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Evaluating the safety of malaria treatment in glucose-6-phosphate dehydrogenase-deficient individuals: evidence and tools to support malaria elimination

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Evaluating the safety of malaria treatment in glucose-6-phosphate dehydrogenase-deficient individuals: evidence and tools to support malaria elimination

by

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DISSERTATION

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A version of Chapter 1 of this dissertation has been published online ahead of print in PLoS ONE.[1]

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The final co-authors listed in each publication directed and supervised the research that forms the basis for the dissertation chapters.

The published material is substantially the product of Eugénie Aude Marguerite Poirot's period of study at UCSF and was primarily conducted and written by her. The work she completed for these published manuscripts are comparable to a standard dissertation chapter.

Approved:  Roly Gosling, MD, PhD, Dissertation Chair

EVALUATING THE SAFETY OF MALARIA TREATMENT IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE-DEFICIENT INDIVIDUALS: EVIDENCE AND TOOLS TO SUPPORT MALARIA ELIMINATION

Eugénie Aude Marguerite Poirot

Abstract

There have been remarkable achievements in the global fight against malaria over the past decade, with a growing number of countries moving towards malaria elimination. Sustaining these gains and eliminating malaria will require the right set of interventions and strategies that target the entire *Plasmodium falciparum* (*P. falciparum*) parasite reservoir, including both the mosquito vector and the human host. Primaquine is the only available drug that clears mature *P. falciparum* gametocytes in humans, thereby preventing transmission to the mosquito vector. However, the drug's oxidative capacity to cause hemolytic effects in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, has limited its deployment because of safety concerns. G6PD deficiency is an X-linked enzyme defect that affects over 400 million people worldwide and is relatively common in malaria endemic areas. To reduce *P. falciparum* malaria transmission in eliminating settings and areas threatened by artemisinin resistance, the World Health Organization (WHO) recommends adding a 0.25 mg/kg dose of primaquine, lowered from a previously higher 0.75 mg/kg dose, to standard artemisinin-based combination therapy (ACT) without G6PD testing. This policy change, issued in 2012, was based on data gathered passively from limited pharmacovigilance systems and

lacks substantive evidence to support the safety of the recommendation. The objective of this dissertation was to evaluate the safety of malaria treatment in G6PD-deficient individuals and contribute evidence to support the programmatic use of primaquine for falciparum malaria.

The study designs and populations presented in this dissertation include: 1) a secondary analysis of data from a double-blind, placebo-controlled trial of preventive antimalarial treatment in asymptomatic infants (n = 1557) in Tanzania (Chapter 1); 2) a pilot study evaluating a pharmacovigilance safety monitoring tool for the rollout of single low-dose primaquine among confirmed, uncomplicated malaria cases (n = 102) in Swaziland (Chapter 2); and 3) a pooled analysis of individual-level patient data in malaria-infected patients (n = 604) from two randomized controlled trials in Senegal and Tanzania and two active pharmacovigilance programs in Bangladesh and Swaziland (Chapter 3).

In the trial of asymptomatic infants in Tanzania, findings demonstrated that treatment with an antimalarial drug known to have oxidative potential like primaquine reduced hemoglobin levels at 7 and 14 days post-treatment, relative to placebo and other antimalarial treatments. Also, at day 7, G6PD deficiency was associated with higher odds of moderate anemia (hemoglobin <8 g/dL) and greater absolute reductions in hemoglobin. However, there was no evidence that G6PD deficiency exacerbated the adverse effects of oxidative treatment. Measuring the risk of adverse effects in G6PD-deficient individuals associated with oxidative treatment is challenging due to limited data.

The pilot study of the Primaquine Roll Out Monitoring Pharmacovigilance Tool (PROMPT) served to facilitate the systematic collection of primaquine safety data. Results illustrated the feasibility and acceptability of implementing active safety monitoring to support surveillance of possible adverse events, including clinically important drops in hemoglobin, following primaquine treatment. Data that include sufficient G6PD-deficient individuals through the wider adoption of PROMPT can allow for pooled analyses powered to reliably detect statistically significant increased risks of hemolysis.

In the pooled analysis of data collected using PROMPT, results supported the addition of a single dose of 0.25 mg/kg primaquine to ACT in the treatment of uncomplicated *P. falciparum* malaria. An analysis of randomized clinical trial data among G6PD-deficient individuals found that the addition of 0.25 mg/kg primaquine to ACT did not significantly increase risk of hemolysis. An analysis using all available data showed that falls in hemoglobin following treatment appeared greater in G6PD-deficient individuals compared to G6PD-normal individuals but that doses ≤0.5 mg/kg, (double the WHO recommended 0.25 mg/kg dose) did not result in greater hemolysis in G6PD-deficient versus G6PD normal individuals.

Taken together, the studies outlined in this dissertation serve to better characterize the safety profile of primaquine to support its safe use as a gametocytocide for *P. falciparum* malaria elimination.

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Chapter 1: Risks of hemolysis in glucose-6-phosphate dehydrogenase deficient infants exposed to chlorproguanil-dapsone, mefloquine and sulfadoxine-pyrimethamine as part of Intermittent Presumptive Treatment of malaria in Infants

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Abstract

Introduction: Chlorproguanil-dapsone (CD) has been linked to hemolysis in symptomatic glucose-6-phosphate dehydrogenase deficient (G6PDd) children. Few studies have explored the effects of G6PD status on hemolysis in children treated with Intermittent Preventive Treatment in infants (IPTi) antimalarial regimens. We sought to examine the joint effects of G6PD status and IPTi antimalarial treatment on incidence of hemolysis in asymptomatic children treated with CD, sulfadoxine-pyrimethamine (SP), and mefloquine (MQ).

Methods: A secondary analysis of data from a double-blind, placebo-controlled trial of IPTi was conducted. Hemoglobin (Hb) measurements were made at IPTi doses, regular follow-up and emergency visits. G6PD genotype was determined at 9 months looking for SNPs for the A-genotype at coding position 202. Multivariable linear and logistic regression models were used to examine hemolysis among children with valid G6PD genotyping results. Hemolysis was defined as the absolute change in Hb or as any post-dose Hb <8 g/dL. These outcomes were assessed using either a single follow-up Hb on day 7 after an IPTi dose or Hb obtained 1 to 14 or 28 days after each IPTi dose.

Findings: Relative to placebo, CD reduced Hb by approximately 0.5 g/dL at 7 and within 14 days after an IPTi dose, and by 0.2 g/dL within 28 days. Adjusted declines in the CD group were larger than in the MQ and SP groups. At day 7, homo-/hemizygous genotype was associated with higher odds of Hb <8 g/dL (adjusted odds ratio = 6.7, 95% CI 1.7 to 27.0) and greater absolute reductions in Hb (-0.6 g/dL, 95% CI -1.1 to 0.003). There was no evidence to suggest increased reductions in Hb among homo-/hemizygous children treated with CD compared to placebo, SP or MQ.

Conclusions: While treatment with CD demonstrated greater reductions in Hb at 7 and 14 days after an IPTi dose compared to both SP and MQ, there was no evidence that G6PD deficiency exacerbated the adverse effects of CD, despite evidence for higher hemolysis risk among G6PDd infants.

Introduction

Substantial progress has been made in malaria control over the last decade, with many malaria endemic countries now planning for malaria elimination [1]. The path to malaria elimination is multi-faceted, requiring the detection of clinical cases and targeting of asymptomatic infections where parasite reservoirs are likely to persist and perpetuate onward transmission [2]. Antimalarial drugs play a central role in this endeavor; however, certain antimalarial drugs that are key to many control and elimination strategies are unsafe among patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency and can cause hemolysis.

G6PD deficiency is the most common X-linked enzyme deficiency in humans, affecting more than 400 million people worldwide [3]. Due to the X-linked nature of this deficiency, females can be homozygous or heterozygous while males can only be hemizygous for the gene. In consequence, partial inactivation of one female X chromosome or lyonization in somatic cells, result in varying enzyme activity among heterozygous females depending on the proportion of G6PD normal and G6PD deficient (G6PDd) red cells in their blood [4]. G6PD deficiency is relatively common in historically malaria endemic countries. This overlap is not a coincidence as evidence suggests that G6PD deficiency arose through natural selection by malaria; the parasite appears to undergo adaptive changes in G6PDd cells to confer protection against malaria [5].

While variants of G6PD deficiency appear to provide partial protection against malaria [6-8] it can also cause hemolysis after exposure to certain triggers, such as the ingestion of certain foods (fava beans), infection (Hepatitis viruses A and B, cytomegalovirus, pneumonia, and typhoid

fever) and exposure to oxidant drugs [3, 9-13]. Drug-induced G6PD deficiency-related hemolysis has been reported to follow therapy with a range of antimalarial drugs, including primaquine, methylene blue, and the sulphone drug dapsone [14]. Additionally, dapsone is used for a variety of indications [15], including in the treatment of leprosy, varied skin conditions, and more recently *Pneumocystis carinii* [16], especially in patients with HIV infection, but can also be used in combination with antimalarial drugs (pyrimethamine, proguanil and chlorproguanil) for malaria chemoprophylaxis and treatment [17].

In the late 1990s, chlorproguanil-dapsone (CD) was developed by a public-private partnership as a low-cost treatment for uncomplicated *Plasmodium falciparum* (*P. falciparum*) malaria in response to concerns of growing resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) in Africa [16, 18]. CD was later withdrawn from the market in 2008 following demonstration of post-treatment hemolytic anemia in G6PDd patients in two phase III trials [19, 20]. Prior to this, in 2004, Gosling *et al.* undertook a study to examine the protective efficacy and safety of three antimalarials – SP, mefloquine (MQ) and CD in an area of high SP resistance in northeast Tanzania in search of alternative regimens to SP for Intermittent Preventive Treatment for malaria in infants (IPTi) [21]. At the time, SP was used as first line treatment for uncomplicated malaria nationally in Tanzania. Findings from this study - the Kilimanjaro IPTi Drug Options Trial - showed that IPTi with the long acting drug MQ substantially reduced the incidence of clinical episodes of malaria in children, but neither SP nor CD showed signs of any protective efficacy against clinical malaria. High levels of SP resistance markers owing to evidence of increasing selection for individual dihydrofolate reductase (*Pfdhfr*) and

dihydropteroate synthetase (*Pfdhps*) mutations offers an explanation for this lack of efficacy in episodes of clinical malaria [22]. Regarding the safety of these three antimalarial drugs, more deaths were observed in infants in the CD and MQ groups than in the SP or placebo groups. The study also noted that children in the CD group experienced greater declines in mean hemoglobin concentrations and had a higher risk of moderate anemia (hemoglobin <8 g/dL) 7 days after an IPTi dose. Children in the CD group also had a shorter time to first or only episode of moderate anemia than did those in the placebo or other drug groups [21]. Despite these patterns, none of the analyses specifically documented the effect of G6PD status on hemolysis and other adverse events, including hospitalization, blood transfusion or death, in children treated with these three IPTi antimalarial regimens. To our knowledge, this analysis is the first to thoroughly examine the effects of G6PD deficiency in the context of IPTi in asymptomatic children under the age of one year.

To address this gap, using existing data from the Kilimanjaro IPTi Drug Options Trial, we explored differences in susceptibility to hemolysis and other adverse events among asymptomatic G6PDd infants treated with three antimalarial drugs in sub-Saharan Africa. Here, we examine the joint effects of G6PD status and IPTi antimalarial treatment on incidence of hemolysis. We hypothesized that incidence of hemolysis is increased among homo-/hemizygous infants treated with CD compared to placebo and other antimalarials, especially within 7 days following treatment. First, we contrast absolute changes in hemoglobin levels up to 7, 14 and 28 days after an IPTi dose by treatment. Then we estimate the impact of G6PD status on hemolysis among all infants who received active antimalarial treatment and assess for evidence of

modification of the effects of IPTi treatment on hemolysis by G6PD status. Finally, we assess G6PD genotype effects on incidence of other adverse event episodes up to two years of age following IPTi.

Methods

Study design and study site

This is a secondary analysis of data from a clinical trial that took place between 2004 and 2008. The drug trial has been described in detail elsewhere [21]. Briefly, the trial tested the protective efficacy and safety of three antimalarial regimens for IPTi. The trial was a randomized, double-blind placebo-controlled trial of SP, CD, and MQ, compared to placebo, conducted at neighboring moderate- and low-transmission sites in northeast Tanzania. Children aged 8-16 weeks who attended clinics for WHO's Extended Program on Immunization (EPI) were eligible for inclusion. Enrolled children who met the inclusion criteria were randomly assigned to receive full treatment doses of SP, CD, MQ, or placebo, given alongside routine immunizations at approximately 2, 3, and 9 months of age. Blood samples were collected before children were given their first and third course of IPTi. Blood was also collected on filter paper bloodspots (Whatman 3MM) using the buffy coat. Hemoglobin measurements were made at IPTi doses, regular follow-up and emergency visits. A total of 800 infants had their hemoglobin concentration systematically measured on day 7 after IPTi drug administration, including the first 200 infants to receive the first course of IPTi and the second 200 infants to receive the third course at each site. Every child was followed up at home on days 2 and 3 following treatment. During these visits, health workers assessed for possible adverse events and checked for

adherence to drug regimens. In addition, all children were followed up at 10, 18, and 24 months of age. Additional follow-up visits at 11 and 12 months of age were done on random samples (**Figure 1.1**).

G6PD (A-) genotyping

G6PD screening was conducted using samples collected at routine visits at 9 months, focusing on the G6PD deficiency allele 202A G6PD A-, the most common in sub-Saharan Africa. Whole blood samples were collected and centrifuged on the day of collection. Plasma, buffy coat and red cells were stored at -20^{o}C locally and transferred to the central laboratory for G6PD testing at a later date. Filter paper bloodspots (Whatman 3MM) for each patient were prepared using the collected buffy coat. DNA extraction from the impregnated filter papers was done using the Chelex method as previously described [23]. The extracted DNA samples were genotyped using a simple high-throughput sequence-specific oligonucleotide probe (SSOP)-ELISA method described by Enevold *et al.* [24] to detect the most prominent single nucleotide polymorphisms (SNPs) in the G6PD genes (B, A and A-).

Ethics

The clinical protocol of the original study was approved by the National Medical Research Coordination Committee of the National Institute for Medical Research of Tanzania (NIMR-MRCC) and by the London School of Hygiene and Tropical Medicine ethics committee and registered with ClinicalTrials.gov (identifier: NCT00158574).

Study population

For this analysis, only children whose G6PD status could successfully be determined were included.

Variables and definitions

Homozygous females and hemizygous males were considered G6PDd and analyzed together, because of small numbers. Heterozygous G6PD females were analyzed as a separate category.

The primary safety endpoint considered for this analysis was incidence of hemolysis. We used hemoglobin as a surrogate for hemolysis using two measures. First, hemolysis was defined as absolute change in hemoglobin, from the day of the most recent IPTi dose. We also defined hemolysis as any post-dose hemoglobin measurement <8 g/dL (a measure of moderate anemia). For each outcome definition, we evaluated hemolysis using two approaches. The first restricted the analysis to children who had their hemoglobin concentration systematically measured on day 7 after an IPTi dose. The second considered any follow-up hemoglobin measurements obtained from 1 to 14 or 28 days after each IPTi dose administration, provided it was obtained before the next IPTi dose. The time frames for follow-up hemoglobin measurements were chosen to reflect what is known about the duration of drug-induced hemolytic episodes. Typically, within 24-72 hours of drug dosing, clinically detectable hemolysis and jaundice become apparent, worsening until days 7-8 [3]. Studies have shown that hemoglobin concentrations after use of oxidant drugs

are typically lowest on day 7 [19, 25, 26]. Once such drugs are discontinued, hemoglobin concentrations begin to recover after 8-10 days [3].

We also assessed treatment and genotype effects on incidence of adverse events (malaria, hospitalizations, blood transfusions, and death) throughout the study period.

Directed acyclic graphs were used to identify potential confounders of G6PD status. In contrast to many conventional epidemiologic risk factors, genotype is not affected by most risk factors for hemolysis or malaria. However, G6PD deficiency does vary by sex, a potential risk factor for study outcomes. Moreover, genotype varies by distance above sea level (and accordingly site), a risk factor for malaria if not hemolysis, probably due to the selective pressure of malaria endemicity on the source population. Accordingly, genotype effect estimates were adjusted for these potential confounders.

Statistical analysis

All analyses were performed using STATA 13.1 (STATA Corporation, College Station, TX, USA).

Linear and logistic models were used for continuous and binary hemolysis outcomes measured only once for each infant. For repeated continuous and binary outcomes, mixed effects linear and logistic models with random intercepts were used. Analyses exploring G6PD genotype effects on hemolysis were restricted to children in the active arms, motivated by the hypothesis that adverse

effects of G6PD deficiency would only be observed in infants receiving active treatment. Analyses were adjusted for sex and elevation, either directly or using inverse weighting by way of propensity scores for rare binary and failure time outcomes. Propensity scores, an alternative approach to standard adjustment for covariates, are useful when a binary or categorical exposure is common, but the binary or failure time outcome is rare, and when there are a large number of potential confounders that must be accounted for [27]. In analyses of changes in hemoglobin, we also adjusted for treatment assignment, dose number, weight and elevation. For each hemolysis outcome, we assessed genotype-treatment interactions.

Poisson models were used to estimate genotype effects on incidence of hemolysis and secondary adverse event outcomes, adjusting for treatment, gender, site, and elevation, with follow-up censored at 24 months of age or on the date of exit for children lost to follow-up due to migration, refusal, or exclusion. As in the main trial analysis, children were considered not at risk for malaria for 21 days after receipt of treatment with an antimalarial drug, not at risk for hemolysis for 28 days after a hemolytic episode [28], and not at risk for hospitalization for 7 days after hospital admission.

Results

Baseline characteristics

A total of 2419 children were enrolled in the original trial, of whom 1842 (76%) were screened for G6PD deficiency. Among those screened, 1557/1842 (85%) were successfully genotyped and included in this analysis. G6PD results were not obtained for the remaining 285 patients (15%)

either because samples were not received or DNA extraction was not successful. Among the genotyped children, 1129 (73%) returned for at least one follow-up hemoglobin measurement within 28 days of an IPTi dose. In addition, a randomly selected 447 (28%) returned for a day 7 follow-up hemoglobin measurement (**Figure 1.2**).

Demographic and clinical characteristics at enrolment among children with valid genotyping results were generally similar across genotypes, with a few exceptions (**Table 1.1**). Sex differences ($p<0.001$) are explained by the fact that the heterozygous genotype only exists in females. Children from the high transmission site (Korogwe) ($p = 0.003$) and those who lived at lower median elevations from sea level ($p=0.002$) were more likely to be homo-/hemizygous. Homo-/hemizygous children also displayed lower median hemoglobin levels ($p=0.004$) and slightly higher median weight values at enrolment ($p = 0.005$). Compared to children with successful G6PD genotyping results, included in this analysis, excluded children (those with no or invalid G6PD genotype, n = 855) were more likely to be male ($p = 0.004$), lived closer to the nearest clinic ($p = 0.002$), and displayed higher bednet coverage ($p = 0.02$) (**Table 1.2**).

