VOLUME 00 NO 00

Consequences of restricting antimalarial drugs to rapid diagnostic test-positive febrile children in south-west Nigeria

Catherine Olufunke Falade[1], Adebola Emanuel Orimadegun[2], Obaro Stanley Michael[1], Hannah Odunola Dada-Adegbola[3], Oluwatoyin Oluwafunmilayo Ogunkunle[2], Joseph Ayotunde Badejo[1], Roland Ibenipere Funwei[1,4], IkeOluwapo Oyeneye Ajayi[5], Ayodele Samuel Jegede[6], Olusola Daniel Ojurongbe[7], James Ssekitooleko[8], Ebenezer Baba[8], Prudence Hamade[9], Jayne Webster[10] and Daniel Chandramohan[10]

1 *Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria*
2 *Department of Paediatrics, College of Medicine, University of Ibadan, Ibadan, Nigeria*
3 *Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
4 *Department of Pharmacy Technician Studies, Bayelsa State College of Health Technology, Otuogidi, Nigeria*
5 *Department of Epidemiology & Medical Statistics, College of Medicine, University of Ibadan, Ibadan, Nigeria*
6 *Department of Sociology, Faculty of The Social Sciences, University of Ibadan, Ibadan, Nigeria*
7 *Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Osogbo, Nigeria*
8 *Malaria Consortium Regional Office for Africa, Kampala, Uganda*
9 *Malaria Consortium, London, UK*
10 *London School of Tropical Medicine and Hygiene, London, UK*

Abstract

OBJECTIVES To investigate the consequence of restricting antimalarial treatment to febrile children that test positive to a malaria rapid diagnostic test (MRDT) only in an area of intense malaria transmission.

METHODS Febrile children aged 3–59 months were screened with an MRDT at health facilities in south-west Nigeria. MRDT-positive children received artesunate–amodiaquine (ASAQ), while MRDT-negative children were treated based on the clinical diagnosis of non-malaria febrile illness. The primary endpoint was the risk of developing microscopy-positive malaria within 28 days post-treatment.

RESULTS 309 (60.5%) of 511 children were MRDT-positive while 202 (39.5%) were MRDT-negative at enrolment. 18.5% (50/275) of MRDT-positive children and 7.6% (14/184) of MRDT-negative children developed microscopy-positive malaria by day 28 post-treatment ($\rho = 0.001$). The risk of developing clinical malaria by day 28 post-treatment was higher among the MRDT-positive group than the MRDT-negative group (adjusted OR 2.74; 95% CI, 1.4, 5.4). A higher proportion of children who were MRDT-positive at enrolment were anaemic on day 28 compared with the MRDT-negative group (12.6% *vs*. 3.1%; $\rho = 0.001$). Children in the MRDT-negative group made more unscheduled visits because of febrile illness than those in MRDT-positive group (23.2% *vs*. 12.0%; $\rho = 0.001$).

CONCLUSION Restricting ACT treatment to MRDT-positive febrile children only did not result in significant adverse outcomes. However, the risk of re-infection within 28 days was significantly higher among MRDT-positive children despite ASAQ treatment. A longer-acting ACT may be needed as the first-line drug of choice for treating uncomplicated malaria in high-transmission settings to prevent frequent re-infections.

keywords consequences, malaria RDT for malaria diagnosis

Introduction

Malaria continues to be a major cause of morbidity and mortality in sub-Saharan Africa in spite of the scaling-up of interventions for its control [1]. In 2013, it was estimated that 100 million cases of malaria and more than 300 000 malaria-related deaths occurred in Nigeria [2]. In areas with a high burden of malaria, early diagnosis and effective treatment of childhood fevers remains one of the key malaria control strategies [3–6]. Accurate parasite-based diagnosis at all levels of the healthcare delivery system is crucial in order to promote early and

effective treatment of childhood malaria [7]. This will also lead to early identification and appropriate management of non-malarial childhood fevers by encouraging institution of further laboratory tests for identification of causes of non-malarial fevers such as bacterial infections. Furthermore, a confirmative diagnosis of malaria is essential to support an effective surveillance system in areas where the prevalence of malaria has been decreasing [8]. The emergence and spread of strains of *Plasmodium falciparum* resistant to chloroquine and sulfadoxine-pyrimethamine, which necessitated the use of the more expensive ACTs, further underscores the need for the confirmation of malaria diagnosis, in order to facilitate more targeted treatment [9].

