recommended Seasonal Malaria Chemoprevention (SMC) previously referred to as Intermittent Preventive Treatment in children (IPTc), is recommended in areas of highly seasonal malaria transmission across the Sahel sub region. The recommendation is based on results from 7 studies on SMC conducted in areas of highly seasonal transmission of malaria. However, the WHO has recommended a cut-off based upon molecular markers, stating that sulphadoxine-pyrimethamine (SP) should not be implemented when the prevalence of the *Plasmodium falciparum* dihydropteroate synthetase (*pfdhps)* 540E mutation among infections exceeds 50%. Therefore, the study reviews the evidence for ongoing surveillance of SP and amodiaquine (AQ) on the resistance molecular markers in support of the use of large scale of SMC.

MATERIALS AND METHODS

SMC definition

Seasonal Malaria Chemoprevention (SMC), previously referred to as Intermittent Preventive Treatment in children (IPTc), is defined as the intermittent administration of full treatment courses of an antimalarial medicine during the malaria season to prevent malarial illness with the objective of maintaining therapeutic antimalarial drug concentrations in the blood throughout the period of greatest malarial risk.

The efficacy of SMC

Eight randomized controlled trials (Cissé et al., 2006; Dicko et al., 2008; Kweku et al., 2008; Bojang et al., 2010; Dicko et al., 2011; Konaté et al., Sesay et al., 2011; Zongo et al., 2015) in children aged between 3 and 59 months during the rainy season comparing treatment doses of amodiaquine-sulphadoxine-pyrimethamine (AQ-SP) at monthly or two monthly intervals versus no treatment conducted in several countries in West Africa were included in the analysis for protective efficacy.

The end points for the analysis were: uncomplicated clinical malaria (defined as fever or a history of fever plus any level of P. falciparum parasitaemia) during the period of drug administration and one month following the last SMC course, severe malaria (defined as per the WHO definition (WHO, 2010) during the period of drug administration and one month following the last SMC course, moderate anaemia (haemoglobin (Hb) <8g/dL) at the cross-sectional survey at the end of the intervention period (approximately one month following the last SMC course) and all-cause mortality during the period of drug administration and one month following the last SMC course. The summaries of the conclusions of the evidence review by the Technical Expert Group (TEG) are as follows. Monthly or bimonthly administered SMC regimens (irrespective of the drug used) showed a protective effect of SMC against clinical malaria of 78% [95%CI: 69% to 84%, p<0.001]. A slightly higher protective effect against clinical malaria was found when the analysis was restricted to monthly-administered SMC (all drugs) [protective efficacy (PE) =83%, 95%CI: 78% to 87%, p<0.001] or monthly-administered SP+AQ only [PE=83%, 95%CI: 72% to 89%, p<0.001].

The benefit was observed also in areas with good ITN coverage. Monthly-administered SMC using any drug regimen had a protective efficacy (PE) of 61% (95% CI: 15% to 82%, p=0.02) against severe malaria, defined as an episode of malaria which met the WHO definition of severe malaria or which resulted in hospital admission. A higher PE against severe malaria was demonstrated using monthly administered SP+AQ alone [PE=77%, 95% CI: 45% to 90%, p<0.001]. All regimens gave a PE against moderate anaemia (Hb <8g/dl) of 20% [95% CI: -5% to 38%, p=0.11] and 29% [95% CI: -11% to 54%, p=0.14] respectively. There were no serious adverse events reported attributed to SMC in over 900,000 treatment courses. Only a small number of deaths were observed in the eight controlled studies during the intervention period limiting possible evaluation of the effect of SMC against all-cause mortality, although the results are consistent with a protective effect and do not exclude a substantial benefit. The both strategies of regimens gave a pooled protective efficacy against all cause mortality of 18% (95% CI: -69% to 61%, p=0.58) and 34%, (95% CI: -73% to 75%, p=0.40) respectively.

A high level of protection against uncomplicated clinical malaria (defined as fever or a history of fever with parasitaemia at any density) was maintained for 4 weeks after the administration of each treatment with SP+AQ; thereafter protection decayed rapidly. The cumulative efficacy over 21 days was 91% and over 28 days it was 86%. This duration of protection was also demonstrated for severe malaria (mainly cerebral malaria and severe anaemia). Age based dosing schemes used either a half or whole tablet. There was no association between efficacy and the dose of SP given, however there was an association between AQ dose and malaria incidence, the effect being most marked in children under 2 years of age. There is evidence of a moderate increase in the incidence of vomiting when the threedoses of AQ given exceed the maximum recommended value (>15 mg/kg daily).