Treatment effects on declines in hemoglobin up to 7, 14, and 28 days after an IPTi dose

In analyses adjusting for genotype, sex, dose number, site, and elevation, treatment with CD reduced hemoglobin levels by approximately 0.5 g/dL 7 days after an IPTi dose, by a similar amount within 14 days, and by 0.2 g/dL within 28 days, compared to placebo (**Table 1.3**). Adjusted declines in the CD group were also generally larger than in the MQ and SP groups, which did not differ from placebo.

Genotype effects on hemolysis up to 28 days after an IPTi dose among children given active treatment

In analyses examining the impact of G6PD status on hemolysis among 329 children in the active treatment arms, adjusting for sex and elevation using inverse weighting, we found a strong association of homo-/hemizygous genotype with Hb <8 g/dL 7 days after IPTi treatment (adjusted odds ratio = 6.7, 95% CI 1.7 to 27.0, p = 0.01) (**Table 1.4**). This was an uncommon outcome, with only 6 events (2%) in the normal group (n=281), 4 events (17%) in the homo-/hemizygous group (n=24), and no events in the heterozygous group (n=29) (p = 0.01). On day 7, there was also borderline evidence of a greater average decline in hemoglobin among homo-/hemizygous children compared to normal children (-0.6 g/dL, 95% CI -1.1 to 0.003, p=0.05). In contrast, we found little or no evidence for a genotype effect on either outcome within 14 or 28 days of an IPTi dose.

Effect modification by G6PD status on incidence of hemolysis up to 28 days after an IPTi dose

In analyses stratified by genotype and adjusting for dose number, sex, site, weight and elevation, treatment with CD reduced hemoglobin by nominally larger amounts in the homo-/hemizygous group (range: 0.49 – 1.31 g/dL) than among normal genotype children (range: 0.18 – 0.48 g/dL) (**Table 1.5**), but there was no persuasive evidence for treatment-genotype interaction in any of these analyses (p>0.05, test for interaction of CD vs. placebo and homo-/hemizygous vs. normal). Due to few events, we were unable to explore effect modification by G6PD status on Hb <8 g/dL at day 7 or within 14 or 28 days of an IPTi dose.

Genotype effects on adverse event episodes up to two years of age

During a median follow-up time of 21.8 months, 314 children experienced at least one episode of clinical malaria, for a total of 707 episodes, including 598 in the normal genotype group, 67 among heterozygous children, and 42 among the homo-/hemizygous. There were also a total of 954 hospitalizations; 362 participants had one hospitalization, 155 had two, and 85 were hospitalized three or more times. There was no evidence for differences in malaria incidence or hospitalizations by G6PD genotype. Thirty-four children received blood transfusions, including three who required more than one transfusion, for a total of 38 events. Seven children died, including two in the homo-/hemizygous group (one in the CD arm); the remaining deaths were among G6PD normal children. These outcomes were too uncommon for meaningful statistical analysis (**Table 1.6**).

Discussion

Our analysis of the effects of antimalarial regimens and G6PD status on hematologic parameters in asymptomatic children treated with IPTi showed that treatment with CD caused reductions in hemoglobin at 7 and 14 days after an IPTi dose, relative to placebo, SP, and MQ. We also found evidence for higher hemolysis risk among G6PDd infants. Although we found no clear evidence that G6PD deficiency exacerbated the adverse effects of CD, the study was not powered to detect an interaction between these two factors.

The transient but statistically significant decrease in hemoglobin after initiation of antimalarial treatment is consistent with previous studies examining the hematologic variations in pediatric uncomplicated *P. falciparum* malaria and corresponding trends during post-treatment follow-up [29]. Furthermore, the hemolytic potential of CD in this context has been widely documented and clinically important decreases in hemoglobin or related adverse events requiring medical intervention post-treatment have been noted [19, 20, 30, 31]. Our analysis also revealed a strong association of homo-/hemizygous G6PD genotype with post-dose incidence of anemia (Hb <8 g/dL) 7 days after IPTi treatment. Homo-/hemizygous infants also displayed borderline significant evidence of greater absolute declines in hemoglobin compared with normal infants at day 7. This is consistent with expectations and the clinical-hematological picture of drug-induced acute hemolytic anemia in G6PDd patients [16, 32, 33].

Drug induced hemolysis is self-limiting in individuals with G6PD deficiency [34]. In this study we show that hemoglobin levels are temporarily reduced with hemoglobin declines appearing less pronounced within 28 days. Research has shown that the mechanism for this is explained through the destruction of older red blood cells during drug exposure that are the most enzyme deficient while young erythrocytes with nearly normal levels of G6PD are resistant to destruction [34, 35].

Some limitations to our analysis should be noted. As previously described, many analyses were underpowered, in particular for treatment-genotype interactions, due to the low frequency of homo-/hemizygous and heterozygous genotypes. Assessing the hematological effects of

antimalarials is complicated especially when the risk of adverse reactions is confined to a relatively small subgroup of patients – those with G6PD deficiency - and demonstrating a statistically significant trend is unlikely. Further, we conducted multiple comparisons, potentially inflating the overall type-I error rate. Additionally, analyses were restricted to children who survived and remained in follow-up to 9 months of age, and had valid G6PD results, with the potential to induce selection bias and limit the generalizability of our findings. However, baseline characteristics of the children with valid G6PD results were generally similar to those who were excluded from the analysis. An additional limitation is that G6PD deficiency was determined by screening human DNA for a single SNP in the G6PD gene (G202A), although mutations other than G6PD A-, could have affected hemolysis risk and explain part of the observed results. However, this is unlikely to be important, because G6PD A- accounts for 90% of G6PD deficiency in Africa [3].

These limitations render it challenging to find true subgroup effects, partially explaining why there is currently limited data concerning the hemolytic risk associated with diverse antimalarial drug regimens across G6PD genotypes in most populations. This analysis was underpowered to detect interaction; nonetheless, the direction of trends is consistent with expectations that IPTi with CD may produce greater reductions in hemoglobin among homo-/hemizygous children compared to other antimalarials. CD was withdrawn from the market in 2008 due to post-licensure hemolytic toxicity in patients with G6PD deficiency; however, dapsone is still used in the prevention and treatment of a variety of diseases [15-17, 36] and can precipitate life-threatening hemolysis in individuals with G6PD deficiency. In addition, two 8-aminoquinoline

drugs with similar safety profiles, primaquine and tafenoquine, are attracting much interest as chemotherapeutic and prophylactic antimalarial agents against the liver stages of *P. vivax* and *P. falciparum*. Both drugs also cause oxidant hemolysis in individuals with G6PD deficiency, and as malaria control programs begin to consider the use of primaquine or tafenoquine (if approved and licensed), identifying subgroups for which antimalarial treatment may be harmful or likely to have the largest benefit will become increasingly important. While this may prove challenging given the rarity of G6PD deficiency, even if fixed by design, pooled analyses with larger samples of G6PDd individuals could offer new insights. Interaction analyses can enable us to substantiate clinically important differences in hematological effects for oxidative antimalarial treatments across G6PD genotypes, and approaches to examine such trends in this important patient population are needed.

Conclusion

This re-analysis of data from the Kilimanjaro IPTi Drug Options Trial among nearly 1600 children from northeastern Tanzania showed that treatment with CD as well as G6PD deficiency were associated with declines in hemoglobin and increased risk of moderate anemia. However, despite a fairly large sample, the study was underpowered to determine whether the adverse effects of CD are exacerbated among infants with G6PD deficiency, primarily because these deficient G6PD genotypes were uncommon. Combining data from a series of similar, well-conducted primary studies could offer a way to answer this question and optimize the targeting of treatment, which is increasing in importance as systematic treatment with drugs with

hemolytic potential, such as the 8-aminoquinolines, primaquine and tafenoquine, becomes more widespread.

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Table 1.1. Demographic and clinical data at enrolment among G6PD genotyped children with successful G6PD genotyping results

Characteristic	Normal (n = 1324)	Heterozygous (n = 119)	Homo-/hemizygous (n = 114)	P-value
Age (weeks)	9.0[8.0-10.0]	9.0[8.0-10.0]	9.0[9.0-10.0]	0.3
Weight (kg)[a]	5.5[5.1-6.0]	5.5[5.0-5.9]	5.7[5.2-6.2]	0.005[f]
Hemoglobin (g/dL)[b]	10.7[9.9-11.5]	10.7[10.0-11.4]	10.2[9.3-11.3]	0.004[f]
Elevation from sea level (m)[c]	359[315-580]	355[311-565]	329[310-541]	0.002
Distance to nearest clinic (km)[d]	2.2[1.1-4.6]	2.2[1.0 – 4.9]	1.7[0.9-4.2]	0.2
Witnessed bednet coverage	1169(88.3)	104(87.4)	101(88.6)	1.0
Reported ITN coverage	724(54.7)	69(58.0)	67(58.8)	0.6
Rural residence[e]	819(62.0)	78(65.6)	70(61.4)	0.7
Girls	630(47.6)	119(100.0)	40(35.1)	[-][g]
Korogwe	700(52.9)	65(54.6)	79(69.3)	0.003
Treatment				
Placebo	333(25.2)	33(27.7)	29(25.4)	0.8
SP	330(24.9)	34(28.6)	25(21.9)	
CD	333(25.2)	25(21.0)	28(24.6)	
MQ	328(24.8)	27(22.7)	32(28.1)	

Data are median [IQR] or n (%). *CD* chlorproguanil-dapsone, *G6PD* glucose-6-phosphate dehydrogenase, *MQ* mefloquine, *SP* sulfadoxine-pyrimethamine
[a]Data missing for 3 patients in the normal group
[b]Data missing for 17 patients in the normal group, 1 patient in the homo-/hemizygous group, and 1 patient in the heterozygous group
[c] Data missing for 2 observations in the normal group
[d]Data missing for 2 patients in the normal group
[e]Data missing for 2 patients in the normal group
[f]A parametric test was used after assessment of normality
[g]The probability of a heterozygote being female is 1; the event is certain to occur and cannot be explained by chance

Table 1.2. Demographic and clinical data at enrolment among children from entire cohort

Characteristic	Children with G6PD PCR results (n = 1557)	Children without PCR results (n = 855)	P-value
Age (weeks)	9.0[8.0-10.0]	9.0[8.0-10.0]	0.7
Weight (kg)[a]	5.5[5.1-6.0]	5.5[5.2-6.1]	0.6
Hemoglobin (g/dL)[b]	10.6[9.9-11.4]	10.7[10.0-11.5]	0.4[f]
Elevation from sea level (m)[c]	354[314-573]	366[315-580]	0.2
Distance to nearest clinic (km)[d]	2.1[1.1-4.6]	1.8[1.0-3.8]	0.002
Witnessed bednet coverage	1374(88.3)	781(91.4)	0.02
Reported ITN coverage	860(55.2)	486(56.8)	0.4
Rural residence[e]	967(62.1)	498(58.3)	0.08
Girls	789(50.7)	381(44.6)	0.004
Korogwe	844(54.2)	432(50.5)	0.08
Treatment			
Placebo	395(25.4)	209(24.4)	1.0
SP	389(25.0)	213(24.9)	
CD	386(24.8)	216(25.3)	
MQ	387(24.9)	217(25.4)	

Data are median [IQR] or n (%). *SP* sulfadoxine-pyrimethamine, *CD* chlorproguanil-dapsone, *G6PD* glucose-6-phosphate dehydrogenase, *MQ* mefloquine, *PCR* polymerase chain reaction

[a]Data missing for 2 patients with PCR results and 1 patient without PCR results

[b]Data missing from 19 patients with PCR results and 11 patients without PCR results

[c]Data missing for 2 patients with PCR results and 7 patients without PCR results

[d]Data missing for 2 patient with PCR results and 7 patients without PCR results

[e]Data missing from 2 patients with PCR results and 4 without PCR results

[f]A parametric test was used after assessment of normality

Table 1.3. Adjusted treatment effects on changes in hemoglobin 7, 14, and 28 days after an IPTi dose

	Adjusted between-group differences (g/dL) [95%CI]								
	7 days after an IPTi dose			*14 days after an IPTi dose*			*28 days after an IPTi dose*		
Treatment	**Estimate**	**P-value[a]**	**Overall p-value[b]**	**Estimate**	**P-value [a]**	**Overall p-value[b]**	**Estimate**	**P-value[a]**	**Overall p-value[b]**
Placebo	Ref		0.01	Ref		<0.001	Ref		0.07
SP	0.00 [-0.34 to 0.34]	1.0		0.06 [-0.22 to 0.34]	0.7		-0.07 [-0.28 to 0.13]	0.5	
MQ	0.10 [-0.26 to 0.45]	0.6		-0.03 [-0.31 to 0.26]	0.8		0.08 [-0.13 to 0.29]	0.5	
CD	-0.45 [-0.80 to -0.09]	0.01		-0.50 [-0.79 to 0.22]	0.001		-0.19 [-0.40 to 0.01]	0.07	

Adjusted for genotype, dose number, sex, site, weight (kg) and elevation (m). *CD* chlorproguanil-dapsone, *CI* confidence interval, *IPTi* Intermittent Preventive Treatment in infants, *MQ* mefloquine, *SP* sulfadoxine-pyrimethamine

[a]P-values for adjusted treatment effects compared to chlorproguanil-dapsone

[b]P-values for overall adjusted treatment effects

Table 1.4. Adjusted genotype effects on changes in hemoglobin up to 7, 14, and 28 days after an IPTi dose among treated infants

	Adjusted between-group difference (g/dL) [95% CI]								
	7 days after an IPTi dose			*14 days after an IPTi dose*			*28 days after an IPTi dose*		
Hemolysis endpoint	**N**	**Estimate**	**P-value**	**N[Nobs]**	**Estimate**	**P-value**	**N[Nobs]**	**Estimate**	**P-value**
Absolute declines[a]									
Normal	281	Ref		409[450]	Ref		706[913]	Ref	
Heterozygous	29	-0.08 [-0.6 to 0.5]	0.8	39[43]	-0.09 [-0.6 to 0.4]	0.7	70[87]	-0.2 [-0.5 to 0.2]	0.3
Homo-/hemizygous	24	-0.6 [-1.1 to 0.003]	0.05	34[39]	-0.4 [-0.8 to 0.1]	0.1	62[79]	-0.3 [-0.6 to 0.1]	0.1
	Adjusted odds ratio [95% CI]								
	7 days after an IPTi dose			*14 days after an IPTi dose*			*28 days after an IPTi dose*		
	N	**Estimate**	**P-value**	**N[Nobs]**	**Estimate**	**P-value**	**N[Nobs]**	**Estimate**	**P-value**
Declines to less than 8 g/dL[b]									
Normal	281	Ref		451[530]	Ref		769[1136]	Ref	
Heterozygous[c]	29	-	-	40[49]	0.9 [0.2 to 3.7]	0.8	74[101]	0.6 [0.2 to 2.1]	0.5
Homo-/hemizygous	24	6.7 [1.7 to 27.0]	0.01	35[41]	2.9 [1.03 to 8.4]	0.04	66[93]	1.3 [0.5 to 3.2]	0.6

Analyses were restricted to children in the active arms on the assumption that adverse effects of G6PD deficiency are only observed in infants receiving active treatment.
N[Nobs] = number of unique infants[number of observations]. *CI* confidence interval, *IPTi* Intermittent Preventive Treatment in infants
[a]Adjusted for intervention arm, dose number, sex, site, weight (kg), and elevation (m).
[b]Adjusted for sex and elevation (m) using inverse probability to treatment weights.
[c]All observations were dropped due to no events.

Table 1.5. Adjusted treatment effects on changes in hemoglobin 7, 14, and 28 days after an IPTi dose, by G6PD genotype

		Adjusted between-group differences (g/dL) [95%CI]								
		7 days after an IPTi dose			*14 days after an IPTi dose*			*28 days after an IPTi dose*		
Genotype	**Treatment**	**Estimate**	**P-value[a]**	**P-value, test for interaction[b]**	**Estimate**	**P-value[a]**	**P-value, test for interaction[b]**	**Estimate**	**P-value[a]**	**P-value, test for interaction[b]**
Normal	Placebo	Ref		0.9(0.8)	Ref		0.5(0.2)	Ref		0.7(0.4)
	SP	-0.03 [-0.40 to 0.35]	0.9		0.09 [-0.22 to 0.40]	0.6		-0.03 [-0.25 to 0.20]	0.8	
	MQ	0.02 [-0.37 to 0.41]	0.9		-0.08 [-0.39 to 0.23]	0.6		0.07 [-0.16 to 0.30]	0.5	
	CD	-0.44 [-0.82 to -0.06]	0.02		-0.48 [-0.79 to -0.18]	0.002		-0.18 [-0.40 to 0.05]	0.1	
Heterozygous	Placebo	Ref			Ref			Ref		
	SP	0.38 [-0.68 to 1.44]	0.5		0.39 [-0.49 to 1.27]	0.4		-0.05 [-0.70 to 0.60]	0.9	
	MQ	0.48 [-0.74 to 1.70]	0.4		0.70 [-0.34 to 1.74]	0.2		0.07 [-0.66 to 0.79]	0.9	
	CD	-0.40 [-1.68 to 0.88]	0.5		-0.19 [-1.20 to 0.83]	0.7		-0.12 [-0.85 to 0.60]	0.7	
Homo-/hemizygous	Placebo	Ref			Ref			Ref		
	SP	-0.40 [-2.07 to 1.27]	0.6		-0.89 [-2.07 to 0.29]	0.1		-0.69 [-1.51 to 0.13]	0.1	
	MQ	0.41 [-1.06 to 1.87]	0.6		-0.34 [-1.51 to 0.83]	0.6		0.15 [-0.65 to 0.95]	0.7	
	CD	-0.65 [-2.10 to 0.80]	0.4		-1.31 [-2.43 to -0.19]	0.02		-0.49 [-1.25 to 0.28]	0.2	

Adjusted for dose number, sex, site, weight (kg) and elevation (m). *CD* chlorproguanil-dapsone, *CI* confidence interval, *G6PD* glucose-6-phosphate dehydrogenase, *IPTi* Intermittent Preventive Treatment in infants, *MQ* mefloquine, *SP* sulfadoxine-pyrimethamine

[a]P-values for adjusted treatment effects by G6PD genotype compared to chlorproguanil-dapsone