The current Nigerian national treatment guidelines for diagnosis and treatment of malaria, in line with the WHO directive [9,10], recommend that, where possible, malaria diagnosis should be confirmed with microscopy or rapid diagnostic test prior to starting treatment. The major advantage of this treatment policy is that patients with malaria will receive timely treatment, and those with non-malaria febrile illnesses can be investigated and managed appropriately early [6,11]. As microscopy is often not available in primary healthcare facilities, malaria rapid diagnostic tests (MRDT), which require minimal training to use and a short waiting time for the results, are the viable options for parasite-based diagnosis in lower-level health facilities [10,11]. However, false-positive and false-negative results from MRDT are not uncommon. Malaria antigenemia clears very slowly, and so false-positive MRDT results may occur within 2–4 weeks of a successfully treated malaria infection [12–14]. Cross-reactivity of some heterophile antigen, such as in patients with rheumatoid arthritis, may also lead to a false-positive result [15]. False-negative results usually occur at low parasite densities, but also occasionally at significantly high parasite densities, in what is referred to as 'the prozone effect' [16]. Although uncommon, false-negative results have also been observed where short molecules of the HRP-2 gene have been deleted [14,17] or where overwhelming infection and high levels of antigen have bypassed the collection mechanism of the device.

A major concern in implementing MRDT-based treatment for children with febrile illness is the risk of a false-negative result leading to a missed opportunity for administering appropriate treatment. Restricting the use of ACT to MRDT-positive children would deny the MRDT-negative children, who would have received an antimalarial treatment based upon presumptive diagnosis of malaria, the benefit of the post-treatment chemoprophylactic effect. This could, in theory, lead to increase frequency and severity of clinical malaria in children who are denied ACT on account of negative MRDT results. There is also concern for patients with no or little malaria immunity, particularly infants who would be left without antimalarial therapy due to a false-negative RDT that the risk of developing severe malaria may be high [18]. However, a study from Benin reported that treating only MRDT-positive children for malaria provided adequate management of fevers among children aged 5–15 years [19].

A better understanding of treatment outcomes of MRDT-negative children has the potential to enhance the acceptability of MRDTs by healthcare workers and caregivers of children. Thus, there is a need to investigate treatment outcomes of MRDT-based diagnosis of febrile illnesses in children under 5 years old. This study, therefore, addresses the question of whether or not limiting ACT treatment to MRDT-positive febrile children only results in significant adverse outcomes within 28 days post-treatment.

Methods

Study design and subjects

This prospective cohort study compared the risk of malaria during the 28-day post-treatment period among MRDT-positive children who received an ACT (MRDT-positive group), and MRDT-negative children who did not receive antimalarial treatment (MRDT-negative group) at enrolment. Children aged 3–59 months presenting with fever (axillary temperature ≥ 37.5 °C) or history of fever within 48 h at the study health facilities were enrolled. Participants were enrolled consecutively from the middle of November 2013 to the middle of November 2014. The following children were excluded from the study: (i) resided > 15 km away from the study health facilities; (ii) parent/caretaker refused informed consent; (iii) child had a history of allergy to artesunate or amodiaquine; (iv) had a danger sign or symptoms/signs of severe malaria as defined by the WHO [20].

Study area and sites

The study was carried out in an urban secondary healthcare facility (St Mary's Hospital, Eleta, Ibadan) and in a rural primary health centre (Idi-Ayunre in Oluyole LGA, Oyo State). St. Mary's Hospital is used by a wide range of socio-demographic groups from the city of Ibadan, while Idi-Ayunre PHC (located 35 km away from Ibadan) is used mainly by the population from the surrounding villages. All laboratory investigations except MRDT

testing were carried out at the University College Hospital, Ibadan; the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan; and the Molecular biology Laboratory of Ladoke Akintola University, Oshogbo (LAUTECH).