To ensure maximum efficacy balanced with tolerability, and for effective wide-scale deployment, a dosing scheme using either a half or a whole tablet is ideal. For AQ, a regimen of ½ of a 153 mg tablet should be used in infants <12 months old, and a full tablet in those aged 12 to 59 months. Use of a similar age regimen for SP tablets ensures that the majority of children receive the recommended minimum SP dose of 25/1.25 mg/kg. Both drugs have been used for decades for malaria treatment, and SP is currently used for intermittent preventive treatment of malaria in pregnancy and in infancy. Both AQ and SP are also used in combination with artesunate as artemisinin-based combination therapy, which is used for the treatment of uncomplicated malaria in many endemic countries. Mild side effects may occur, of which the commonest is vomiting associated with intake of AQ. Severe side effects include severe skin reactions and blood dyscrasia, but they

are rare. In Senegal, where nearly 800 000 treatment courses of SP + AQ within SMC have been given to children, no serious adverse events attributable to these drugs were observed during intensive pharmacovigilance based on spontaneous reporting.

Data extraction

A systematic search for studies reporting efficacy and safety of SMC in children was conducted using PubMed, Web, Clinicaltrials and WHO database, and abstracts from congresses were not included in this study.

RESULTS AND DISCUSSION

Choice of drug regimen, frequency of doses and age at administration

The combination of SP + AQ was chosen for SMC for the following reasons: In clinical trials, SP + AQ conferred greater protection than other drug combinations (Sokhna et al., 2008). The use of the two drugs in combination limits the risk for selection for resistance to either SP or AQ used as mono-therapy. SP and AQ retain their efficacy in areas of Sahel and sub-Sahel with seasonal transmission where SMC is appropriate (WHO, 2010). The SP + AQ regimen is safe, well tolerated and relatively cheap. The combination of SP + AQ does not include artemisinin derivatives. Therefore, artemisinin based combinations can be reserved for treatment of clinical cases in which the rapid action of an artemisinin derivative is most useful.

SMC is recommended in areas of highly seasonal malaria transmission throughout the Sahel sub-region. A complete treatment course of SP + AQ should be given to children aged 3 to 59 months at monthly intervals, beginning at the start of the transmission season, up to a maximum of four doses during the malaria transmission season (provided both drugs retain sufficient antimalarial efficacy). The recommended dosing schedule by age is: infants 3 to 11 months old: half of a 153 mg tablet of AQ base given once daily for 3 days and a single dose of half a 500/25 mg tablet of SP; and children 12 to 59 months: a full tablet of 153 mg AQ base given once daily for 3 days and a single dose of a full tablet of 500/25 mg SP. The single dose of SP is given only on the first day, at the same time as the first dose of AQ. The target areas for implementation are those in which: malaria transmission and the majority (> 60%) of clinical malaria cases occur during a short period of about 4 months; the clinical attack rate of malaria is greater than 0.1 attack per transmission season in the target age group; and SP + AQ remains efficacious (> 90% efficacy). SMC should not be given to: a child with an acute febrile illness or to severely ill children unable to take oral medication; an HIV-positive child receiving cotrimoxazole prophylaxis; a child who has received a dose of either SP or AQ during the past month; and a child who is allergic to either SP or AQ. Breakthrough malaria infections that occur during SMC administration should not be treated with drug regimens containing either SP or AQ.

Timing, delivery and importance of adherence to the 3-day regimen

SMC should be implemented during the high malaria transmission period, when the incidence of malaria is high. It should be administered to children aged 3 to 59 months at 1-month intervals (SMC cycle) up to a maximum of four cycles in a year (SMC round). SMC with SP + AQ provides a high degree of protection for up to 4 weeks, and protection decreases rapidly thereafter. It is therefore important to respect a one month interval between SMC cycles in order to achieve a high level of protection and to minimize selection for malaria parasites resistant to SP + AQ. The period of administration of SMC should be chosen to target the period when children are most at risk for malaria attacks. For example, SMC was delivered in August, September and October in field trials in Burkina Faso (Konaté et al., 2011) and Mali (Dicko et al., 2011); while in Senegal (Cissé et al., 2006); it was given in September, October and November, covering the period of highest risk for malaria.