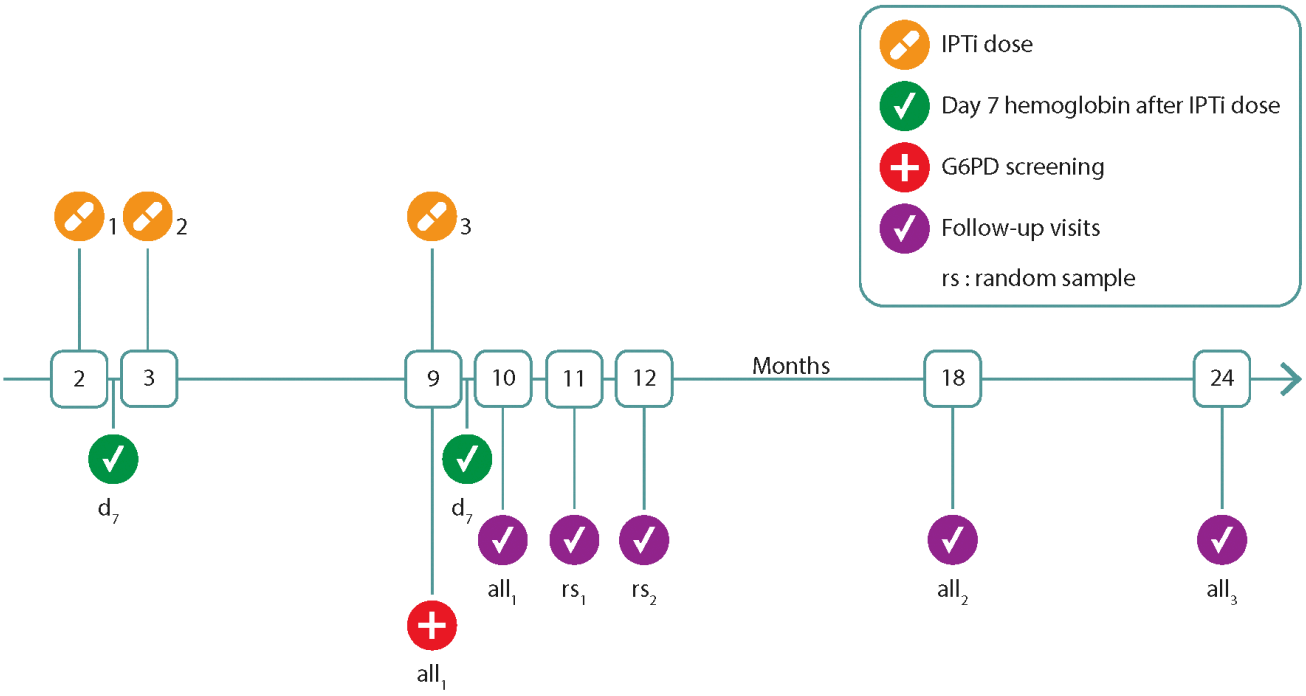
[b]P-values for overall interaction in stratified analyses(p-values for interaction of chlorproguanil-dapsone vs. placebo and homo-hemizygous vs. normal)

Table 1.6. Comparison of secondary outcomes of IPTi according to G6PD genotype using multivariable Poisson regression

	Number of events	PYAR	Unadjusted Incidence per 1000 infant-months [95% CI]	Marginal incidence per 1000 infant-months [95% CI]	Conditional IRR	P-value
All episodes of malaria						
Normal	598	27.8	21.5[19.9-23.4]	23.6[20.5-26.7]	Ref	
Heterozygous	67	2.5	26.5[20.8-33.6]	31.9[18.2-45.7]	1.4[0.9-2.1]	0.2
Homo-/hemizygous	42	2.4	17.6[13.0-23.8]	14.6[6.6-22.5]	0.6[0.4-1.1]	0.09
All-cause hospital admissions						
Normal	793	27.8	28.5[26.6-30.6]	28.7[26.3-31.1]	Ref	
Heterozygous	77	2.5	30.3[24.2-37.9]	35.4[25.9-44.9]	1.2[0.9-1.6]	0.2
Homo-/hemizygous	84	2.4	35.2[28.4-43.6]	33.7[24.5-42.8]	1.2[0.9-1.6]	0.3
Blood transfusions						
Normal	32	27.8	1.2[0.8-1.6]	1.2[0.8-1.7]	Ref	
Heterozygous	2	2.5	0.8[0.2-3.1]	0.9[-0.4-2.3]	0.7[0.2-3.4]	0.7
Homo-/hemizygous	4	2.4	1.7[0.6-4.5]	1.4[-0.2-3.0]	1.1[0.3-3.7]	0.9
All-cause deaths						
Normal	5	27.8	0.2[0.07-0.4]	0.2[0.02-0.4]	Ref	
Heterozygous	0	2.5	0 [-]	0 [-]	0 [-]	[-]
Homo-/hemizygous	2	2.4	0.8[0.2-3.3]	0.7[-0.3-1.6]	3.4[0.6-19.3]	0.2

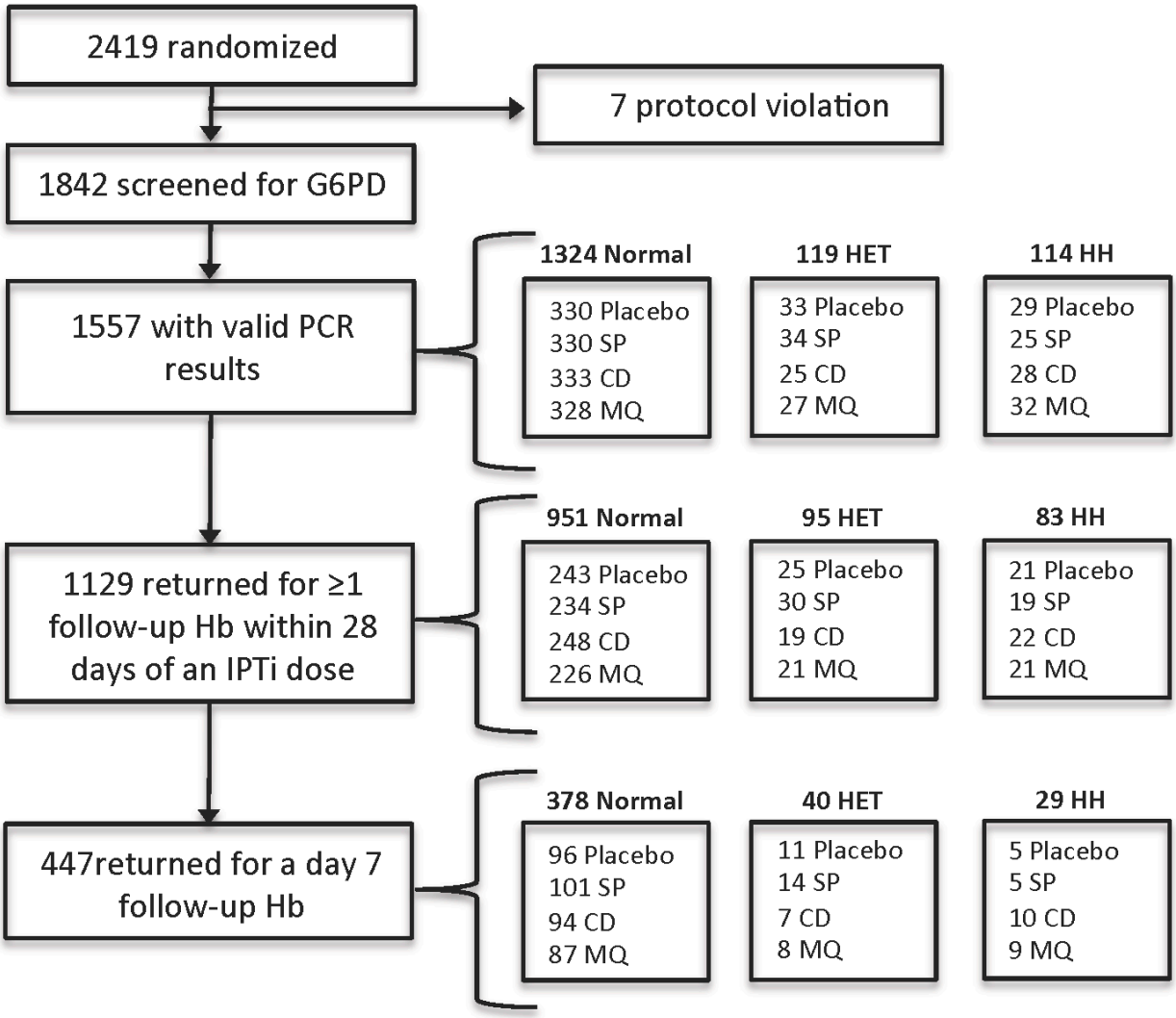
Adjusted for intervention, sex, site and elevation. *CI* confidence interval, *G6PD* glucose-6-phosphate dehydrogenase, *IPTi* Intermittent Preventive Treatment in infants, *IRR* incidence rate ratio, *PYAR* person-year at risk per 1000

Figure 1.1. Trial timeline for participants of the IPTi trial in Tanzania



G6PD glucose-6-phosphate dehydrogenase, *IPTi* Intermittent Preventive Treatment in infants

Figure 1.2. Trial profile for the entire cohort (both sites combined)



CD chlorproguanil-dapsone, *G6PD* glucose-6-phosphate dehydrogenase, *HET* heterozygous, *Hb* hemoglobin, *HH* homo-/hemizygous, *IPTi* Intermittent Preventive Treatment in infants, *MQ* mefloquine, *PCR* polymerase chain reaction, *SP* sulfadoxine-pyrimethamine

Chapter 2: Development of a pharmacovigilance safety monitoring tool for the rollout of single low-dose primaquine and artemether-lumefantrine to treat Plasmodium falciparum infections in Swaziland: a pilot study

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Abstract

Introduction: Countries remain reluctant to adopt the 2012 WHO policy on single low-dose (0.25 mg/kg) primaquine (SLD PQ) for *Plasmodium falciparum* transmission blocking due to concerns over drug-related hemolysis risk, among glucose-6-phosphate dehydrogenase-deficient (G6PDd) people, without evidence demonstrating that it can be safely deployed in their setting. Pharmacovigilance methods provide a systematic way of collecting safety data and supporting the rollout of SLD PQ.

Methods: We developed and piloted the Primaquine Roll Out Monitoring Pharmacovigilance Tool (PROMPT), comprised of: (1) a standardized form to support the surveillance of possible adverse events since SLD PQ treatment; (2) a patient information card to enhance awareness of known side effects of SLD PQ use; and (3) a database compiling recorded information. Data on patient characteristics, malaria diagnosis and treatment are collected. Blood samples are taken to measure hemoglobin (Hb) and test for G6PD deficiency. Active follow-up includes repeat Hb testing and adverse event monitoring on or near day 7. A 13-month prospective pilot study in two hospital facilities in Swaziland alongside the introduction of SLD PQ generated preliminary evidence on the feasibility and acceptability of PROMPT.

Results: PROMPT was well received by nurses as a simple, pragmatic approach to active surveillance of SLD PQ safety data. Of the 102 patients enrolled and administered SLD PQ, none were G6PDd. 93 (91.2%) returned on or near day 7 for follow-up. Four (4.6%) patients had falls in Hb≥25% from baseline, none of whom presented with signs or symptoms of anemia. No patient's Hb fell below 7 g/dL and none required a blood transfusion. Of the 11 (11%) patients who reported an event over the study period, three were considered serious and comprised of two

deaths and one hospitalization; none were causally related to SLD PQ. Four non-serious adverse events were considered definitely, probably, or possibly related to SLD PQ.

Conclusion: Improved pharmacovigilance to monitor and promote the safety of the WHO recommendation is needed. The successful application of PROMPT demonstrates its potential as an important tool to rapidly generate locally acquired safety data and support pharmacovigilance in resource-limited settings.

Introduction

As malaria programs move towards elimination, stopping onward transmission from detected cases becomes increasingly important. The most commonly used antimalarials are artemisinin derivatives, which destroy early developing *Plasmodium falciparum* (*P. falciparum*) gametocytes (stages I to III) and all blood stages of human malaria species [1]. However, most symptomatic cases present with measurable and transmissible levels of mature gametocytes (stages IV and V), as determined by Pfs25 RNA-based quantitative nucleic acid sequence-based amplification (Pfs25 QT-NASBA), which can infect mosquitoes after artemisinin-based combination therapy (ACT) [2-4]. The 8-aminoquinoline compounds are the only class of drugs effective against mature *P. falciparum* gametocytes. Primaquine (PQ) is currently the only licensed antimalarial in this class of drugs.

Since its approval by the FDA in 1952, PQ has been used as 14-day anti-relapse treatment against *P. vivax* and *P. ovale* species [5, 6]. In addition, in combination with an effective ACT, a single dose of PQ can prevent onward transmission of *P. falciparum* [1, 7, 8]. However, PQ can cause hemolysis in people who are deficient in glucose-6-phosphate dehydrogenase (G6PD) [9, 10]. The extent of an individual's susceptibility to and severity of PQ-induced hemolysis depends on dose and duration of exposure and degree of G6PD deficiency [9, 11-13].

Previously, the World Health Organization (WHO) recommended the addition of a single 0.75 mg/kg dose of PQ to ACTs for the clearance of *P. falciparum* gametocytes. However, low uptake of this recommendation and concerns over the potential for hemolytic toxicity in G6PD-deficient

(G6PDd) individuals prompted the issue of a new WHO policy, recommending the use of a lower 0.25 mg/kg dose of PQ in areas threatened by artemisinin resistance and in settings targeting malaria elimination. This 0.25 mg/kg dose should be given to all patients with parasitologically-confirmed *P. falciparum* malaria together with an ACT, except for pregnant women and infants, without G6PD testing [14]. Despite evidence suggesting that the risks for hemolysis are low at the 0.25 mg/kg PQ dose, it is a common concern that PQ, even at low doses, may result in severe hemolysis in G6PDd individuals [15]. There remains limited data to suggest the safety of this recommendation and countries have been reluctant to implement the policy without more evidence demonstrating that it can be safely deployed in their setting.

The use of standardized, prospective pharmacovigilance methods for PQ provides an opportunity to generate data that can be used to confirm or refute safety concerns. To date, pharmacovigilance on PQ is weak and relies predominately on passive reporting. In most countries in sub-Saharan Africa, passive reporting systems, which lack an appropriate denominator of persons exposed to treatment, are limited or non-existent [16]. From a total of 1,429 reports (4,560 events) submitted to the Uppsala Monitoring Center, the WHO's international drug monitoring program, in which PQ was suspected to be a causative or interacting factor for the event, 89% were reported from Thailand and only one report (3 events) came from Africa [17]. Monitoring the use of PQ offers a mechanism to more accurately quantify the risk of hemolysis associated with PQ administration, especially in patients who are G6PDd. Unlike other antimalarial drug regimens where adverse drug reactions may be unknown or poorly defined, requiring weeks of follow-up, the objectives for monitoring PQ use are

straightforward; signs of hemolytic anemia, our main safety concern, can be more specific (i.e., dark urine), easily measurable (by hemoglobin concentration) and attainable within a week of drug administration through active surveillance methods.

To support the safe rollout of SLD PQ, we developed the Primaquine Roll Out Monitoring Pharmacovigilance Tool (PROMPT) in collaboration with the National Malaria Control Programme (NMCP) in Swaziland. The objective of this study was to assess the feasibility and perceived acceptability of using a simple pharmacovigilance tool to collect standardized safety data in those prescribed SLD PQ therapy for the treatment of *P. falciparum* malaria.

Methods

This project involved two phases: (1) a tool development phase during which the components of PROMPT were developed and (2) a pilot phase, when intended end-users in Swaziland participated in testing PROMPT alongside the introduction of SLD PQ.

Tool development

Tools

Through an interactive process, involving a literature review and discussions with national and international experts, we developed a tool comprised of three parts: (1) a standardized form (on either a paper or electronic platform) collecting a minimum set of essential data elements to support the surveillance of possible adverse events since SLD PQ treatment; (2) a patient

information card to enhance awareness of known side effects of SLD PQ use; and (3) a database compiling recorded information.

Data collection form

Data collection begins once the prescriber has decided to treat confirmed, uncomplicated malaria cases with PQ (day 0), in addition to the standard blood schizonticide recommended by that particular country. The prescriber collects basic socio-demographic data (age, sex, and weight), contact information, patient and family history data (e.g., severe anemia, G6PD deficiency, blood transfusions), malaria diagnosis information, and drug dosage and frequency data.

For those involved with active surveillance, a blood sample is collected to measure hemoglobin or hematocrit and to screen for G6PD deficiency. Patients are asked to return for a scheduled follow-up visit on or near day 7 because drug-induced hemolysis and jaundice are clinically detectable within the first 7 days [9]. At this time, a repeat hemoglobin or hematocrit measure is taken to gauge the degree and speed of decline from the baseline value prior to starting SLD PQ. Information is also gathered about possible adverse events and serious adverse events, defined according to International Conference of Harmonization guidelines [18]. The form comprises of a short list of possible events (e.g., nausea, vomiting, dark urine, diarrhea, etc.) as well as space for recording any other signs or symptoms external to the list provided. Specifically, data on all new onset or worsening signs or symptoms, since SLD PQ treatment (i.e., treatment emergent adverse events), regardless of causality, are collected. Patients involved with active monitoring who miss their day 7 assessments are phoned within a week to ask about adverse events. All

adverse events reported as part of passive or active follow-up, record the following information: description of the adverse event, date of onset, severity grade, actions taken, its relatedness to PQ and/or the blood schizonticide used, date resolved, outcome of AE, and other drugs (including traditional medicines) taken along with PQ at the time of treatment and in the two-week period prior to the onset of the event. Severity grades follow the WHO Toxicity Grading Scale for Determining the Severity of Adverse Events [19]. In addition, for those presenting with suspected PQ-induced hemolysis, urine color is recorded using a Hillmen color chart designed to assess the presence of hemoglobinuria (**Figure 2.1**) [20]. A colorimetric level of 5 or above is considered evidence of hemoglobinuria.

Patient information card

At the time of prescribing PQ (day 0), patients are given a patient information card with oral and written instructions on how to identify the signs and symptoms of known side effects of PQ (e.g., gastrointestinal upset, dark urine, backaches) and to monitor the color of their urine (**Figure 2.2**). The card also contains instructions of what to do and a telephone number to call if side effects are experienced. Patients are instructed to immediately return to the clinic should they experience any adverse reactions following PQ treatment.

Database

Data from health facilities are uploaded into a secure country-level database. In settings where electronic data collection is feasible the database can assist with managing follow-up visits and remind study participants and caregivers of follow-up appointments, including producing a

telephone follow-up call list. Anonymized data from end-users can then be fed to a global database where data can be combined and analyzed.