Sample size

The primary endpoint for estimating the sample size was the risk of clinical malaria within 28 days of initiating treatment at the time of enrolment among MRDT-negative children, compared with that in MRDT-positive children. We assumed that the relative risk of clinical malaria (RR) in MRDT-negative febrile children who were not given ACT, compared with MRDT-positive children who were given ACT, will be 2.0. A previous study in Ibadan showed that 7% of children aged 6 months to 10 years treated with artesunate–amodiaquine (ASAQ) had clinical malaria by day 28 [21]. Thus, to detect a RR of 2 at 95% significance and 90% power, a sample size of 430 per group will be required. Assuming the loss to follow-up at day-28 post-treatment would be about 10%, we estimated that the study will require 956 febrile children. However, the study was able to enrol only 511 children over the study period.

Enrolment and follow-up procedures

Eligible children were screened for malaria using an HRP II-based malaria RDT (MRDT) [SD Bioline™, Standard Diagnostics Inc, Yongin-si, Gyeonggi-do, Korea]. At enrolment, data on demographic and socio-economic characteristics, the history of current and past illnesses and any previous treatments received were collected. Children were examined by a medical officer, and relevant clinical findings were documented. In addition to conducting a MRDT, blood samples were collected for malaria microscopy, PCR and for investigating other causes of febrile illness.

MRDT-positive children were treated with a fixed-dose preparation of artesunate plus amodiaquine (ASAQ™; Sanofi Aventis, Bridgewater, NJ, USA) at standard dosage under supervision on days 0, 1 and 2. MRDT-negative children were treated with an appropriate drug regimen based upon the diagnosis of non-malarial febrile illness. Both MRDT-positive and MRDT-negative cohorts were followed up at the health facilities on days 1, 2, 3, 7, 14, 21 and 28 in order to assess the resolution of clinical symptoms and signs. Capillary blood samples were collected on days 7, 14, 21 and 28 post-treatment for repeat haematocrit, MRDT and thick blood smear for malaria parasites detection and quantification. MRDT-negative children who became MRDT-positive during follow-up or unscheduled visits were treated with ASAQ™.

Laboratory procedures

Dried thick blood smears were stained with 10% fresh Giemsa at pH 7.2 and were read by two independent microscopists blinded to the MRDT results for presence and quantification of malaria parasites. Parasite density was calculated by counting asexual malaria parasites against roughly 200 white blood cells (WBC) using an assumed total white cell count of 8000/mm^3. A blood smear was considered negative if no parasites at asexual stage were seen after 100 high power fields had been screened. Discordant results were read by a senior investigator (COF); otherwise, the mean of parasite densities by the two microscopists was recorded as the final reading.

DNA was extracted using QIAamp DNA Mini kit™ (QIAGEN Sciences, Germantown, MD, USA) according to the manufacturer's instructions. Genotype of each *P. falciparum* isolate was characterised based upon the fragment size of alleles of Merozoite surface protein-1 (*msp-1*), *msp-2* and Glutamate Rich Protein (*glurp)* after amplification of 18srRNA by nested PCR, to detect *Plasmodium specie*. Infections were defined as polyclonal if parasites in matched primary and post-treatment samples from the same patient showed more than one allele of K1, MAD20 or RO33 and FC27 or IC1/3D7 families of *msp-1 and msp-2,* respectively. If an isolate had one allele at each of the families, the clone number was taken to be one. Absence of allelic identity in the three allelic families of *msp-1* (K1, MAD20 and RO33) or the two families of *msp-2* in matched primary and post-treatment samples indicated a newly acquired infection.

Blood culture for bacteraemia was carried out using the Bactec Technique [22]. Isolation and sensitivity testing of bacteria from midstream urine, throat, ear and skin swabs were carried out using standard techniques.

Study consent and ethical approval

Ethical approvals for the study protocol were obtained from University of Ibadan/University College Hospital Ethical Review Committee, the Oyo State Ministry of Health Ethics Committee and The London School of Tropical Medicine and Hygiene IRB. Participation in the study was voluntary and based on written or witnessed verbal informed consent of parent or caregiver of children who fulfilled the inclusion criteria. The study conformed to the principles embodied in the Declaration of Helsinki.