The method of delivery must be such that > 95% of eligible children receive SMC at monthly intervals during the period of highest malaria risk. This strict timing is best suited for community delivery, in which community health workers reach each household once a month, and a sufficient number of health workers can be deployed in each area to treat all children over a period of 3–4 days; community case management schemes are also suitable, in which health workers living in a village deliver SMC a few days each month. SMC drugs can be dispensed door-to-door or by gathering children at a pre-agreed location in each area of residence.

Combining SMC with community case management has particular advantages: there are more opportunities for catching up missed doses; breakthrough cases can be diagnosed and treated, providing information about the effectiveness of SMC; and use of the same person to deliver SMC and to provide diagnoses and treatment is more economical. SMC can also be delivered in programs at health facilities, e.g. in outreach clinics of the expanded program of immunization. Field trials have shown, however, that such programs are less effective in achieving high coverage. SMC provides protection for up to 1 month after each complete (3-day) course. It is therefore important that children receive SMC each month during the main risk period and complete the course each month in order to obtain the maximum degree of protection. Good adherence also reduces the risk for selecting drug resistant parasites.

Health workers should give the dose of SP and the first dose of AQ to the children under their direct observation

and should advise the children's caregivers on how to give the second and third doses of AQ to the child at home. Adherence to the full regimen should be one of the main messages in advocacy and behaviour change incommunication during the launching and promotion of SMC. The importance of adherence should also be stressed in communication activities at each monthly cycle.

Areas suitable for implementation and likely cost

SMC is recommended for use in areas with highly seasonal malaria transmission, and it is likely to be most cost-effective where the burden of malaria is highest in children. The suitability of an area for SMC is determined by the seasonal pattern of rainfall, malaria transmission and the burden of malaria. SMC is recommended for deployment in areas: where more than 60% of the annual incidence of malaria occurs within 4 months; where there are measures of disease burden consistent with a high burden of malaria in children (incidence 10 cases of malaria among every 100 children during the transmission season); where SP and AQ retain their antimalarial efficacy.

Evaluation of the cost of delivering SMC in large field trials shows that the greatest costs are for delivering the drugs and the incentives paid to health workers. In the Gambia, the cost of SMC delivery by village health workers was estimated to be $1.63 (USA) per child per year (Bojang et al., 2011). In Senegal, where SMC was delivered by community health workers paid a daily rate and supervised by the health post nurse, the overall cost at 46 health posts was estimated to be $0.5 (USA) per child per month, or approximately $1.50 (USA) per child per year.

The cost of SMC is similar to those of other malaria control interventions. Analysis of the costs of delivering SMC suggest that in areas where the incidence of malaria in children in the target age group is above 0.2 attacks of malaria per transmission season, SMC will be a highly cost-effective intervention as assessed by both the cost of a case and a disability-adjusted life year (DALY) prevented. DALYs averted were calculated by combining the burden of disease averted due to less malaria morbidity (as a function of malaria incidence, disease duration and impact on quality of life) and less malaria mortality (as a function of malaria incidence, case fatality rate and average life expectancy at age 1 year). In areas where the incidence of clinical attacks of malaria in children is between 0.1 and 0.2 attacks per transmission season, SMC may still be an attractive option although relatively more expensive. At an incidence rate of less than 0.1 clinical attacks per transmission season, SMC is unlikely to be a cost effective intervention. SP + AQ are safe and well-tolerated when used at the recommended doses and regimens.

Effects on immunity

The distribution of malaria morbidity and mortality within communities depends directly upon transmission intensity. Traditionally, the level of malaria endemicity has been classified by reference to the spleen rate (the proportion of children with an enlarged spleen in a sample of the population) in malarious areas (Bruce-Chwatt, 1980). More recently, however, it has been recognized that the entomological inoculation rate (EIR), which is the number of infectious mosquito bites received per person per unit of time (Macdonald, 1957), is a more direct measure of transmission intensity (Beier et al., 1999).