Sample size considerations

Sample size calculations were based on two scenarios for detecting a 25% or greater within-in person reduction in hemoglobin after PQ treatment in G6PD-deficient persons using a one sample paired t-test with a 0.05 level of significance (one-sided). A 25% or greater reduction was regarded as the minimum clinically important threshold that would signify caution for implementing the addition of SLD PQ to ACT for the treatment of *P. falciparum* infections. Under the first scenario, in program settings where G6PD screening is available at the individual level, seven G6PD-deficient individuals would provide 80% power to detect a 25% or greater within-person reduction in hemoglobin, assuming a mean hemoglobin of 10 g/dL before treatment, a standard deviation of 2 g/dL for the within-person change in hemoglobin, and up to 25% loss to follow-up. In the second scenario, in program settings where G6PD screening is unavailable and status is unknown, a program with an estimated 10% G6PD deficiency population prevalence would, under the same assumptions, need to treat a minimum of 70 individuals with PQ (**Appendix, Tables 2.1-2.4**).

Pilot phase

Study area

In response to a request from the Ministry of Health (MoH), PROMPT was first piloted in the Lubombo and Manzini regions of the Kingdom of Swaziland alongside the introduction of SLD

PQ. Good Shepherd Mission Hospital, a rural, regional referral hospital in Lubombo, and Raleigh Fitkin Memorial Hospital, a hospital in Manzini run by the Catholic mission and government were both purposively selected by the NMCP to represent facilities with the highest burden of malaria cases. Lubombo and Manzini reported a total of 266 and 164 malaria cases respectively this past year (March 2014 to April 2015) [22]. Malaria transmission is highest in the lowlands of the Lubombo region and peaks in the rainy season between November and May. *Plasmodium falciparum* is the predominant parasite species accounting for over 99% of malaria cases. One-third of Swaziland's population is at risk of infection, with residents in the Lubombo region near the eastern borders with Mozambique and South Africa at greatest risk.

Pre-piloting

Stakeholder engagement and workflow integration

The pilot was tested following discussions with key stakeholders, including the MoH, an experienced database developer, infectious disease specialists with pharmacovigilance expertise, and local health workers (nurses, laboratory technologists, nurse matrons and doctors) over 6 months. Consultations with the database developer allowed us to build a user-friendly, electronic data collection tool configured to country capacity. Infectious disease and pharmacovigilance specialists offered guidance on how to standardize and define the data elements (and minimum reporting criteria) collected. Health workers reviewed the steps necessary to use PROMPT and increased awareness around its use. We observed each facility and talked with clinic staff to best integrate PROMPT into the existing clinical workflow and capture confirmed malaria cases.

Training for the pilot evaluation

Nurses from both facilities received a two-day training workshop followed by seven days of pre-pilot on-site training. Staff from the NMCP, health facility doctors and nurse matrons were also invited to the group training to enable additional support to trained health workers during study implementation. Half way through study implementation (August 2014) trained health workers received a one-day refresher training. Both trainings focused on illustrating routine study procedures. The health workers were trained in data reporting procedures and in electronic data collection. Each facility received job aids (e.g., flowcharts and figures) to offer cues and direction on key procedures. Instruction on identifying the signs and symptoms of acute hemolytic anemia, the main adverse effect of SLD PQ use, was also given. Training objectives also included opportunities for the study team to complete tool procedures in a pre-testing environment and to solicit feedback from the team on components of PROMPT needing further refinement.

Pilot-testing PROMPT

Recruitment

All patients prescribed standard doses of first-line artemether-lumefantrine (AL) for confirmed, uncomplicated *P. falciparum* malaria over one year of age were eligible to receive SLD PQ. Patients were not deemed suitable for SLD PQ treatment if they had any of the following: evidence of severe malaria [23]; history of allergies to study drugs; acute anemia, defined as hemoglobin <8 g/dL; were pregnant or breastfeeding. Patients selected for inclusion also had to

agree to follow-up phone calls and/or visits and had no intentions of leaving Swaziland for a 14-day follow-up period.

Procedures

Prior to SLD PQ administration, G6PD deficiency screening was performed using the CareStart G6PD deficiency screening kit (Access Bio. Inc., New Jersey, USA, LOT No. GP13E01; GP13C01) and hemoglobin was measured using HemoCue 201+ photometers (HemoCue, Angelholm, Sweden). Patients were then treated with the WHO recommended single dose of PQ (0.25 mg/kg) (Primaquine®, Micro Labs Ltd Hosur, Bangalore, India) using 7.5 mg PQ tablets, according to four weight bands established following discussions with country doctors and senior officials from the NMCP (**Table 2.5**). Primaquine tablets were analyzed by high-performance liquid chromatography to measure the concentration of active pharmaceutical ingredient (API) against a reference standard; tested tablets possessed over 95% API. Patient were given primaquine with the first dose of AL together with food and then directly observed for 30 minutes. If a patient vomited within 30 minutes, treatment was re-administered. Those who vomited a second time were excluded from further SLD PQ dosing.

At the time of prescribing SLD PQ, trained nurses collected data as outlined in the data collection form and provided patients with a patient information card. Patients were scheduled for follow-up visits on or near day 7 and received SMS text messages reminders two days prior to scheduled visits and on day 7. Patients were also encouraged to follow-up at unscheduled times should they feel unwell. Patients were asked general open-ended questions to elicit events

and determine if they had experienced any problems since being treated with SLD PQ at follow-up visits. Responses were probed to solicit further details on reported events. All adverse and serious adverse events reported by participants were examined by hospital physicians and recorded by trained nurses. If medically indicated, patients were closely monitored and appropriate referrals were made according to national protocol. Patients were telephoned within a week of missed visits to collect information on adverse events. Reporting of adverse events followed local ethics and UCSF Institutional Review Board guidelines.

Questionnaire data from each visit were entered into Samsung T211 Galaxy Tab 3 (7.0) 3G (Samsung, Seoul, Korea) tablets running Android OS to enable automated data collection in the health facilities (**Figure 2.3)**. The tablets were synchronized directly to a secure country-level database for real-time review. The data were checked by a co-investigator for inconsistencies and corrected by contacting the study nurses. The database also served to manage follow-up visits and remind subjects and caregivers of follow-up appointments via automated reminders using SMS text messaging. In addition, the database produced a telephone follow-up call list for nurses, accessible directly from the tablets, to track and follow-up with patients who missed their scheduled visit.

Data analysis plan

Outcome measures and definitions

Tool evaluation – feasibility and perceptions of acceptability

To assess feasibility, we measured: (1) the proportion enrolled among those eligible and (2) the proportion of patients completing study procedures, looking closely at day 7 follow-up adherence. We also collected process measures (e.g., visit length, provider attitudes about clinic burden, satisfaction with counseling sessions with patients, etc.), and reactions to and ease or challenges of study procedures, using a semi-structured questionnaire to investigate perceptions related to acceptability among trained nurses. Discussions (one per hospital facility) took about 60 minutes to complete. A moderator collected handwritten or electronic notes.

Safety assessment of single low-dose primaquine

To assess the safety of introducing SLD PQ the hematologic response and occurrence of adverse events in those receiving SLD PQ therapy were investigated. Hemoglobin concentrations (defined in g/dL) were expressed as the absolute and relative change from baseline values (day 0). Anemia was classified according to WHO guidelines [24] (6-59 months Hb<11 g/dL; 5-11 years Hb<11.5 g/dL; 12-14 years Hb <12.0 g/dL; ≥15 years, non-pregnant women Hb <12.0 g/dL; pregnant women Hb <11.0 g/dL; ≥15 years men Hb <13.0 g/dL).

Data Analysis

Descriptive statistics were used to present the demographic characteristics of the study population and their baseline clinical and biological characteristics. Data were presented in terms of mean and standard deviations or median and interquartile range for continuous variables and percentages for categorical variables. All patients with complete hemoglobin values who returned for follow-up within 10 days after treatment were included in our assessments of hematological changes. Categorical data were compared using the chi-square test or a Fisher's exact test, when applicable. Analyses of reported adverse events were based on descriptive summaries of frequency, severity, and relatedness to SLD PQ. Quantitative data were analyzed using STATA 13.0 (© StataCorp, College Station, TX, USA). Qualitative data from interview notes were summarized after familiarization of the data using an iterative approach. Emergent themes from the data were identified based on frequency of appearance and summarized.

Ethical considerations

The study was approved by review committees at the Swaziland MoH (reference MH/599C/ FWA 000 15267) and University of California, San Francisco (IRB no. 13-11626), and granted non-engaged status by the United States Centers for Disease Control and Prevention. Participants or a parent or guardian for children less than 7 years of age gave written, informed consent; children between the ages of 7 and 17 years gave assent following national guidelines.

Results

Feasibility

Over a 13-month pilot period (March 2014 to April 2015), 166 confirmed, uncomplicated malaria cases from two hospital facilities in Lubombo (77) and Manzini (89) regions were reported through Swaziland's Health Management Information System and Immediate Disease Notification System. Of those reported, 112/166 (67.5%) patients were seen by four nurses trained in the use of PROMPT and screened for inclusion. Ten patients were excluded: one tested positive for pregnancy, seven had hemoglobin concentrations less than 8 g/dL, and two declined to participate. A total of 102 patients (59 from Lubombo and 43 from Manzini) were enrolled and administered SLD PQ and 98/102 (96.0.%) were followed up with 93/102 (91.2%) successfully completing all follow-up procedures. This included obtaining a repeat hemoglobin measurement and collecting data on all adverse events experienced since taking SLD PQ (**Figure 2.4**). Four patients (3.9%) were lost to follow-up with no data collected after taking SLD PQ at the health facility. Demographic characteristics did not differ significantly between those who were lost to follow-up and those who were retained, except for foreign nationality. Those lost to follow-up were more likely to be of foreign nationality.

Patients were scheduled to return on or near day 7 for a follow-up visit; however, the timing of scheduled post-treatment follow-up visits varied. Patients returned for a follow-up visit as early as 4 days post-treatment to as late as 23 days post-treatment. Nonetheless, adherence to visits on or near day 7 was good, with 66/102 (64.7%, 95% CI 54.6 to 73.9%) returning promptly on day

7 and 87/102 (85.3%, 95% CI 76.9 to 91.5%) returning within 10 days (range: 4-10) post-treatment for a follow-up visit.

Perceptions of acceptability

All four trained nurses participated in informal feedback discussions to offer initial perceptions of PROMPT's acceptability and to suggest ways to improve upon the existing tool. The standardized data collection form using handheld electronic devices was popular. Nurses noted several benefits of using an electronic data entry system over paper for monitoring the safety of SLD PQ. All nurses agreed that this included the time saved on data entry and half of nurses felt that the electronic devices kept patient records confidential and accessible only by trained nurses and staff. There was also general agreement amongst the nurses that the average staff time required for screening and enrolment (27.5 minutes; range: 20-45 minutes), and follow-up visits (12.5 minutes; range: 10-15 minutes) per patient was acceptable. This period of time excludes the time taken for retrieving missing or additional information.

These discussions also revealed their views on counseling sessions with patients on G6PD deficiency and known side effects associated with SLD PQ use. All nurses agreed that the patient information cards were a useful component of PROMPT that facilitated the performance of study procedures and conveyed key messages. However, nurses agreed that the language used to communicate G6PD deficiency and adverse effects of SLD PQ use to patients was challenging to develop. In addition, nurses highlighted challenges with the adverse event reporting process, including challenges with identifying cases as relevant to report. Nurses more frequently

reported on events that were either severe or life-threatening in nature or expected signs and symptoms of SLD PQ use (i.e., dark-colored urine, anemia, jaundice).

The main challenge described when using an electronic platform was server connectivity; while nurses were still able to enter information and store data while the server was offline, data could not be synchronized and uploaded to the country-level database. This limited capacity to monitor and analyze data in real-time and resulted at times in delayed or duplicated SMS text messages being sent to patients.

Baseline characteristics of participants enrolled in PROMPT

In our cohort of enrolled *P. falciparum* malaria patients treated with SLD PQ who completed follow-up (n = 93), the mean age was 25 years, ranging from 13 months to 68 years (**Table 2.6)**. Overall, 17.2% of participants were under five years of age. Anemia was frequent before treatment: 26 patients (28.0%, 95% CI: 19.1 to 38.2%) were mildly anemic and 36 (38.7%, 95% CI: 28.8 to 49.4%) were moderately anemic. The majority of patients less than 15 years (n = 32) were anemic at baseline (93.8%, 95% CI: 79.2 to 99.2%). None of the enrolled patients were G6PDd at baseline (95% upper confidence limit [UCL]: 3.6%). One child screened for inclusion was G6PDd; however, the child was excluded and not given SLD PQ based on a hemoglobin concentration less than 8 g/dL. The baseline characteristics of the study populations were similar between the two hospital facilities (all $p > 0.05$) [data not shown].

Safety assessment of single low-dose primaquine

Hemolysis

Out of the 93 patients who successfully completed all follow-up procedures, 87 patients returned for at least one follow-up hemoglobin visit within 10 days of receiving SLD PQ with 58 (66.7%) experiencing declines in hemoglobin concentration post-treatment. The mean absolute reduction in hemoglobin concentration within 10 days, relative to day 0 was 0.6 g/dL (95% CI: -0.9 to -0.3 g/dL; p = 0.0008) (**Table 2.7**). The relative mean decrease in hemoglobin was 3.7% (95% CI: -6.4 to -1.0; p = 0.007). Few patients (11/87, 12.6%) dropped 2 g/dL or more relative to day 0 (range: 2.1 – 5.9 g/dL). The maximum absolute decrease in hemoglobin concentration was 5.9 g/dL though this individual patient started with a high hemoglobin concentration of 20.1 g/dL that was likely due to dehydration at baseline. Four patients (4.6%, 95% CI: 1.3 to 11.4%), all male adults with a starting hemoglobin concentration greater than 13 g/dL, experienced fractional drops in hemoglobin of 25% or greater from baseline; the median absolute and relative drops in hemoglobin among these four patients were 4.9 g/dL and 30.5%, respectively. Two patients (2.3%, 95% CI: 0.3 to 8.1%) dropped below 8 g/dL; both were moderately anemic at baseline. No patient who fell below 8 g/dL or experienced falls in hemoglobin of 25% or greater post-treatment was clinically symptomatic with signs of anemia or dark urine (macroscopic hemoglobinuria or Hillmen urine score ≥5). None of the patients dropped below 7 g/dL (95% UCL: 4.2%).

Description of reported adverse events

In general, SLD PQ was well tolerated by patients. Out of all the patients who received SLD PQ and returned for follow-up, 89% (87/98) of patients did not report any adverse event since receiving treatment. Of the 11 (11%) patients who reported an event, three were considered serious using ICH guidelines and comprised of two deaths and one hospitalization; none were classified as related to SLD PQ (**Table 2.8**). In all three serious cases, the underlying clinical diagnosis was unclear and reported as a multitude of symptoms. One death was attributed to the patient's pre-existing immune-compromised condition. This patient, presenting with fever, chills, diarrhea, and vomiting, was diagnosed with uncomplicated *P. falciparum* malaria, testing positive by rapid diagnostic test (RDT) and microscopy. He was admitted to hospital as a precaution to monitor his diarrhea, and treated with AL plus SLD PQ, and panadeine. The patient's diarrhea continued the next day. He was confused. Diclofenac was added to his treatment. Two days after symptom onset, the patient died. Another patient presenting with complaints of weakness, jaundice, and coughing, was prescribed AL based on signs and symptoms, despite testing negative for malaria by RDT and microscopy. When the patient returned to the clinic to pick up his AL treatment (two days after event onset), the patient was incorrectly enrolled into the study and treated with AL plus SLD PQ. Despite antimalarial therapy, the patient's condition continued to deteriorate. Upon active follow-up by telephone, the study nurses learned of the patient's death. A verbal autopsy suggested that the patient's health had deteriorated to the point where the patient was unable to pass urine. He died within 10 days of initial presentation. One patient, with complaints of diarrhea, vomiting, headache, and

dizziness, was admitted and hospitalized one day after treatment with SLD PQ but recovered within 48 hours of onset.

None of the patients vomited in the 30 minutes following drug administration. The most frequently reported events in all 11 patients were dark urine (36%), vomiting (27%), fever (18%), and diarrhea (18%). Four non-serious adverse events were considered definitely, probably or possibly related to SLD PQ. Of the non-serious adverse events, all recovered except for one whose outcome remains unknown despite multiple telephone attempts at follow-up. This patient, following referral to one of the study facilities, reported malaise and worsening of symptoms, after receiving the recommended higher dose of PQ (30 mg), following a diagnosis of vivax malaria confirmed by microscopy.

Dissemination and impact

Key findings from the safety assessment were disseminated and recommendations were made (**Figure 2.5**) to key stakeholders during the NMCP's Swaziland Malaria Elimination Advisory Group General Committee meeting in January 2016. Results and recommendations on safety, dosing, and the management of adverse events were shared to prepare for countrywide adoption of the WHO policy. Regional trainings launching the new diagnosis and treatment guidelines followed. As of December 2015, SLD PQ has been available at all three levels of Swaziland's health system. This includes both public and private clinics, health centers, and referral hospitals,

making Swaziland the first country in sub-Saharan Africa to roll out SLD PQ for malaria elimination.

Discussion

This study is, to our knowledge, the first to demonstrate the feasibility and acceptability of performing active safety monitoring using a simple pharmacovigilance tool in those prescribed SLD PQ therapy for the treatment of *P. falciparum* malaria. Results suggest that PROMPT, achieved its objective, and successfully yielded standardized safety data, on the range and frequency of adverse events, including hematologic changes, experienced after SLD PQ therapy. These findings are supported by the following observations.

Firstly, PROMPT promoted and facilitated the capture of treatment emergent adverse events after initiation of SLD PQ therapy with a known denominator of the number of people exposed to the drug (n = 102). Calculating adverse drug reaction frequencies or incidence rates to examine risk for particular treatments is impossible with current systems that rely on passive spontaneous reporting [16]. Importantly, patients with signs or symptoms suggestive of PQ-induced hemolysis or other serious event were, for the most part, promptly identified and monitored within a week of drug administration. Despite two deaths, unrelated to the intake of SLD PQ, and one patient who was lost to follow-up after receiving a higher dose of PQ (30 mg) for vivax malaria, all *P. falciparum* patients who presented with an adverse event recovered. Moreover, repeat hematologic testing ensured that clinically important drops in hemoglobin (i.e., fractional drops in hemoglobin ≥25% from baseline or hemoglobin concentrations <7 g/dL post-

treatment) were detected. No patient's hemoglobin fell below 7 g/dL and none required a blood transfusion.