In The Gambia and Kenya, the risks of severe malaria (cerebral malaria or severe malaria anemia) in childhood were reported to be lowest among populations with the highest transmission intensities, and the highest disease risks were observed among populations exposed to low to moderate intensities of transmission (McElroy et al., 1997; Snow et al., 1997). Snow et al. (1997) argued that interventions that diminish the risk of infection might actually increase the risk of poor clinical outcomes. That interpretation provoked some controversy (Molineaux, 1997). The difficulty was in grappling with an apparent paradox: a greater risk of infection yields a lower risk of disease, and attacking the risk of infection yields a lower risk of disease. The study asserts that no paradox exists and that the viewpoints are reconcilable when put in the context of quantitative risk of infection and disease and of naturally acquired immunity (NAI). The thresholds of exposure leading to clinical immunity or to a high risk of severe disease are not necessarily superimposed. An intervention that pushes the attack rate below the threshold of risk of severe disease does not necessarily cross below the threshold of exposure needed to sustain acquired clinical immunity. However, there must be a threshold of exposure to sustain clinical immunity, and it can be crossed by interventions that diminish the risk of infection. If this threshold is crossed, an increase in susceptibility to less frequent episodes of infection may occur.

Other data on the effect of intermittent preventive treatment (IPT) and ITNs on rebound of malaria, or the lack thereof, are also consistent with this hypothesis; specifically, it has been proposed (Sutherland et al., 2007) that the extended period of protection observed in some (Schellenberg et al., 2005) but not all (Aponte el al., 2007) studies following cessation of intervention may be due to a situation (such as a partially effective drug) which allows for low-level and persistent parasitaemia and, consequently, prolonged stimulation of the immune system. It is also possible that the extended protective effect noted with the RTS,S vaccine in the field (Vekemans et al., 2008) may be due to the induction of blood-stage immunity in some vaccines as a result of leaky RTS,S-elicited protection (Wipasa et al., 2007). An

alternative explanation to an extended protective efficacy following cessation of intervention (IPT, insecticide treated bednets(ITN), or vaccine) may be a decrease in the malaria transmission rate during the study period as a result of a very successful intervention (Gosling et al., 2008).

There are few studies of the effects of IPTi on immunity. Quelhas et al. (2008) showed that there is no difference in IgG and IgM against erythrocyte antigens (MSP-1_{19}, AMA- 1 and EBA-175) between children who received SP-IPTi or placebo and that in some children IgG levels were increased in the SP-IPTi group. By contrast, Schreiber et al. (2007) showed a decrease of antibodies to *P. falciparum* lysate after a single dose of SP in one of the IPTi studies. The first study was designed to look at correlates of immunity during an IPTi study with follow up to 24 months of age, whereas the second was designed to detect exposure to blood stage infection following a single dose of SP. In addition the first study took place in Mozambique (Mayor et al., 2008) where the frequency of resistant alleles to SP was higher than in the second study in Ghana (Marks et al., 2004). It is assumed that children will be exposed to malaria in between doses of IPTi when the drug concentration falls below the required levels for effective prophylaxis and will thus develop immunity. This appears to be true as there has not been a significant increase in the overall number of cases of clinical malaria in any of the trials during the period after IPTi administration.

However, in one trial, there was an increased incidence of high parasitaemia malaria (Chandramohan et al., 2007) and in another an increased risk of severe anaemia cases in the second year of life (Mockenhaupt et al., 2007). Stratifying for risk of malaria infection by using entomological data and other risk factors such as house type, ITN use, maternal education, distance to health facility and more may allow for improved assessment of differences in immunity comparing children with similar exposure.

Estimating the population and burden in SMC areas

The geographical area mapped by rainfall seasonality, as defined earlier, in malaria endemic areas led to an estimate of 39 millions children under 5 years of age at risk of malaria in areas suitable for implementation of SMC, 24.9 million in the Sahel or sub-Sahel and 14.1 million in southern and eastern Africa. On the basis of this population at risk, the method used in the World Malaria Report 2008 (WMR) (WHO, 2008) gave a total burden estimate of 33.7 million cases per year in children under 5 years of age in sites suitable for SMC, 24.1 million cases in the Sahel and sub-Sahel, and 9.6 million per year in the rest of Africa. The burden estimate using a prevalence-incidence relationship derived by the Malaria Atlas Project (MAP) (Patil et al., 2009) was 12 million malaria cases, 8.5 million of these in the Sahel and sub-Sahel. Mortality estimates using a fixed case fatality rate were 151,552 (108,506 in the Sahel and sub-Sahel) for the WMR burden estimate and 53,953 (38,474 in the Sahel and sub-Sahel) for the MAP burden estimate. Use of a population-based mortality rate, as described by Rowe et al. (2006) gave an estimate of 314,283 deaths from malaria (221,811 in the Sahel and sub-Sahel). Applying a higher case fatality rate of 10 per 1,000 gave similar estimates to those produced using the method of Rowe et al. (2006).

The burden in SMC areas above minimum incidence thresholds

Applying lower prevalence thresholds to the map of areas suitable for SMC produced smaller population estimates: 28.9 million children under 5 years of age at risk in areas with incidence greater than 0.1 episodes per child during the transmission peak (21 million in the Sahel and sub-Sahel) and 24.9 million children at risk in areas with 0.2 episodes per child during the transmission peak (18.9 million in the Sahel and sub-Sahel). Corresponding morbidity and mortality estimates were slightly lower but remained substantial, particularly in the Sahel and sub-Sahel. The most stringent incidence threshold of 0.2 episodes per child during the transmission peak resulted in an estimate of 25.7 million malaria cases and 115,704 deaths (18.9 million cases and 85,225 deaths in the Sahel and sub-Sahel).

Estimating the potential public health impact of SMC

Using the WMR estimate of 33.7 million malaria cases per year in children under 5 years of age in the areas mapped as suitable for SMC, and 151,552 deaths per year (applying the fixed case fatality rate [CFR] to the incidence estimate), SMC is predicted to have a considerable impact. Restricting estimates to areas with incidence greater than 0.2 cases per child per year made only relatively minor changes. Even if the study approach has resulted in a 50% overestimate of the malaria burden in SMC areas, the potential impact of SMC could still be substantial, with ~5 million cases and 20,000 deaths averted if the intervention was widely deployed. Malaria cases and malaria deaths potentially averted in all areas suitable for SMC, and in areas with incidence > 0.2 cases per child per year, with varying assumptions about efficacy, monthly coverage and the fraction of the burden occurring during the SMC period. 75% was considered the best estimate of the fraction of the total annual malaria burden occurring during the SMC period in SMC areas. Solid lines show impact for 3 SMC courses, assumed to have 65% efficacy as detailed in the text. The malaria burden estimate used for these calculations

was that using the WMR method: 33,677,976 malaria cases in all SMC areas with stable endemic P. falciparum, and 25,712,319 derived from the population mapped by rainfall in areas with estimate of malaria incidence > 0.2/child/year. Deaths refer to the constant-case fatality rate of 4.5 per 1,000, applied to the incidence estimates.

Monitoring the efficacy of sulphadoxine–pyrimethamine and amodiaquine

SMC with SP and AQ may increase drug pressure on the malaria parasite population, which could lead to selection of drug-resistant parasites and the spread of resistance to SP and/or AQ. Therefore, monitoring the estimated efficacy of SP and AQ during SMC is important. Unfortunately, there is currently no recommended way of estimating the efficacy of SP and AQ. A baseline assessment of resistance would be helpful, and surveys should be carried out at 2–3 year intervals in representative locations with techniques such as molecular markers of resistance to SP and in vitro assays of the sensitivity of *P. falciparum* to AQ and SP. Indirect methods, such as monitoring the impact of SMC with SP and AQ on the prevalence of malaria infection or clinical malaria over time, might also be useful for detecting declining efficacy of SP and AQ, which could lead to surveys of markers of resistance or in vitro assays for confirmation. Collaboration between national malaria control programs, local research institutions, who and other organizations involved in monitoring antimalarial drug resistance is highly recommended.

SMC drug resistance area

Genetic mutations associated with parasite resistance to SP and AQ can be assessed by molecular biological techniques such as polymerase chain reaction, single sequence oligonucleide probing or sequencing. *Pfdhfr* (51, 59 and 108) and *pfdhps* (437 and 540) mutations are known to be good indicators of SP resistance, and recent reports indicate that mutations at both *pfcrt* and *pfmdr1* are good markers of resistance to AQ. The areas in which SMC with SP and AQ is suitable are those in which the efficacy of the combination remains > 90% (Cairns et al., 2012). Resistance to SP or AQ will reduce the efficacy of SMC in protecting children against clinical malaria, although the relation between the degree of resistance and the effectiveness of SMC has not yet been clearly defined. There is, however, a threat that deployment of SMC with SP and AQ will increase drug pressure on the malaria parasite and lead to increased resistance to the combination. It is therefore essential to continue to monitor the development of resistance to SP and AQ both *in vivo* and *in vitro*.