Second, we show high adherence (>90%) to visits on or near day 7 and demonstrate the acceptability of our approach among trained nurses. The use of simple approaches –real-time electronic data collection, SMS text reminders, follow-up phone calls, and patient information cards - encouraged patients to return to clinics and helped ensure the timely reporting of safety data to a central repository. This is in line with prior research illustrating how sensitization and training of health workers and consumers can be strong incentives for reporting and adherence to scheduled visits [25]. Furthermore, there is growing evidence on the impacts of mobile health (mHealth) interventions on health outcomes in low- and middle-income countries [26]. Pharmacovigilance and post-marketing surveillance of the safety and quality of medicines has been proposed as one of six areas within malaria control where the use of text messaging technology could improve routine delivery of health services in Africa [27]. Some settings may however not have the resources to support pharmacovigilance strategies that rely on advanced technologies, active follow-up, and the literacy of participants. Alternative strategies that are feasible and appropriate using paper forms with less-intense monitoring and patient information cards formatted for illiterate individuals using pictorial methods should be adopted.

This study did however face challenges that are inherent to establishing pharmacovigilance systems with reliable reporting processes [16]. Underreporting, likely due to a lack of understanding as to what constitutes an adverse event, appears to have been higher than

anticipated. Results reveal that patients and providers may have selectively reported adverse events potentially associated with SLD PQ and suggestive of hemolysis (e.g., dark urine and paleness/jaundice). In addition, there was no baseline assessment of symptoms in order to reduce the workload for healthcare providers. Thus, while procedures specified that an abnormality present at baseline should not be recorded unless it increased in severity, doing so relied on patients recalling exactly when symptoms started at least one week prior. Recall is usually better in the short-term [28]; however, certain events may have been dismissed, especially those less severe, unexpected or thought to be a clinical manifestation of malaria, despite having an onset post-SLD PQ administration and which should, by definition, have been reported. Methods for eliciting information could have also varied and influenced the detection of adverse events. Future research should explore factors that influence patterns of reporting to better accommodate these challenges.

There are limitations to the study also worth noting. Sampling was restricted to two hospital facilities in a low transmission African setting in relatively healthy participants with hemoglobin concentrations of 8 g/dL or greater, and no enrolled patient was G6PDd, which limits the generalizability of our findings. Furthermore, by not enrolling any G6PDd individuals, we were unable to achieve the targeted G6PDd sample size. This could be due to a low prevalence of G6PD deficiency in this malaria-infected population or due to a multitude of factors that affect the performance of G6PD tests [29]. False normal readings due to biological conditions, including recent hemolytic events, that leave patients with a high proportion of young red blood cells with high G6PD enzyme function and normal phenotype and/or borderline colorimetric

results that produce subjective and variable readings could explain why all patients in this population tested G6PD normal. Confirmatory testing would be required to avoid misclassification of results and examine this in the future. The qualitative rapid test can only reliably identify completely deficient phenotypes.

This study also relied on the views of four trained nurses to produce opinions on tool acceptability thus restricting the breadth of opinions and experiences captured. Nonetheless, perceptions did demonstrate promise that PROMPT would be well received on a larger scale. Additional interviews among end users would allow us to capture a broader range of views in the future.

Expanding PROMPT's use beyond Swaziland presents an opportunity to address these limitations and improve the systematic collection of safety data where data are needed. Data that include G6PDd participants are currently being collected from three additional countries across Asia and Africa (Bangladesh, Senegal, and Tanzania) with differing levels of risk associated with PQ therapy. As data from these sites increases in size, it will be possible to pool individual patient data, directly compare, and determine the risks of PQ therapy with ever-greater accuracy. These new insights could serve to inform the highest safe dose of SLD PQ in G6PDd individuals that is critical to defining the therapeutic dose range and guiding appropriate dosing regimens [30].

PROMPT could also extend to support pharmacovigilance activities under diverse contexts and select populations. For example, subjects could be followed up at additional time points (e.g., days 14 and 28) to assess outcomes after PQ treatment of longer duration for *P. vivax* or *P. ovale*. Women of childbearing age inadvertently exposed to PQ in very early, unsuspected pregnancy could also be identified at around 3 months and prospectively followed at regular intervals (e.g., every 3 months or so until several weeks post-natal) to assess pregnancy and birth outcomes. Data on the use of PQ in pregnancy remains limited and further study on the safety of PQ in pregnancy are needed. Finally, these tools could be expanded beyond PQ to other drugs for a variety of other diseases.

As access to SLD PQ increases, a larger evidence-base to support the safety of the latest WHO recommendation will be needed. Several countries, in the Asia Pacific, including Bhutan, Indonesia, Malaysia, Myanmar, Thailand, the Philippines, and Vietnam include SLD PQ in their *P. falciparum* treatment guidelines, albeit in different doses and schedules. And demand for its use is growing rapidly in parts of Africa with Botswana, Ethiopia, Namibia, South Africa, Swaziland, Zanzibar, and Zimbabwe who have written SLD PQ into treatment guidelines for falciparum malaria [31]. Although evidence suggests that the risk of hemolytic toxicity in G6PDd individuals treated with a 0.25 mg/kg single dose of PQ for falciparum malaria is low [15], no medicine is absolutely free from risk. Obtaining safety data on the use of SLD PQ in settings that extend beyond small and selected populations that are characteristic of randomized controlled clinical trials to reflect real-life usage in programmatic settings will help reduce uncertainties that surround the safety of SLD PQ. PROMPT holds potential for becoming a

practical tool for generating locally acquired safety data and supporting pharmacovigilance in resource-limited settings, however, wider adoption is needed to further evaluate and refine its use.

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Appendix: Sample size guide

To determine what constitutes a "sufficient" sample size for the proposed pilot, a number of considerations must be taken into account.

For settings where the patient's G6PD status is known prior to treatment with primaquine:

Table 2.1. Number of persons needed to treat to have sufficient power to detect a given within-person percent reduction in hemoglobin

Minimum percent reduction in hemoglobin that you want to be able to detect after treatment with primaquine	Number of G6PD-deficient persons needed to treat
15%	11
20%	7
25%	5
50%	3

[1]Detects a within person change in hemoglobin between Days 0 and 7 with 80% power, and a significance level of 0.05 (one-sided), assumes mean of 10 g/dL before treatment, a standard deviation of the within person change in hemoglobin of 2 g/dL, and no loss to follow-up

The sample sizes in **Table 2.1** assume that G6PD screening is available at the individual level. If the program wants to know whether primaquine treatment is associated with a 25% or greater drop in hemoglobin (e.g., a drop from 10 g/dL to 7.5 g/dL or lower), the program would need to measure the change in hemoglobin in at least 5 G6PD-deficient individuals before and after treatment with primaquine. A sample of 5 G6PD-deficient individuals would give the program 80% power to detect a 25% or greater average within-person drop in hemoglobin as statistically significant. Programs that want to be able to detect a smaller average within-person drop in hemoglobin as significant, for example a 15% drop, would need to test a larger number of people (**Table 2.1**).

For settings where the patient's G6PD status is unknown prior to treatment with primaquine:

In settings where individuals' G6PD status is unknown and G6PD screening is unavailable, programs can use the population prevalence of G6PD deficiency to estimate the number of individuals that need to be treated with primaquine and have pre-post treatment hemoglobin measurements. Let's assume that the population prevalence of G6PD deficiency is 1% and the program wants to be able to detect a 25% or greater within-person drop in hemoglobin following treatment with primaquine. Using **Table 2.1**, we know that the program would need to measure the change in hemoglobin in at least 5 G6PD-deficient individuals before and after treatment with primaquine. Assuming G6PD-deficient individuals are randomly distributed among the population, the program would need to measure the change in hemoglobin among at least 500 individuals of unknown G6PD status before and after treatment with primaquine (**Table 2.2**). By evaluating 500 individuals, the program can assume that 1% of those 500, or 5 people, were G6PD-deficient. The numbers presented in **Table 2.2** are derived by dividing the number needed to treat in **Table 2.1** by the estimated population prevalence of G6PD (5/0.01 = 500).

Table 2.2. Number of persons needed to treat to have sufficient power to detect a given within-person percent reduction in hemoglobin

Minimum percent reduction in hemoglobin that you want to be able to detect after treatment with primaquine	Numbers of individuals needed to treat with primaquine by the estimated population prevalence of G6PD deficiency			
	1%	**5%**	**10%**	**15%**
15%	1,100	220	110	74
20%	700	140	70	47
25%	500	100	50	34
50%	300	60	30	20

[1]Detects a within person change in hemoglobin between Days 0 and 7 with 80% power, and a significance level of 0.05 (one-sided), assumes mean of 10 g/dL before treatment, a standard deviation of the within person change in hemoglobin of 2 g/dL, and no loss to follow-up

For settings where the patient's G6PD status is known prior to treatment with primaquine with 25% loss to follow-up:

One further consideration might be the loss to follow-up of individuals who are treated with primaquine. It may not be reasonable to expect that all people who are treated with primaquine will return 7 days later for a post-treatment hemoglobin measurement. Using the example above, if we assume that the program will lose up to 25% of individuals between the time of treatment and the time of their follow-up hemoglobin measurement, programs may want to treat and evaluate an additional 25% of people. For example, if the program plans to measure the change in hemoglobin in at least 5 G6PD-deficient individuals before and after treatment with primaquine, the program would want to treat 7 individuals to ensure that 5 have both a pre- and post-treatment hemoglobin measurement. The numbers in **Table 2.3** are derived by dividing the number of individuals needed by 1 minus the estimated loss to follow-up. For example, 5/(1-0.25) = 6.7, or 7 G6PD-deficient individuals. Similar calculations are done in **Table 2.4** for settings where G6PD status is unknown.

Table 2.3. Number of persons needed to treat to have sufficient power to detect a given within-person percent reduction in hemoglobin assuming 25% loss to follow-up

Minimum percent reduction in hemoglobin that you want to be able to detect after treatment with primaquine	Number of G6PD-deficient persons needed to treat
15%	15
20%	10
25%	7
50%	4

[1]Detects a within person change in hemoglobin between Days 0 and 7 with 80% power, and a significance level of 0.05 (one-sided), assumes mean of 10 g/dL before treatment, a standard deviation of the within person change in hemoglobin of 2 g/dL, and up to 25% loss to follow-up

For settings where the patient's G6PD status is unknown prior to treatment with primaquine with 25% loss to follow-up:

Table 2.4. Number of persons needed to treat to have sufficient power to detect a given within-person percent reduction in hemoglobin, assuming 25% loss to follow-up

Minimum percent reduction in hemoglobin that you want to be able to detect after treatment with primaquine	Numbers of individuals needed to treat with primaquine by the estimated population prevalence of G6PD deficiency			
	1%	**5%**	**10%**	**15%**
15%	1,500	300	150	100
20%	1000	200	100	67
25%	700	140	70	47
50%	400	80	40	27

[1]Detects a within person change in hemoglobin between Days 0 and 7 with 80% power, and a significance level of 0.05 (one-sided), assumes mean of 10 g/dL before treatment, a standard deviation of the within person change in hemoglobin of 2 g/dL, and up to 25% loss to follow-up

Table 2.5. Dosage chart for single low-dose primaquine

Weight (kg)	Number of tablets	Dose (mg)	Dose range (mg/kg)
10-15	0.5	3.75	0.375 – 0.25
16-30	1	7.5	0.469 – 0.25
31-45	1.5	11.25	0.363 – 0.25
>45	2	15	< 0.333

Table 2.6. Baseline characteristics (n = 93)

Characteristic	% (n/N) or median [IQR]
Female	46% (43/93)
Participants from Manzini district	59% (55/93)
Age (years)	27 [5-38]
Weight (kg)	58 [20-71]
Baseline hemoglobin concentration (g/dL)	11 [10-13]

Data are presented as % (n/N) or median [IQR]. *IQR*, interquartile range

Table 2.7. Baseline and follow-up values and changes in hemoglobin concentration within 10 days post-treatment in participants who received AL + PQ (n = 87)

Characteristic	Mean (SD) [range]
Baseline hemoglobin (g/dL)	11.6 (2.3) [8 – 20.1]
Follow-up hemoglobin (g/dL)	11.0 (1.8) [7.2 – 15.8]
Absolute change in hemoglobin (g/dL)	-0.6 (1.6) [-5.9 - 3]
Percent change in hemoglobin (g/dL)	-3.7 (12.7) [-31.4 – 30.9]

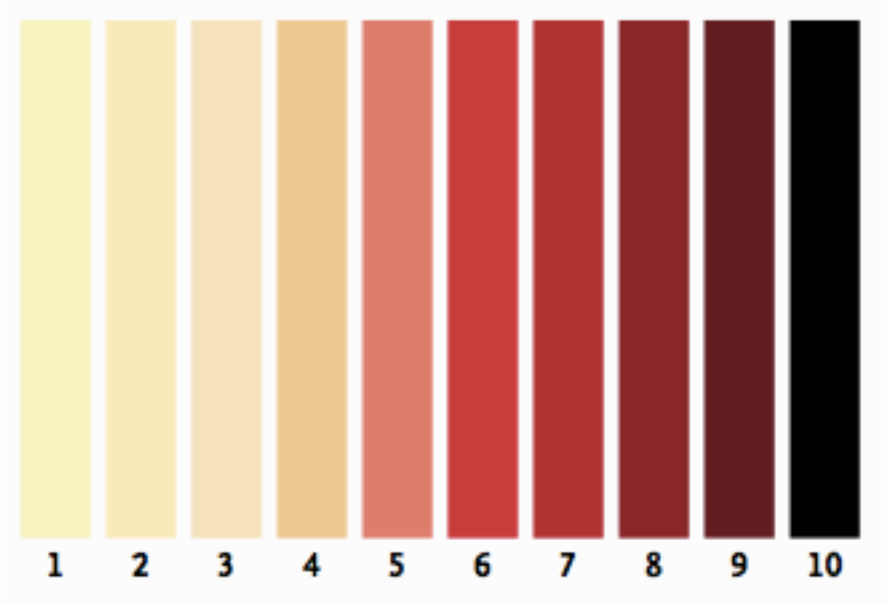
Primaquine was given on day 0 of treatment, together with dose 1 of artemether-lumefantrine. Mean absolute change defined as the hemoglobin mean on the day of follow-up minus the hemoglobin mean at day 0. Mean relative percentage change between day 0 (t1) and follow-up (t2) defined as [(hb(t2) – hb(t1))/hb(t1)] x 100. *AL* artemether-lumefantrine, *Hb* hemoglobin, *PQ* primaquine, *SD* standard deviation

Table 2.8. Detailed summary of adverse events and serious adverse events* as reported after exposure to SLD PQ together with AL

Sex	Age (years)	Baseline Hb	Follow-up Hb	Adverse event	Severity Grade	Actions Taken	Outcome
Male	32	14.9	13.2	Dark urine	Moderate	Urinalysis and CBC	Recovered
Male	28	15.7	15.8	Dark urine	Moderate	None	Recovered
Female	63	12.6	9.6	Unwell (worsening post-dose)	Moderate	None	Unknown
Female	18	9.2	-	Anemia, back pain, fever, and vomiting after meals	Moderate	Prescribed drug	Recovered
*Female	31	14.1	12.5	Diarrhea, vomiting, headache, and dizziness	Moderate	Hospitalization	Recovered
Male	5	9.3	8.0	Dark urine (Grade: 8)	Severe	None	Recovered
Female	30	14.9	13.6	Nausea	Moderate	None	Recovered
*Male	60	12.8	-	Fever, chills, diarrhea and vomiting	N/A	Prescribed drug	Fatal
Male	44	9.8	11.2	Dark urine (Grade: 5)	Mild	None	Recovered
*Male[a]	28	19.5	-	Weak, jaundice, coughing	N/A	Prescribed drug	Fatal
Female	3	10.5	11.6	Stomach cramps	Mild	Unknown	Recovered

Dashes represent missing values. *AL* artemether-lumefantrine, *CBC* complete blood count, *Hb* hemoglobin, *SLD PQ* single low-dose primaquine.
[a]Received antimalarial treatment despite testing negative for malaria by rapid diagnostic test and microscopy.

Figure 2.1. Urine color scale designed to assess the degree of hemoglobinuria.



Dark-colored urine with a colorimetric of 5 or above was considered evidence of hemoglobinuria. Reproduced with permission from (19), Copyright Massachusetts Medical Society.

Figure 2.2. Example of a patient information card in English

PROMPT

Primaquine Roll Out Monitoring Tool

Family Name |__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__|

First (Given) Name |__|__|__|__|__|__|__|__|__|__|__|__|__|

G6PD test result: ☐Normal ☐Deficient

Patient Instructions

You have received treatment for malaria. Please return if you do not improve or notice new problems. Please check the color of your urine (by passing urine into a white or clear container) while taking malaria medications. If you notice dark to very dark urine (reddish-brown) or experience nausea, vomiting, stomach or back pain, please return to the health facility with this card or call the following number:

You are scheduled to return to the clinic for a follow up visit in 7 days on __ __ / __ __ / __ __ (dd/mm/yy).

----------perforation----------

(BACK)

(logo (s))

Provider Instructions

Health Facility Name: _____

Contact No. for NMCP:

Should the patient return with signs or symptoms of commonly reported adverse events associated with primaquine, please complete a patient encounter form. If patient presents to a different health facility from the health facility where original treatment was provided, please contact the National Malaria Control Programne (NMCP).