Anti-folate drug action and resistance mechanisms

The anti-folate class of drugs consists of compounds that bind enzymes necessary for parasite folate biosynthesis. The most widely used anti-malarial drugs within this class are sulphadoxine-pyrimethamine (SP) and, more recently, chlorproguanil-dapsone. The pyrimethamine portion of SP and chlorcycloguanil, the active metabolite of chlorproguanil, bind the enzyme dihydrofolate reductase (DHFR) (Dieckmann et al., 1986). Sulphadoxine and dapsone bind the enzyme dihydropteroate synthase (DHPS) (Triglia et al., 1999).

Mutations in the dhfr and dhps genes of P. falciparum parasites have been associated with decreased parasite sensitivity to the anti-folate drugs. A change from wild-type Ser108 to Asn108 (S108N) in dhfr is sufficient to cause low-level pyrimethamine resistance both *in vitro* and *in vivo* (Reeder et al., 1996). This single mutation can increase *in vitro* resistance to pyrimethamine by 100-fold relative to wild-type (Reeder et al., 1996). The progressive addition of mutations altering Cys50 to Arg (C50R), Asn51 to Ile (N51I), Cys59 to Arg (C59R), and Ile164 to Leu (I164L) in dhfr can yield higher levels of SP resistance *in vitro* and *in vivo*(Sirawaraporn et al., 1997). Genotypes consisting of multiple mutations in the dhfr gene have evolved in different parts of the world [Table 6] and are most often associated with higher levels of resistance than the single mutant genotypes. The triple dhfr mutant genotype consisting of N51I, C59R, and S108N shows *in vitro* resistance to pyrimethamine that is 225 times higher than a wild-type lab strain (Nzila-Mounda et al., 1998) and has demonstrated strong association with *in vivo* SP treatment failure (Kublin et al., 2002; Happi et al., 2005).

The Ala16 to Val (A16V) and Ser108 to Thr (S108T) mutations in dhfr confer resistance tocycloguanil but not pyrimethamine (Foote et al., 1990). The addition of the N51I, C59R, and I164L mutations in the presence of S108N confers high levels of resistance to both pyrimethamine and cycloguanil (Sirawaraporn et al., 1997). Mutations in codons Ser436 to Ala or Phe (S436A/F), Ala437 to Gly (A437G), Lys540 to Glu (K540E), Ala581 to Gly (A581G), and Ala613 to Ser or Thr (A613S/T) in dhps have been shown to affect parasite susceptibility to the sulpha drugs including sulphadoxine and dapsone (Berglez et al., 2004).

The dhps A437G mutation alone predicted clinical failure of SP in parts of Kenya (Omar et al., 2001). Data from various malaria endemic areas suggest asymmetric selection of resistant genotypes starting with mutations in dhfr and followed by those in dhps (Sibley et al., 2001). Multiple mutant dhps genotypes have also evolved in different parts of the world. The double dhps mutant genotype consisting of A437G and K540E is highly associated with *in vivo* clinical failure independently (Alker et al., 2008). However, the quintuple mutant genotype consisting of the double dhps mutant genotype

(A437G, K540E) in combination with the dhfr triple mutant genotype (S108N, N51I, C59R) is a better predictor of clinical failure than either multiple mutant genotype alone (Mugittu et al., 2004).

Prevalence of drug resistance before and after SMC implementation

A concern of any form of community wide drug administration is that it will encourage the emergence of drug resistant parasites. In the study conducted in Senegal (Niakhar region), the prevalence of the dhfr triple mutation and the dhps mutation increased substantially in both IPTc and placebo arms during the intervention period (Cissé et al., 2006). At the post-intervention survey, the proportion of parasitaemic children carrying parasites with the dhfr triple mutation and the dhps mutation was higher in children in the SP+AS arm than in the placebo arm (dhfr: SP+AS 95%, placebo 75%, p = 0.01 and dhps: SP+AS 86%, placebo 44%, p=0.001). However, as the prevalence of parasitaemia in the children who received IPTc was overall much lower than that in children in the placebo group, the estimated prevalence of drug resistant parasitaemia among study children was lower in the SP+AS arm than in the placebo arm (dhfr: SP+AS 13%, placebo 28% and dhps: SP+AS 12%, placebo 16%).