If found, please return to NMCP

Figure 2.3. Screenshot images of data collected using Samsung T211 Galaxy Tab 3 (7.0) 3G tablets

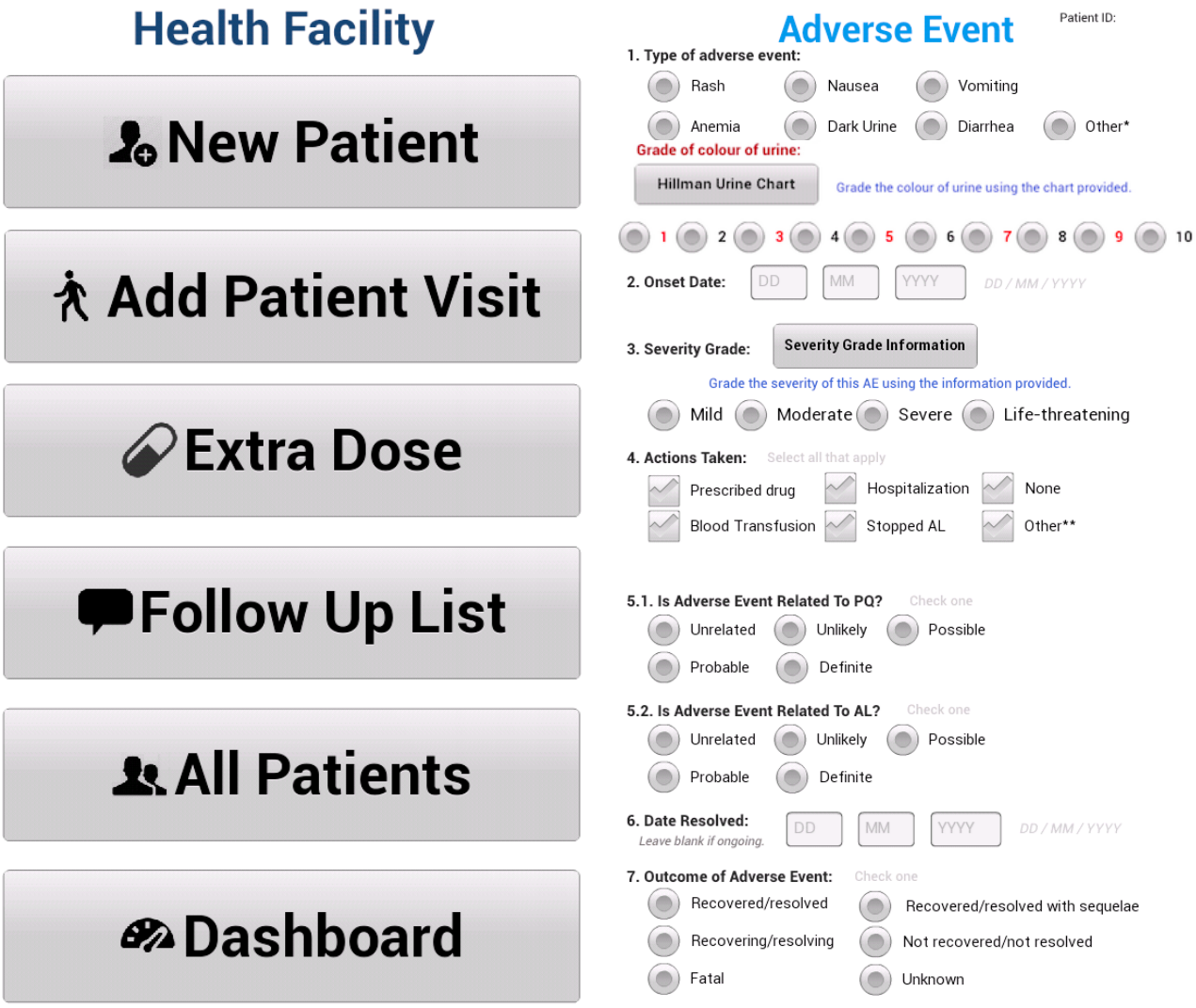
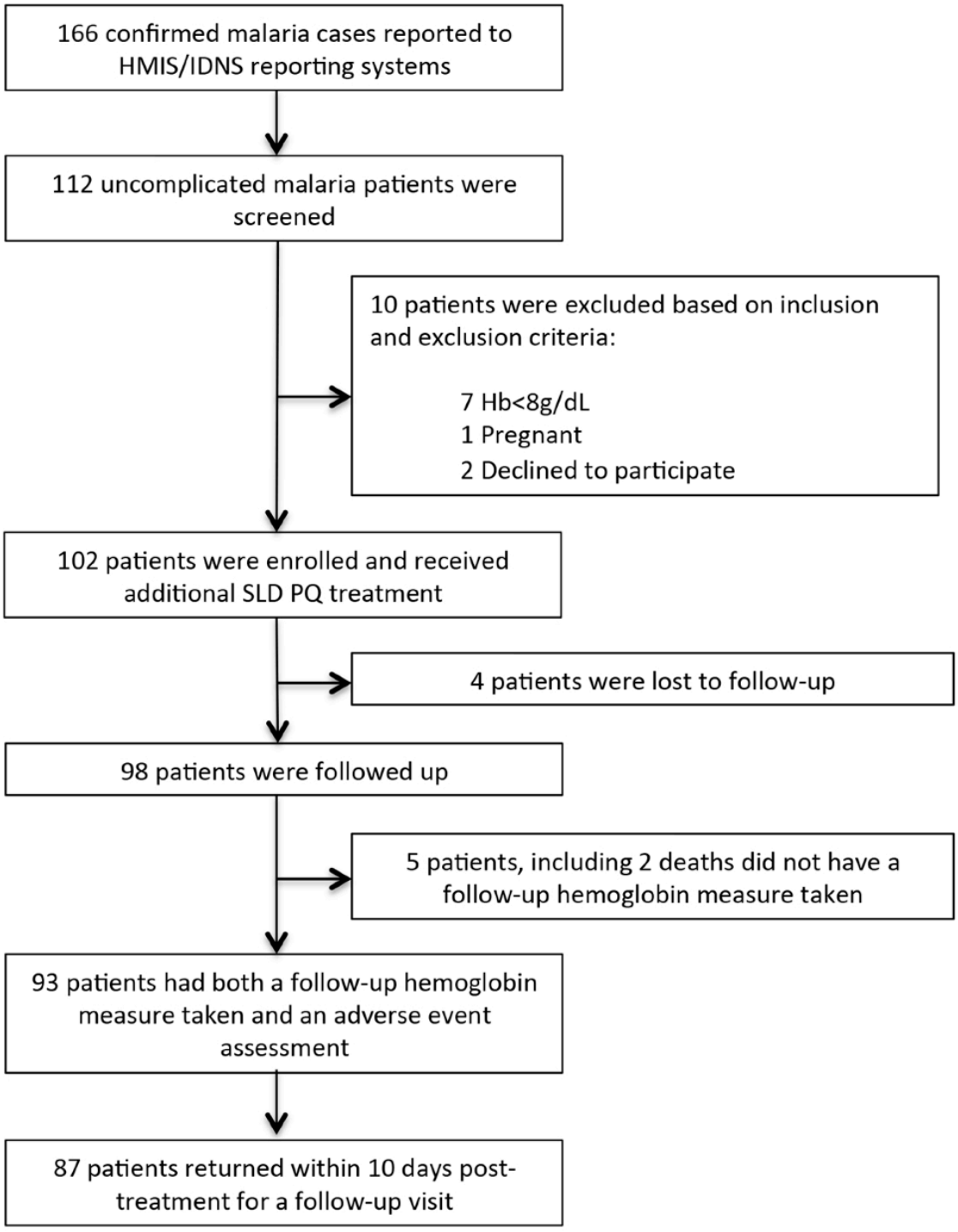


Figure 2.4. Cohort profile



Hb hemoglobin, *HMIS/IDNS* Health Management Information System/Immediate Disease Notification System, *SLD PQ* single low-dose primaquine

Figure 2.5. Summary of key findings and recommendations

Findings	
1.	Routine treatment with AL plus SLD PQ in patients with uncomplicated malaria, Hb concentrations ≥8 g/dL, and classified as G6PD-normal by point-of-care test, was well-tolerated.
2.	Four (4.6%) patients had falls in Hb greater than 25% from baseline, none of whom presented with signs and symptoms of anemia. No patient's Hb fell below 7 g/dL and none required a blood transfusion.
3.	Eleven patients reported an event. Three were considered serious, including two deaths and one hospitalization; none were classified as related to SLD PQ. Four non-serious cases were considered definitely, possibly or probably related to SLD PQ. Appropriate measures were, for the most part, in place to clinically monitor patients presenting with suspected cases of PQ-induced hemolysis efficiently.
Recommendations	
Safety 	1. Treat confirmed, uncomplicated falciparum infections with SLD PQ and exclude pregnant women, infants <6 months, breastfeeding women of infants <6 months, and those with severe or concomitant disease. Caution should be exercised in individuals with a personal or family history of hemolysis or in persons concurrently receiving other potentially hemolytic drugs or drugs that interact with PQ. If possible, G6PD testing should be made available, at least at referral hospitals, should cases of hemolysis arise and the underlying cause of hemolysis needs to be identified.
Dosing 	1. Provide clear guidance on drug administration criteria and dosage to all prescribers of SLD PQ. If possible, SLD PQ should be given directly as directly observed therapy, together with food. Clear dosing instructions in pictorial format and specific instructions should be given to those who will be prescribing and dosing patients.
Management 	1. Provide medical training to staff on G6PD deficiency and SLD PQ safety to strengthen patient-provider communication and ensure patients receive proper health information. 2. Provide patients with a patient information card, identical or similar to that used for PROMPT, to encourage the monitoring of signs and symptoms of SLD PQ use and self-reporting of adverse events. 3. Assess suspected cases of PQ-induced hemolysis and refer to a higher level of care, as appropriate. 4. Monitor patients treated with SLD PQ for adverse events using the program's existing pharmacovigilance system. 5. Offer training modules to healthcare providers on adverse event reporting to clarify complex topic areas. 6. Report all adverse drug reactions and serious adverse events to the Central Medical Stores.

AL artemether-lumefantrine, *Hb* hemoglobin, *G6PD* glucose-6-phosphate dehydrogenase, *PQ* primaquine, *PROMPT* Primaquine Roll Out Monitoring Pharmacovigilance Tool, *SLD PQ* single low-dose primaquine

Chapter 3: Primaquine treatment and the risk of hemolysis in malaria-infected patients: a prospectively planned pooled analysis of individual patient data

Eugenie Poirot, Eric Vittinghoff, Joelle Brown, and Roland Gosling

Abstract

Introduction: There is a lack of substantive evidence to support the safety of the WHO recommendation to add a single 0.25 mg/kg dose of primaquine (PQ) to *Plasmodium falciparum* treatment to prevent onward transmission in areas eliminating malaria and/or threatened by drug resistance. We undertook a pooled analysis of individual patient data to determine the risk and severity of hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency using data from randomized controlled trials and active pharmacovigilance programs.

Methods: Data from four studies of malaria-infected patients undertaken in Bangladesh, Senegal, Swaziland, and Tanzania were pooled. All studies included at least one arm (or exposure group) treated with PQ in which hemoglobin (Hb) levels were measured at baseline and at least once post-treatment during a scheduled day 7 follow-up visit. Participants were phenotypically screened for G6PD deficiency. Standard and adjusted linear regression analyses accounting for study heterogeneity were used to evaluate the treatment effect of PQ on absolute and fractional differences in Hb in G6PD-deficient patients and to compare differences in Hb changes between G6PD phenotypes by PQ dose received.

Results: The four studies, including two randomized trials and two prospective, observational cohort studies from programmatic settings, provided data for 604 participants (G6PD-deficient, n = 72; G6PD normal, n = 532). In a restricted analysis of 68 G6PD-deficient patients randomized to treatment, the addition of PQ to artemisinin combination-based therapy (ACT) reduced Hb levels by approximately 0.49 g/dL [95% CI: -1.17, 0.2] or 3.97% [95% CI: -9.21, 1.27] relative to baseline compared to ACT alone. There was no statistical difference between treatment groups. Using data from all sites, mean and fractional changes in Hb concentrations from

baseline, were larger in G6PD-deficient subjects compared to G6PD-normal subjects and varied by PQ dose; mean changes in G6PD-deficient subjects ranged from -0.98 g/dL [95% CI: -1.47, -0.50] with ACT alone to 2.75 g/dL [95% CI: -4.22, -1.28] in those treated with PQ doses >0.5 mg/kg. All subjects in the >0.5 mg/kg dose group came from a single site, Bangladesh.

Conclusion: The findings of this pooled analysis are reassuring and provide evidence to suggest that a single dose of 0.25 mg/kg PQ is likely to be safe in G6PD-deficient malaria-infected patients. However, the number of G6PD-deficient subjects were few. A reanalysis should be carried out when more data become available to validate findings using a larger sample. Active pharmacovigilance using common safety endpoints should be encouraged to help collect data on the use of single low dose PQ and guide safe malaria treatment practices.

Introduction

To reduce *P. falciparum* malaria transmission in eliminating settings and areas threatened by artemisinin resistance, the WHO recommends adding a single low dose of 0.25 mg/kg primaquine (PQ) to artemisinin-based combination therapy (ACT) without glucose-6-phosphate dehydrogenase (G6PD) testing [1]. The safety of this low dose was largely based on a review of historical data at higher and multiple doses of PQ in otherwise healthy G6PD-deficient African-American men [2] but direct evidence substantiating its safety in people with malaria in combination with ACTs is lacking [3]. There are concerns related to the safety of PQ because it is known to cause dose-dependent acute hemolytic anemia in individuals who are G6PD-deficient [4]. G6PD deficiency is an X-linked blood disorder common across malaria endemic regions affecting nearly 400 million people worldwide [5, 6].

Countries implementing the WHO PQ policy recommendation for falciparum malaria remain cautious, requesting more evidence to support the safety of PQ in people with G6PD deficiency. Recent studies to date assessing the safety of single low dose PQ in combination with ACTs have shown no evidence of clinically meaningful or statistically significant drops in hemoglobin among either gametocytemic, male adults [7] or non-anemic, asymptomatic children [8] with falciparum infections. However, both studies excluded people deficient in G6PD [9]. Studies examining the safety and efficacy of low dose PQ are likely to be small-scale clinical trials with few, if any, people with G6PD deficiency and are thus unlikely to be sufficiently powered to detect statistically significant increased risks of hemolysis [10]. In order to inform implementing country policy, we undertook a multicenter, pooled analysis of individual patient data from

prospective clinical trials and programmatic settings using common safety endpoints. A pragmatic approach was taken to facilitate the rapid collation of standardized safety data where data on the use of single low-dose PQ is needed to support its rollout. This analysis aimed to determine the risk and severity of hemolysis, firstly; in patients with G6PD deficiency randomized to receive standard malaria treatment (i.e., an ACT) with and without low dose PQ and secondly; in patients treated with PQ with and without G6PD deficiency.

Methods

Study selection criteria

This analysis was part of a multicenter collaboration made up of research institutions, organizations and National Malaria Control Programs (NMCP) and assembled to collect standardized demographic and safety data using the Primaquine Roll Out Monitoring Pharmacovigilance Tool (PROMPT) [11]. Individual data from four studies were preselected for pooling. Any randomized or nonrandomized study involving patients with uncomplicated malaria and known G6PD phenotype status was eligible for inclusion in this pooled analysis. Studies contained at least one arm (or exposure group) treated with PQ in which hemoglobin levels were measured at baseline and at least once post-treatment during a scheduled day 7 follow-up visit.

The four studies, described in detail elsewhere, are summarized in **Table 3.1**. Two randomized trials were undertaken in the districts of Bagamoyo and Kibaha in Tanzania, and in Pikine and Ndoffane in Senegal, and compared routine malaria treatment with an ACT alone to treatment

with an ACT plus single dose PQ. In addition, two prospective, observational cohort studies from programmatic settings conducting active pharmacovigilance of PQ implementation were performed in the districts of Manzini and Lombobo in Swaziland, and in Bandarban in Bangladesh. All studies were conducted between 2014 and 2015 and received ethical approval.

Procedures

Selected studies generated data using a standardized questionnaire at baseline and during follow-up visits. Information gathered from patients included data on patient demographics (including age, sex, and weight), malaria diagnosis, treatment and dose, and the use of concomitant medications through history taking and physical examination. Safety assessments consisted of measuring hemoglobin concentrations at baseline and during follow-up (with a minimum scheduled day 7 follow-up visit), and recording of all treatment emergent adverse events and serious adverse events. Finger prick blood samples were taken to screen for G6PD deficiency and to measure hemoglobin concentrations. G6PD phenotype was determined using the point-of-care CareStart test (AccessBio, USA), except in Bangladesh where venous blood samples were used to acquire semi-quantitative G6PD measurements. In Bangladesh, spectrophotometry (Shimadzu 1800™, Japan) was performed and enzyme activities (in U/gHb) were calculated using recorded hemoglobin concentrations taken at the time of sample collection; enzyme activities were then categorized based on the adjusted male median [12]. All sites used a Hemocue photometer (HemoCue, Angelholm, Sweden) to measure hemoglobin concentrations at baseline and during follow-up. Data were extracted and standardized from each study's database and pooled into a single database of individual patient data.

Statistical analysis

Definitions

G6PD phenotype was defined as either deficient or normal. Treatment dose received (in mg/kg) was categorized as: 0, ≤0.25, >0.25 to ≤0.5, or >0.5 mg/kg. Data were analyzed according to four outcome definitions for hemolysis, based on hemoglobin measurements. Absolute reductions in hemoglobin were calculated as the difference in hemoglobin concentration before and after PQ treatment. Fractional changes in hemoglobin concentration were estimated as the difference in values before and after PQ treatment over the pre-treatment value. Binary measures of hemolysis were defined as either any post-treatment drop in hemoglobin greater than or equal to 25% of the baseline value, or values below 7 g/dL post-treatment. These were chosen to represent clinically important thresholds for increased risk of adverse effects. Although these thresholds have not been clearly established there is evidence suggesting that patients with severe anemia (hemoglobin levels <7 g/dL) have an increased risk of death [13].

Analysis of hematologic changes

Data were reanalyzed at the individual level using standardized definitions and time periods (i.e. a scheduled day 7 visit after a PQ dose) common to all studies. Results may therefore not directly reflect those published elsewhere for each study. Two main analyses were conducted. First, standard and adjusted linear regression models were used to examine the effect of adding single low-dose PQ to routine malaria treatment on absolute and fractional hemoglobin changes in G6PD-deficient subjects randomized to treatment with study site fitted as a fixed effect. Second, models were constructed using data from all sites to estimate the joint effects of G6PD

phenotype and PQ dose on absolute and fractional changes in hemoglobin concentration. Tests for trend were used to assess differences in dose-response between G6PD-deficient and normal subjects. Interactions between day of treatment and treatment arm and site were used to investigate heterogeneity and variation in effect estimates. We also performed sensitivity analyses 1) restricted to participants who returned within 6 to 8 days post PQ treatment, or 2) including any hemoglobin measurements obtained within 10 days of treatment administration.

For the two binary measures of hemolysis, event rates with accompanying exact binomial 95% confidence limits were compared between G6PD-deficient and normal subjects using either a chi-square or Fisher's exact test. All statistical analyses were performed using Stata Version 13 (StataCorp, College Station, Texas, USA).

Results

Data summary

Data from 604/667 (91%) participants across four studies were analyzed, including 366 participants from two trials in Tanzania and Senegal randomized to either ACT alone (artemether-lumefantrine (AL), dihydroartemisinin-piperaquine (DHAP), or artesunate-amodiaquine (ASAQ); n = 188) or in combination with PQ (n = 178) and 238 patients from programmatic settings in Swaziland (n = 85) and in Bangladesh (n = 153) treated with PQ in combination with standard treatment for uncomplicated malaria according to national guidelines. Of those analyzed, 72 (12%) were G6PD-deficient and 532 (88%) were G6PD normal. 62 of 667 (9%) patients were excluded from analysis due to one of the following reasons: missing dose or

G6PD phenotype results (n = 4) or lost to follow-up with incomplete hemoglobin information (n = 58). **Figure 3.1** shows the study profiles for each analysis.

Risk and severity of hemolysis among G6PD-deficient patients randomized to receive standard malaria treatment with and without low dose PQ

68 G6PD-deficient patients were randomized to receive an ACT with and without low dose PQ. Reported baseline characteristics were similar across treatment groups in both trials (**Table 3.2**). Adjusted mean and fractional drops in hemoglobin concentration on day 7 were marginally greater after PQ treatment compared to ACT alone but did not statistically differ between treatment groups. Treatment with PQ reduced hemoglobin levels by 0.49 g/dL [95% CI: -1.17, 0.20], or 3.97% [95% CI: -9.21, 1.27] relative to baseline, compared with those assigned to the ACT alone group (**Table 3.3**).

The magnitude of decline in hemoglobin concentration was not associated with PQ dose (p = 0.2). Sensitivity analyses on timing of return visits yielded similar results, with increased declines in hemoglobin levels in those treated with PQ compared to ACT alone. Treatment effect estimates were slightly smaller when restricted to patients who returned within 6 to 8 days post PQ treatment (mean change, -0.40 g/dL [95% CI: -1.10, 0.30]; fractional reduction, -3.29 % [95% CI: -8.80, 2.21]) or within 10 days of treatment administration (mean change, -0.33 g/dL [95% CI: -0.96, 0.29]; fractional reduction, -2.67% [95% CI: -7.50, 2.20]) [data not shown].

Few G6PD-deficient patients presented with hemoglobin concentrations less than 7 g/dL post-dose or fractional reductions greater than or equal to 25%. Two patients (1/31 (3%) in the ACT plus PQ arm and 1/37 (3%) in the ACT alone) experienced severe anemia (hemoglobin levels <7 g/dL). Four subjects had a 25% or greater drop in hemoglobin after treatment (1/37 (3%) in the ACT arm and 3/31 (10%) in the ACT plus PQ arm) (**Table 3.4**).

Risk and severity of hemolysis among patients with and without G6PD deficiency

Of the 604 participants with complete data who returned for scheduled day 7 follow-up visits, 11% (n = 72) were G6PD-deficient, coming from three sites. No patients were identified as G6PD-deficient in Swaziland. **Table 3.5** shows the demographic and clinical parameters of patients included in this analysis across all four sites. The median age for the pooled data was 9 years, ranging from 1 to 84 and mean hemoglobin at enrolment was 12.2 g/dL [95% CI: 12.0, 12.4]. The median daily dose of PQ varied among those treated for malaria. The median dose among patients receiving a single low dose of PQ together with an ACT for falciparum malaria was 0.25 mg/kg (interquartile range (IQR): 0.25-0.26) across studies in Africa. In Bangladesh, patients received considerably higher doses either as single doses of 0.75 mg/kg for confirmed cases of *P. falciparum* in combination with ACT (median 0.8 mg/kg, IQR: 0.8-0.9) or as multiple lower 0.25 mg/kg doses for 14 days for *P. vivax* or mixed infections (median 5.0 mg/kg, IQR: 4.0-6.6).

Larger declines in hemoglobin were found in G6PD-deficient patients compared to G6PD normal subjects treated with PQ and this varied by dose group (**Table 3.6**). Adjusted mean

changes in hemoglobin ranged from -0.98 g/dL [95% CI: -1.47, -0.50] with ACT alone to -2.75 g/dL [95% CI: -4.22, -1.28] in the >0.5 mg/kg dose groups among G6PD-deficient patients and from -0.63 g/dL [95% CI: -0.90, -0.37] in the >0.25 to ≤0.5 mg/kg to -1.27 g/dL [95% CI: -1.56, -0.99] in the ≤0.25 mg/kg dose groups among G6PD normal subjects (**Table 3.7**, **Figure 3.2**). The same applied for adjusted fractional reductions with hemoglobin drops; the largest fractional changes were found in G6PD-deficient patients treated with PQ, ranging from -9.48 % [95% CI: -15.69, -3.27] in the >0.25 to ≤0.5 mg/kg dose group to -19.03% [95% CI: -30.86, -7.19] in the >0.5 mg/kg dose group.

Though changes in hemoglobin concentration were larger in G6PD-deficient subjects, declines were small at doses ≤0.5 mg/kg. There was no evidence of greater changes in hemoglobin concentration between G6PD phenotypes in the ≤0.25 mg/kg and >0.25 to ≤0.5 mg/kg dose groups. At doses >0.5 mg/kg, compared to those with normal G6PD enzyme function, the small sample of G6PD-deficient subjects had significantly larger mean (-1.89 g/dL [95% CI: -3.38, -0.40]) and fractional (-12.90% [95% CI: -24.89, -0.90]) changes in hemoglobin concentration. When using data across all sites we found evidence of a difference in linear dose-response between G6PD-deficient and G6PD normal patients on absolute and fractional drops in hemoglobin ($P < 0.05$). This interaction was no longer present when data from Bangladesh was excluded ($P = 0.09$).

Five patients treated with PQ (4/380 (1%) and 1/35 (3%) among the phenotypically G6PD normal and deficient subjects, respectively) had hemoglobin concentrations fall below 7 g/dL

(**Table 3.4**). All events presented with lower than average baseline hemoglobin levels (range: 7.60 - 8.80), including 3 G6PD normal subjects treated at doses greater than 0.75 mg/kg. 24 (6%) participants had fractional hemoglobin falls greater than or equal to 25% after treatment with PQ, all of which were not anemic (Hb >10 g/dL) at admission. Of those, fractional falls greater than or equal to 25% occurred in 11% (4/35) phenotypically G6PD-deficient subjects compared to 5% (20/380) G6PD normal subjects. The number of participants falling below 7 g/dL or having declines in hemoglobin of at least 25% did not differ between phenotype groups (Fisher's exact test, $p = 0.4$ and $p = 0.1$, respectively).

Discussion

In this study, we present results from a pooled analysis of both randomized control trial and prospective, active pharmacovigilance data assessing the safety of PQ for the treatment of *P. falciparum* in G6PD normal and deficient subjects in Senegal, Tanzania, Swaziland and Bangladesh. Noting the heterogeneity between study settings, we show that falls in hemoglobin following treatment occur in G6PD normal and to a slightly greater extent in G6PD-deficient subjects. We find that PQ doses equal or less than 0.5mg/kg, double the WHO recommended dose of 0.25mg/kg, do not appear to result in greater hemolysis among G6PD-deficient as compared to G6PD normal subjects. These results are encouraging and support the use of single low dose PQ to patients with uncomplicated *P. falciparum* malaria in combination with an ACT.

The evidence that drove the WHO to change PQ policy in 2012 to a lower dose of 0.25 mg/kg PQ was based on a limited number of studies evaluating the safety of PQ as a single dose *P.*

falciparum gametocytocide, at doses less than 30 mg (equivalent to 0.5 mg/kg for a 60 kg adult), or in G6PD-deficient individuals [14]. To our knowledge, only four studies have evaluated the effect of single dose PQ on changes in hemoglobin concentration at doses less than 0.5 mg/kg [7, 8, 15, 16]. In two recently published studies, malaria-infected participants with normal G6PD enzyme activity in Mali [7] and Burkina Faso [8] were given PQ doses ≤0.5 mg/kg together with an ACT, including the WHO recommended 0.25 mg/kg dose. Both studies found no significant drops in hemoglobin concentration in the 0.25 mg/kg PQ groups compared to controls. However, Goncalves *et al.* observed greater mean drops in hemoglobin levels among children receiving higher 0.4 mg/kg PQ doses (-1.21 g/dL [95 % CI: -1.45, -0.97]) compared to placebo (-0.71 [95% CI: -0.98, -0.44]), implying that mild hemolysis might be occurring in those with normal G6PD enzyme function [8]. This is in contrast to a previous trial undertaken in Uganda in symptomatic malaria infected children with normal G6PD activity where hemoglobin drops in the 0.1 mg/kg, 0.4 mg/kg, and 0.75 mg/kg did not differ significantly compared with placebo [16]. Only one study has investigated the safety of repeated rounds of single dose (0.25 mg/kg) PQ combined with DHAP in 819 subjects with known G6PD status, including G6PD-deficient subjects, on the Myanmar-Thailand border where the more severe Mahidol variant predominates. The mean fractional changes after each PQ treatment in G6PD-deficient subjects (ranging from -5.0% to -4.2%) were greater than G6PD normal subjects (-1.7% to 0.3%) but small and clinically insignificant [15].

The findings presented here also demonstrate that G6PD-deficient subjects treated with PQ doses up to double the WHO recommended 0.25 mg/kg dose might not be at increased hemolytic risk.

First, in our analysis of randomized clinical trial data from Tanzania and Senegal among G6PD-deficient patients, we show that the addition of PQ at a dose of 0.25 mg/kg [dose range: 0.15 – 0.33 mg/kg] to ACT does not significantly increase the risk of hemolysis using any of our measures of hemolysis. Although we cannot exclude that a true association was missed in our small sample of G6PD-deficient patients (n = 68), the confidence intervals from our pooled analysis of continuous outcomes suggest that the average effect is no greater than approximately a 1.2 g/dL drop or a 9.2% fractional reduction. However, the confidence intervals do not exclude larger reductions at the individual level. Second, in our analysis using all available data including pharmacovigilance data from two national programs in Swaziland and Bangladesh, we show patterns consistent with the literature. Falls in hemoglobin levels appear greater in G6PD-deficient subjects compared to G6PD normal subjects but differences between groups are modest, except at doses greater than 0.5 mg/kg. We find no evidence of differences between groups at doses equal to or less than 0.5 mg/kg though it is possible that our sample was too small to detect such differences. Moreover, the statistically significant difference in dose-response between G6PD deficient and normal patients was essentially driven by the uncontrolled data from Bangladesh, where only 4 patients were deficient, and all received PQ doses higher than 0.5 mg/kg. Of a total of 35 G6PD-deficient patients treated with PQ, only one child (3%) had a post-treatment hemoglobin concentration lower than 7 g/dL.

A real strength of this analysis was the inclusion of data from multiple studies and geographical regions using a standardized protocol for the collection of common safety endpoints. However, though adjustments were made to reduce site heterogeneity, study differences, with variations in

doses and dosing schedules, were a limitation. Two sites – Tanzania and Senegal – contributed most of the data on phenotypically G6PD-deficient patients (68/72 (94%)). No G6PD-deficient subjects were identified in Swaziland using the phenotypic CareStart test and all subjects in Bangladesh were treated with PQ doses higher than 0.5 mg/kg. This reflects Bangladesh's national treatment policy where guidelines follow the older WHO recommendation for a single 0.75mg/kg dose of PQ for *P. falciparum* infections. In addition, unlike the sites in Africa, where *P. falciparum* infections make up nearly all cases, in Bangladesh two parasite species - *P. falciparum* and *P. vivax* – coexist. Because *P. vivax* has a dormant liver stage, the treatment of *P vivax* or *P. falciparum / P. vivax* co-infections require a 14 day course of 0.25 mg/kg PQ (3.5 mg/kg total). Accordingly, the data on G6PD-deficient patients treated at PQ doses $\leq$0.5mg/kg were represented entirely from the randomized controlled trials in Tanzania and Senegal.

The variability and accuracy of G6PD phenotype measurements between datasets should also be noted. G6PD phenotype classifications may have been subject to some misclassification. Misclassification is less likely in the data coming from Bangladesh where samples were analyzed using the spectrophotometric assay. On the other hand, although the CareStart test has good sensitivity at detecting severely G6PD deficient individuals (at enzyme activity levels <30% or normal activity), the test does not accurately identify partially deficient heterozygote females of intermediate or mild phenotypes who have higher levels of G6PD activity [17].

In spite of these limitations, by combining and pooling data from numerous studies, we can better characterize the safety of PQ by estimating the risk of drug-induced hemolysis in patients

with known G6PD status. The authors thus chose to include programmatic data in addition to randomized trial data for several reasons. Firstly, the data from national programs represent practical real world data without the restrictive exclusion criteria used in homogenous clinical trials. This includes people wrongly dosed or ineligible to receive PQ. Secondly, PQ has been adopted as policy by programs and is increasingly being recognized as the standard of care for treatment of malaria. Several countries in Asia use high-dose 0.75 mg/kg PQ in their *P. falciparum* treatment guidelines, with demand for the use of PQ at 0.25 mg/kg rapidly growing, especially in parts of Africa [18]. It is therefore important to collect safety data in these countries that reflects the populations receiving treatment and how interventions are actually being used in practice.

This study is the first pooled analysis to specifically look at the risk and severity of hemolysis, at various doses, including the 0.25 mg/kg recommended PQ dose for falciparum malaria. The results presented here support the view that the 0.25 mg/kg recommended PQ dose together with an ACT is likely to be safe in G6PD-deficient malaria-infected patients where risk of hemolytic anemia is highest. These findings may also offer useful insights that would bring us closer to defining a range of dosing for PQ that is both efficacious and safe. The highest tolerable dose in G6PD-deficients may fall somewhere between 0.25 and 0.5 mg/kg. Defining the therapeutic dose range would in turn guide practical dosing regimens in the field and enable the safe delivery of community interventions with PQ [19]. However, this analysis was limited in sample size and statistical power and does not allow us to conclusively state that the 0.25 mg/kg PQ dose is safe. This points to some of the challenges when faced with extremely small numbers of G6PD-

deficient individuals and rare hemolytic events. More pharmacovigilance data from programmatic settings and clinical trial data across diverse epidemiologic settings of hematologic and adverse event data is therefore needed to facilitate a repeat analysis and validate these findings using a larger sample size. Future studies need to include sufficient numbers of G6PD-deficient individuals and hemolytic events to reliably assess the safety of adding single low dose PQ to standard malaria treatment for falciparum malaria. Active pharmacovigilance methods using standardized safety endpoints can help to gather data on the use of single low dose PQ across multiple geographies with diverse G6PD variants and guide safe malaria treatment practices. Without a well-characterized understanding of the safety of PQ, its programmatic use for *P. falciparum* elimination will be challenging.

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Table 3.1. Characteristics of studies included in pooled analysis and demographic and baseline characteristics for uncomplicated malaria patients treated with primaquine

Country	**Bangladesh [20]**	**Tanzania**	**Senegal [21]**	**Swaziland [11]**
District(s)	Alikadam	Bagamoyo-Kibaha	Dakar-Keur Socé	Manzini-Lambobo
Recruitment years	2014	2014	2014-2016	2014-2015
Design	Prospective observational cohort	Randomized two-arm single-blind trial	Randomized two-arm open-label trial	Prospective observational cohort
Comparator	None	ACT + placebo	ACT alone	None
Inclusion criteria				
Sex	Males or nonpregnant, nonlactating females	Males or nonpregnant, nonlactating females	Males or nonpregnant, nonlactating females	Males or nonpregnant, nonlactating females
Age, years	>1 year	>1 year	>18	>1 year
Body weight, kg	≥6 kg	≥10 kg	Not specified	≥10 kg
Hemoglobin	≥8 g/dL	≥8 g/dL	≥8 g/dL	≥8 g/dL
Confirmed malaria infection	Microscopic *P. falciparum* or *P. vivax* mono-infection or *P.v*/*P.f* mixed infection	Microscopic *P. falciparum* mono-infection	Microscopic *P. falciparum* mono-infection	Microscopic or RDT-confirmed *P. falciparum*, *P. vivax* mono-infection or *P.v*/*P.*f mixed infection
Presence of axillary temperature ≥37.5°C or history of fever	Yes (past 24 hours)	Yes (past 24 hours)	Yes	Yes (past 48 hours)
Drug used to treat uncomplicated malaria	6-dose AL regimen for *P. falciparum* or *P. f*/*P. v* mixed infections; 3-dose CQ for *P. vivax*	6-dose AL regimen	6-dose AL regimen, or 3-dose DHAP or ASAQ regimen	6-dose AL regimen or 3-dose QN regimen for *P. falciparum*

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Country	**Bangladesh [20]**	**Tanzania**	**Senegal [21]**	**Swaziland [11]**
Primaquine dosing				
Supervised	No	Yes	Yes	Yes
Concomitant food/milk	Yes	Yes	Yes	Yes
Dosage	According to bodyweight using 15 mg tablets (*P. falciparum*: 6-9 kg, half a tablet; 10-19 one tablet; 20-29 one and a half tablets; 30-39 two tablets; 40-49 two and a half tablets; >50 three tablets; *P. vivax*: 6-9 kg, quarter of a tablet; 10-19 half a tablet; 20->50 one tablet [*P. falciparum*: 0.75 mg/kg; *P. vivax*: 3.5 mg/kg]	Exact weight-based dosing using 15 mg tablets [0.25 mg/kg]	Fixed (one 15 mg tablet) [0.25 mg/kg]	According to bodyweight using 7.5 mg tablets (10-15 kg, half a tablets; 16-30 kg, one tablet; 31-45 kg, one and a half tablets; >45 kg, two tablets [0.25 mg/kg]
Timing with antimalarial treatment	Day 2	Day 0	Day 0	Day 0

AL artemether-lumefantrine, *ACT* artemisinin-based combination therapy, *ASAQ* artesunate-amodiaquine, *CQ* chloroquine, *DHAP* dihydroartemisinin-piperaquine, *G6PD* glucose-6-phosphate dehydrogenase deficiency, *QN* quinine, *P.f plasmodium falciparum P.v plasmodium vivax*

Table 3.2. Demographics and baseline characteristics among G6PD-deficient uncomplicated malaria patients who returned for a scheduled day 7 visit and were randomized to receive single dose primaquine, by site and overall

	Tanzania		Senegal		Overall	
	AL + placebo (n = 19)	AL + PQ (n = 14)	ACT alone (n = 18)	ACT + PQ (n = 17)	ACT alone (n = 37)	ACT + PQ (n = 31)
Female (%)	7 (37)	5 (36)	2 (11)	4 (24)	9 (24)	9 (29)
Age (years)	9 (3 - 23)	9.5 (4 - 13)	23.5 (19 - 30)	26 (21 - 43)	19 (9 - 27)	18 (10 - 29)
Hemoglobin (g/dL)	11.1 [10.2, 11.9]	10.8 [10.1, 11.5]	13.3 [12.5, 14.0]	13.6 [12.9, 14.4]	12.3 [11.6, 13.0]	12.1 [11.5, 12.8]
Anemic (Hb <10 g/dL) (%)	5 (26)	3 (21)	0	0	5 (14)	3 (10)
Asexual parasite levels (parasites/μL)	16280 (960 - 84320)	26360 (840-151200)	21671 (2760 - 39788)	28695 (11621 - 45865)	21420 (1740- 41200)	28695 (1720-57277)

Means and 95% confidence intervals are presented for baseline hemoglobin levels. Data are otherwise number (%) or median (interquartile range). Some percentages do not add up to 100 because of rounding. *AL* artemether-lumefantrine, *ACT* artemisinin-based combination therapy, *G6PD* glucose-6-phosphate dehydrogenase, *Hb* hemoglobin, *PQ* primaquine

Table 3.3. Treatment effect on mean differences and fractional reductions in hemoglobin from baseline among G6PD-deficient uncomplicated malaria patients randomized to receive single dose primaquine who returned for a scheduled day 7 follow-up visit (n = 68)