Data on the prevalence of drug resistance markers in the second year of follow-up was only available for the study conducted in Niakhar. In this study, the difference in the prevalence of markers of resistance to SP was lost at the end of the second year of follow-up (dhfr: SP+AS 88%, placebo 86%, p=0.69 and dhps: SP+AS 64%, placebo 77%, p=0.10). In the Mali (Kati Region), the prevalence of the dhfr triple and dhps mutations was higher in the IPTc arm compared to the placebo arm at the post intervention survey (Dicko et al., 2011). However, the estimated prevalence of drug resistant parasitaemia among study children was comparable in the SP+AQ arm and placebo arms (dhfr triple mutation SP+AQ 5%, placebo 6% and dhps 437 mutation SP+AS 6%, placebo 8%). In a further two studies (Burkina Faso [Bousse region] and Ghana [Hohoe region]), the proportion of parasites carrying SP resistance markers was similar in IPTc and placebo arms at the post-intervention survey (Kweku et al., 2008).

A further safety concern for IPTc is that it will impair the development of natural immunity making children more susceptible to malaria after treatment is stopped. At the time of this review, data on clinical outcomes in the year after administration of IPTc were available for only three studies (Cissé et al., 2006; Dicko et al., 2008; Kweku et al., 2008). None of these studies demonstrated a statistically significant increase in the incidence of clinical malaria during the transmission season following the intervention. Random effect meta-analysis gave a summary effect measure of 1.11 (95% CI 0.99–1.24, p = 0.07). At the end of the malaria transmission season in year 2, the prevalence of anaemia in children who had received SP+AS in Niakhar was higher than in children who had received placebo in year 1 SP+AS 10%, placebo 6%, p = 0.02) (Cissé et al., 2006).

However, the mean hemoglobin concentration (g/dL, 95%CI) was similar in SP+AS and placebo arms (SP+AS: 9.4 (9.3–9.6), placebo: 9.6 (9.4–9.6), p = 0.24). The prevalence of anaemia was similar in IPTc and placebo arms of the Ghana in Hohoe trial at the end of the transmission season in the post- intervention year (SP bimonthly 12%, AS+AQ bimonthly 15%, AS+AQ monthly 10%, placebo 12%) (Kweku et al., 2008).As the prevalence of parasitaemia in the children who received IPTc was overall much lower than that in children in the placebo group, the estimated prevalence of drug resistant parasitaemia among study children was lower in the SP+AS arm than in the placebo arm(Kweku et al., 2008).

Additional study conducted in three health districts in Senegal with 54 health posts with a gradual introduction of SMC show that mutations at codon 540 of pfdhps and codon 164 of pfdhfr were not detected and prevalence of pfcrt, pfmdr1, dhfr triple mutant and pfdhps 437 were 66; 20; 87.5 and 87.5% respectively (Lo et al., 2013). The high prevalence of the *pfdhfr* triple mutation and the *pfdhfr* / *pfdhps* quadruple mutation observed in this study is not very different from the results observed after two years of implementation of SMC with SP in infants in Senegal (Faye et al., 2011). This is consistent with the results obtained in Gabon, in Senegal and in Cameroon (Aubouy et al., 2003; Faye et al., 2007; Nfor et al., 2007). In Burkina Faso, the SMC study show that mutations at codon 540 of pfdhps and codon 164 of pfdhfr were not detected but a significant selection over the course of the study was seen for *pfcrt* 76T (68.5% to 83.0%, *P*=0.04), *pfdhfr* 59R (54.8% to 83.3%, *P* =0.0002), and *pfdhfr* 108N (55.0% to 87.2%, *P*=0.0001), with trends toward selection of *pfmdr1* 86Y, *pfdhfr* N51I, and *pfdhps* 437G (Somé et al., 2014). Pfdhfr and pfdhps mutation associated with antifolate resistance were more prevalent in parasites from children who received SP+AQ than in children who received dihydroartemisinin-piperaquine (DHAPQ) (Zongo et al., 2015).

Conclusions

SMC is a safe method of malaria control that has the potential to avert a significant proportion of clinical malaria episodes in areas with seasonal malaria transmission and also appears to have a substantial protective effect against all-cause mortality. The dhfr-dhps quintuple mutation was not observed after three years of SMC implementation in Senegal study area. Analysis of individual mutations showed that pfmdr1-86Y was more common among children positive for parasites

at the end of the transmission season in SMC areas. However, the absolute prevalence of resistance markers was lower in SMC areas, reflecting the reduction in prevalence due to the intervention, and this is not expected to compromise the efficacy of regimens used for case management.

RECOMMENDATION

Evaluation of the prevalence of markers of drug resistance should be part of routine monitoring and evaluation in areas where SMC is deployed with *in vivo* efficacy of SP+AQ.

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Conflicts of interest

The author has none to declare.

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