				Adjusted between-group difference[a]
		N	Mean difference (SD) [95% CI]	Estimate [95% CI] p
Absolute declines (g/dL)				
	ACT alone	37	-1.02 (1.30) [-1.46, -.59]	Ref
	ACT + PQ	31	-1.54 (1.53)[-2.10, -.98]	-0.49 [-1.17, 0.20] 0.16
Fractional reductions (%)				
	ACT alone	37	-7.98 (10.20) [-11.38, -4.58]	Ref
	ACT + PQ	31	-12.00 (11.30) [-16.17, -7.92]	-3.97 [-9.21, 1.27] 0.14

[a]Linear regression adjusted for site as a fixed effect. *ACT* artemisinin-based combination therapy, *CI* confidence interval; *G6PD* glucose-6-phosphate dehydrogenase, *OR* Odds ratio, *PQ* primaquine, *SD* standard deviation

Table 3.4. Descriptive table of demographic and hematologic parameters among patients who experienced large drops in hemoglobin

No.	Country	Age	Sex	G6PD	Dose (mg/kg)	Hb baseline	Hb day 7	Fractional
Post-dose Hb <7 g/dL								
1	Bangladesh	40	F	Normal	0.91	7.6	6.3	-17.1
2	Bangladesh	13	M	Normal	7.50	7.8	7	-10.3
3	Bangladesh	40	F	Normal	0.94	8.4	6.6	-21.4
4	Tanzania	1	M	Normal	0.25	8.2	7	-14.6
5	Tanzania	4	F	Deficient	0.25	8.2	6.8	-17.1
6	Tanzania	2	F	Normal	0.00	8.8	6.0	-31.8
7	Tanzania	1	M	Deficient	0.00	8.1	6.6	-18.5
Fractional reduction ≥25%								
1	Bangladesh	22	F	Normal	0.87	16.2	11.2	-30.9
2	Bangladesh	38	M	Deficient	0.88	14.5	8.4	-42.1
3	Bangladesh	22	M	Normal	0.85	20.8	12.9	-38.0
4	Bangladesh	7	M	Normal	0.79	11.0	7.5	-31.8
5	Bangladesh	19	M	Normal	0.83	12.2	8.9	-27.0
6	Bangladesh	20	M	Normal	0.75	14.8	9.8	-33.8
7	Bangladesh	12	M	Normal	0.91	14.2	10.6	-25.4
8	Bangladesh	18	M	Normal	0.80	14.2	10.2	-28.2
9	Bangladesh	6	F	Normal	7.00	10.2	7.2	-29.4
10	Bangladesh	26	M	Normal	3.60	12.8	9.0	-29.7
11	Bangladesh	22	M	Normal	0.90	14.3	10.3	-28.0

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12	Bangladesh	28	F	Normal	0.78	12.5	8.8	-29.6
13	Tanzania	6	F	Normal	0.00	12.2	9.1	-25.4
14	Tanzania	30	F	Normal	0.25	13.5	10.1	-25.2
15	Tanzania	9	F	Normal	0.25	12.0	9.0	-25.0
16	Tanzania	49	F	Deficient	0.00	11.2	8.2	-26.8
17	Tanzania	4	F	Normal	0.00	11.2	8.4	-25.0
18	Tanzania	7	F	Normal	0.00	12.9	9.1	-29.5
19	Tanzania	2	F	Normal	0.00	8.8	6.0	-31.8
20	Senegal	52	F	Normal	0.22	12.4	7.8	-37.1
21	Senegal	18	M	Normal	0.00	14.6	10.7	-26.7
22	Senegal	50	F	Normal	0.21	15.7	11.0	-29.9
23	Senegal	22	M	Normal	0.00	15.0	10.4	-30.7
24	Senegal	23	F	Normal	0.00	17.7	11.1	-37.3
25	Senegal	26	F	Deficient	0.21	15.3	9.2	-39.9
26	Senegal	44	M	Deficient	0.21	12.8	9.3	-27.3
27	Senegal	21	M	Normal	0.29	15.4	11.3	-26.6
28	Senegal	40	M	Deficient	0.29	13.5	9.6	-28.9
29	Swaziland	30	M	Normal	0.22	20.1	14.2	-29.4
30	Swaziland	57	M	Normal	0.21	17.3	12.0	-30.6
31	Swaziland	38	M	Normal	0.25	14.0	9.6	-31.4
32	Swaziland	33	M	Normal	0.23	13.2	9.2	-30.3

G6PD glucose-6-phosphate dehydrogenase, *Hb* hemoglobin

Table 3.5. Demographics and baseline characteristics across study sites for patients treated for uncomplicated malaria included in the analysis

	Bangladesh	**Tanzania**	**Senegal**	**Swaziland**	**Overall**
N (%)	153 (25)	211 (35)	155 (26)	85 (14)	604 (100)
Age (years)	19 (11-28)	9 (5-16)	25 (20-35)	26 (5-38)	9 (19-30)
Female (%)	46 (30)	105 (50)	38 (25)	38 (45)	227 (38)
G6PD-deficient (%)	4 (3)	33 (16)	35 (23)	0	72 (12)
Hemoglobin (g/dL)	12.5 [12.1, 12.8]	11.2 [11.0, 11.4]	13.6 [13.4, 13.9]	11.5 [11.0, 12.0]	12.2 [12.0, 12.4]
Anemic (Hb < 10 g/dL) (%)	17 (11)	43 (20)	2 (1)	18 (21)	80 (13)
Primaquine dose (mg/kg)	0.90 (0.8-4.20]	0.25 (0-0.25)	0 (0-0.24)	0.26 (0.21-0.31)	0.25 (0-0.64)

Means and 95% confidence intervals are presented for baseline hemoglobin levels. Data are otherwise number (%) or median (interquartile range). Some percentages do not add up to 100 because of rounding. *G6PD* glucose-6-phosphate dehydrogenase, *Hb* hemoglobin

Table 3.6. Stratum specific-estimates of mean differences and fractional reductions in hemoglobin from baseline by G6PD status among malaria-infected patients who returned for a scheduled day 7 visit, by site and dose received (n = 604)

		Mean difference (g/dL)				Fractional reduction (%)			
Site		**G6PD normal**		**G6PD-deficient**		**G6PD normal**		**G6PD-deficient**	
Dose (mg/kg)	*Total*	*N*	*Estimate [95% CI]*	*N*	*Estimate [95% CI]*	*N*	*Estimate [95% CI]*	*N*	*Estimate [95% CI]*
Bangladesh									
0	1	1	1.10	-	-	1	12.36	-	
>0.5	152	148	-.86 [-1.17, -.55]	4	-2.75 [-4.96, -0.54]	148	-6.13 [-8.69, -3.57]	4	-19.03 [-34.24, -
Tanzania									
0	105	86	-.83 [-1.09, -.58]	19	-.88 [-1.41, -.36]	86	-6.85 [-9.02, -4.68]	19	-7.81 [-12.50, -3.11]
≤0.25	40	35	-.86 [-1.18, -.54]	5	-1.24 [-2.10, -.38]	35	-7.26 [-9.97, -4.55]	5	-12.18 [-19.68, -4.68]
>0.25 - ≤0.5	66	57	-.77 [-1.07, -.48]	9	-1.12 [-1.68, -.57]	57	-6.42 [-9.08, -3.75]	9	-9.56 [-14.66, -4.47]
Senegal									
0	83	65	-1.65 [-1.98, -1.32]	18	-1.17 [-1.84, -.51]	65	-11.49 [-13.69, -9.30]	18	-8.17 [-12.92, -3.41]
≤0.25	45	34	-1.65 [-2.17, -1.15]	11	-1.85 [-3.17, -.54]	34	-11.75 [-15.47, -8.02]	11	-13.13 [-22.57, -3.70]
>0.25 - ≤0.5	27	21	-1.29 [-1.83, -.75]	6	-1.83 [-2.82, -.85]	21	-8.58 [-12.61, -4.54]	6	-13.65 [-20.85, -6.46]
Swaziland[a]									
≤0.25	38	38	-1.19 [-1.78, -.61]	0	-	38	-8.18 [-12.46, -3.89]	0	
>0.25 - ≤0.5	47	47	-.10 [-.43, .23]	0	-	47	-.17 [-3.34, 2.99]	0	
TOTAL	604	532	-.96 [-1.1, -.83]	72	-1.34 [-1.69, -1.00]	532	-7.05 [-8.10, -5.99]	72	-10.34 [-12.93, -7.76]

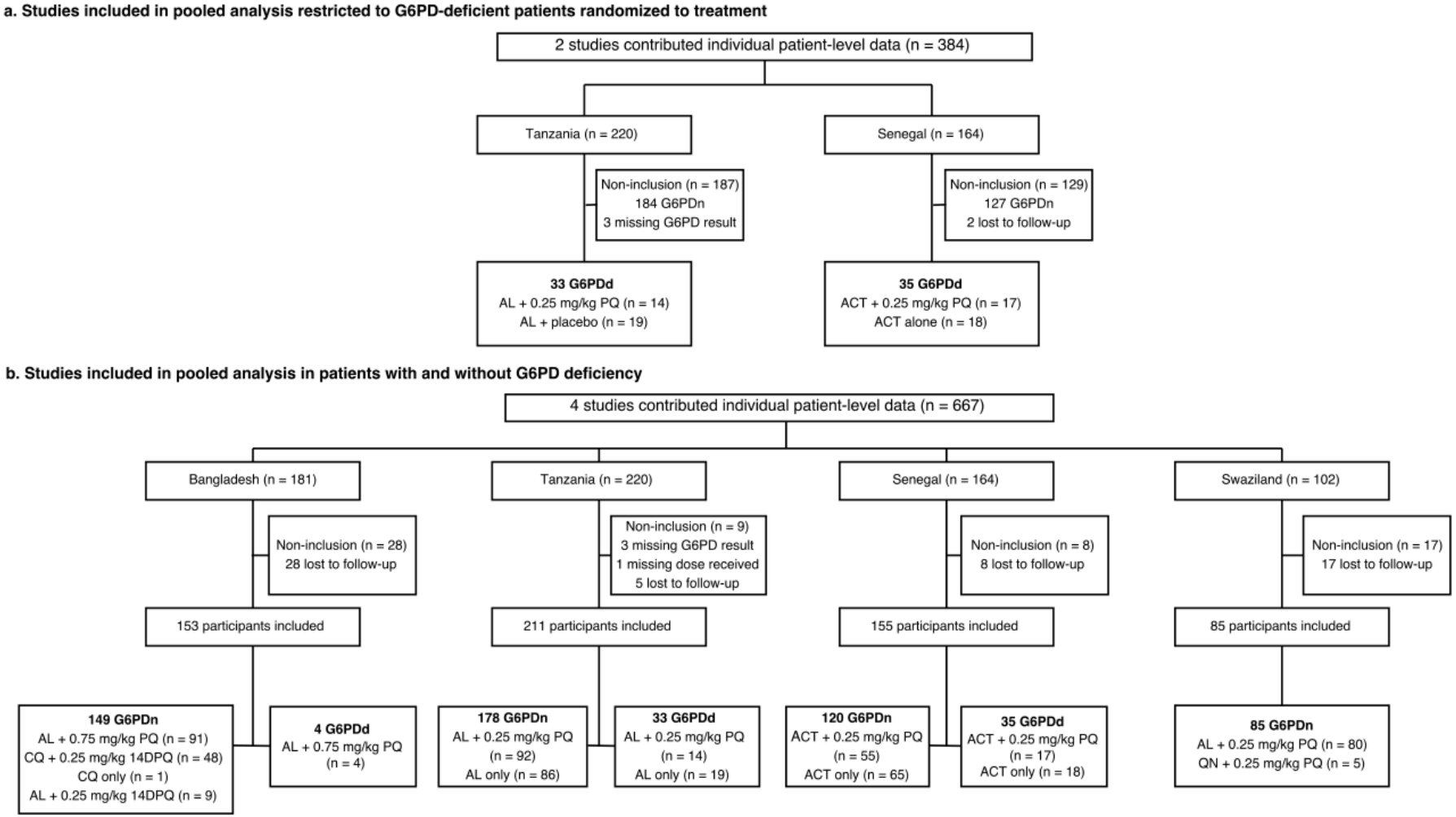
[a]No G6PDd individuals in sample. *CI* confidence interval, *G6PD* glucose-6-phosphate dehydrogenase

Table 3.7. Pooled adjusted analysis of absolute and fractional hemoglobin changes from baseline by G6PD phenotype among malaria-infected patients who returned for a scheduled day 7 visit (n = 604)

	Primaquine dose group (mg/kg)			
	0	*≤0.25*	*>0.25 - ≤0.5*	*>0.5*
Mean difference (g/dL)	Estimate [95% CI]			
G6PD normal	-1.18 [-1.42, -0.94]	-1.27 [-1.56, -0.99]	-0.63 [-0.90, -0.37]	-0.86 [-1.1, -0.62]
G6PD-deficient	-0.98 [-1.47, -0.50]	-1.38 [-2.13, -0.63]	-1.19 [-1.97, -0.42]	-2.75 [-4.22, -1.28]
Adjusted between-group difference	.20 [-.34, .74] p = 0.50	-.11 [-.91, .70] p = 0.80	-.56 [-1.38, .26] p = 0.18	-1.89 [-3.38, -.40] p = 0.01
Fractional reduction (%)	Estimate [95% CI]			
G6PD normal	-8.77 [-10.70, -6.85]	-9.31 [-11.61, -7.02]	-4.64 [-6.76, -2.52]	-6.13 [-8.08, -4.18]
G6PD-deficient	-7.72 [-11.61, -3.82]	-10.82 [-16.84, -4.79]	-9.48 [-15.69, -3.27]	-19.03 [-30.86, -7.19]
Adjusted between-group difference	1.06 [-3.29, 5.40] p = 0.60	-1.51 [-7.98, 4.97] p = 0.60	-4.84 [-11.43, 1.74] p = 0.10	-12.90 [-24.89, -.90] p = 0.04

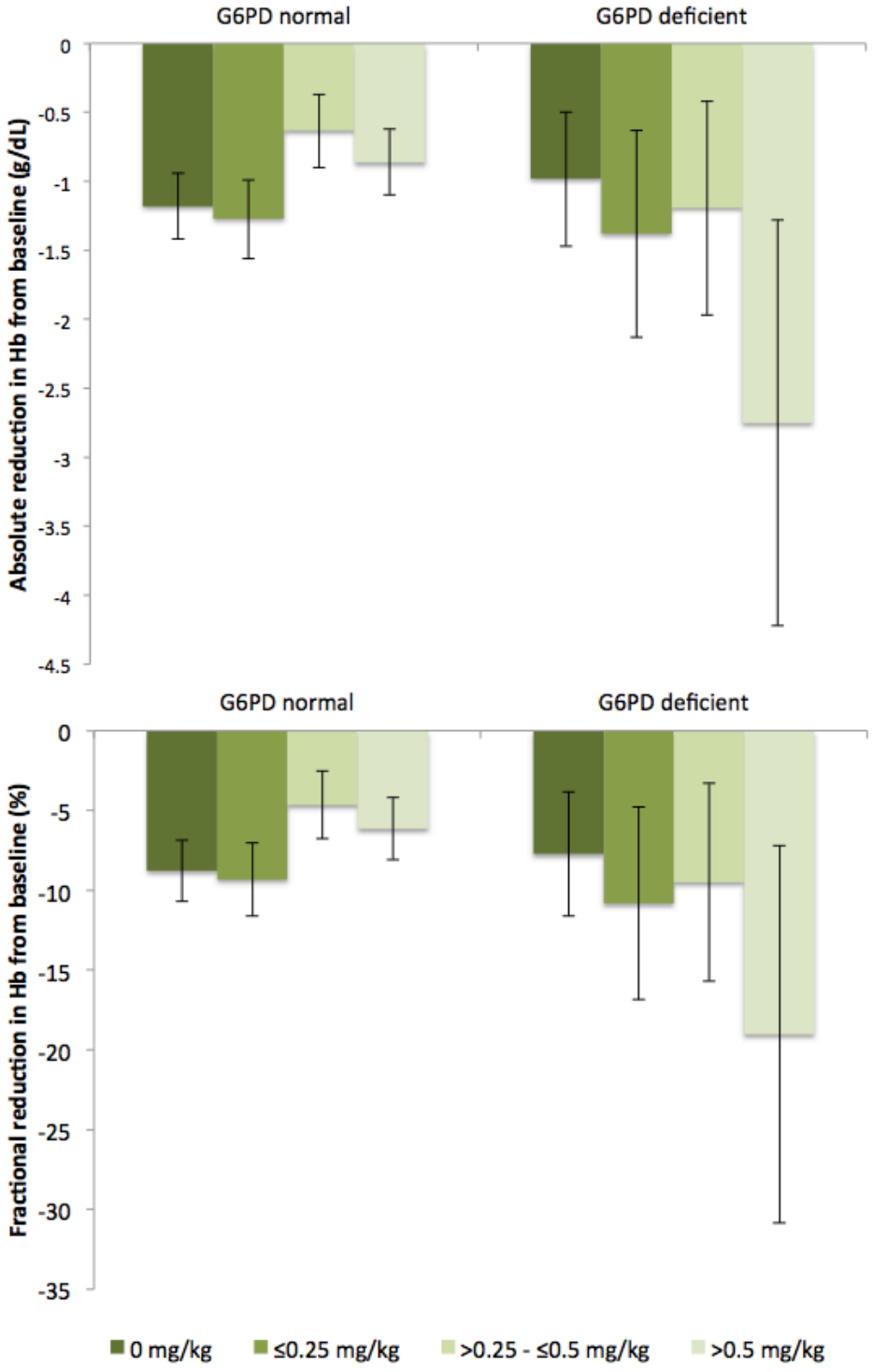
Estimates obtained from linear regression models using site as a fixed effect. P values are comparing G6PD-deficient to G6PD normal individuals. *CI* confidence interval, *G6PD* glucose-6-phosphate dehydrogenase

Figure 3.1. Flow chart of studies providing individual patient data for pooled analyses. a. Pooled analysis restricted to G6PD-deficient patients randomized to treatment (ACT only or ACT plus 0.25 mg/kg PQ). b. Pooled analysis in patients with and without G6PD deficiency



Participants were considered to have complete follow-up if they presented for a scheduled day 7 follow-up visit. *ACT* artemisinin-based combination therapy, *AL* artemether-lumefantrine, *CQ* chloroquine, *QN* quinine, *G6PDd* glucose-6-phosphate dehydrogenase-deficient, *G6PDn* glucose-6-phosphate dehydrogenase normal, *PQ* primaquine, *14DPQ* 14-day primaquine

Figure 3.2. Adjusted mean differences (a) and fractional reductions (b) in hemoglobin by G6PD phenotype and primaquine dose group



G6PD glucose-6-phosphate dehydrogenase deficiency, *Hb* hemoglobin

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