Statistical analysis

All data were double-entered in a database and verified/cleaned using EpiData Version 3.1 [23]. Data analysis was performed using SPSS for Windows 18.0 (IBM Corp., Armont, NY, USA). The primary endpoint was clinical malaria defined as the detection of asexual malaria parasite by microscopy at any time point between days 7 and 28 post-initiation of treatment at enrolment. The result of microscopy was used as reference to assess the performance of MRDT. The socio-economic background of the family was classified using the method described by Oyedeji [24], in which scores were awarded to each child, based on the occupations and educational attainment of the parents or their substitutes. Average scores of 1 and 2 were assigned 'high', a score of 3 was assigned 'middle' while scores of 4 and 5 were assigned 'low'. The Chi-square test was used to compare categorical variables while Student *t*-test was used to test associations between continuous independent variables and the dependent variable. Logistic regression analysis was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of clinical malaria within 28 days of treatment in MRDT-negative *vs*. MRDT-positive children. All covariates with a ρ-value < 0.05 in bivariate analysis were included in the final multivariable logistic model.

With the corresponding results of the microscopy taken as the 'gold standard', the sensitivity and specificity of malaria RDT were calculated as TP/(TP + FN) and TN/(TN + FP), respectively. Positive predictive values (PPV) were calculated as TP/(TP + FP) and negative predictive values (NPV) as TN/(TN + FN), where TP = True positive, TN = True negative, FP = False positive, FN = False negative. Accuracy which is defined as the proportion of the test method that gives a positive result correctly was calculated using the following formula—(TP + TN/TP + TN + FP + FN).

Results

Four hundred and twenty-nine of the 511 (84%) children enrolled completed the study (Figure 1). Of the 82 (16%) children who dropped out, two from the MRDT-positive group were referred to other health facilities for further treatment, parents of 25 children withdrew consent and 55 were lost at the follow-up. The parents of most of the children lost to follow-up moved out of the study area. The dropout rate in the MRDT-positive (17.2%) and MRDT-negative (14.4%) groups did not differ significantly (P = 0.422). The age and gender distribution of the children who dropped out from the MRDT-positive and MRDT-negative groups were also similar.

At enrolment, 309 children (60.5%) were MRDT-positive while 202 (39.5%) were MRDT-negative. A higher proportion of MRDT-positive children was from the rural than the urban site (80.9% *vs*. 19.1%; ρ < 0.001, adjusted OR = 3.58, 95% CI = 2.30, 5.57). A comparison of the socio-demographic characteristics in MRDT-positive and MRDT-negative children is shown in Table 1.

The mean age of MRDT-positive children was higher than that of the MRDT-negative group (29.4 vs 21.9 months; P < 0.001). The proportion of children from the lower social class was higher among the MRDT-positive group than in the MRDT-negative group (35.0% *vs*. 18.8%; P < 0.001).

The proportion of febrile (temp ≥ 37.4°C) children having malaria parasitaemia at enrolment was significantly higher among children in the rural than the urban site (53.4 *vs*. 24.0%; P < 0.001, adjusted OR = 3.2, 95% CI = 2.07, 5.02). The geometric mean parasite densities were 8206 (range, 20–360 000/μL) and 6774 (range, 40–611 600/μL) for the rural and urban site, respectively (ρ = 0.081).

Risk of malaria during 28 days of follow-up post-treatment

Sixty-four of 454 (14.1%) children who completed follow-up had microscopy-positive malaria on or before day 28 (Table 2).

Fifty of 275 (18.5%) children were in the MRDT-positive group, while 14/184 (7.6%) belonged to the MRDT-negative group (ρ = 0.001). The risk of developing clinical malaria (including recrudescence and re-infection) between days 1 and 28 post-treatment was higher among the MRDT-positive group (adjusted OR 2.74; 95% CI 1.4, 5.4) (Table 2). *Plasmodium falciparum* in 30 paired blood spots (taken at enrolment and parasite recurrence) that were successfully extracted and genotyped using *msp-1, msp-2* and *glurp*, were confirmed as re-infections since the parasite profiles of day 0 and recrudescence day were different.

Risk of anaemia and other clinical outcomes

The proportion of children having anaemia (haematocrit < 30%) was higher in the MRDT-positive group at enrolment (37.9% *vs*. 10.0%; ρ = <0.0001) (Table 3).

The proportion of children with anaemia at day 28 post-treatment reduced in both groups. However, a significantly higher proportion of children in the MRDT-positive group were anaemic compared with MRDT-negative children at day 28 post-treatment (12.6% *vs*. 3.1%, ρ = 0.001).

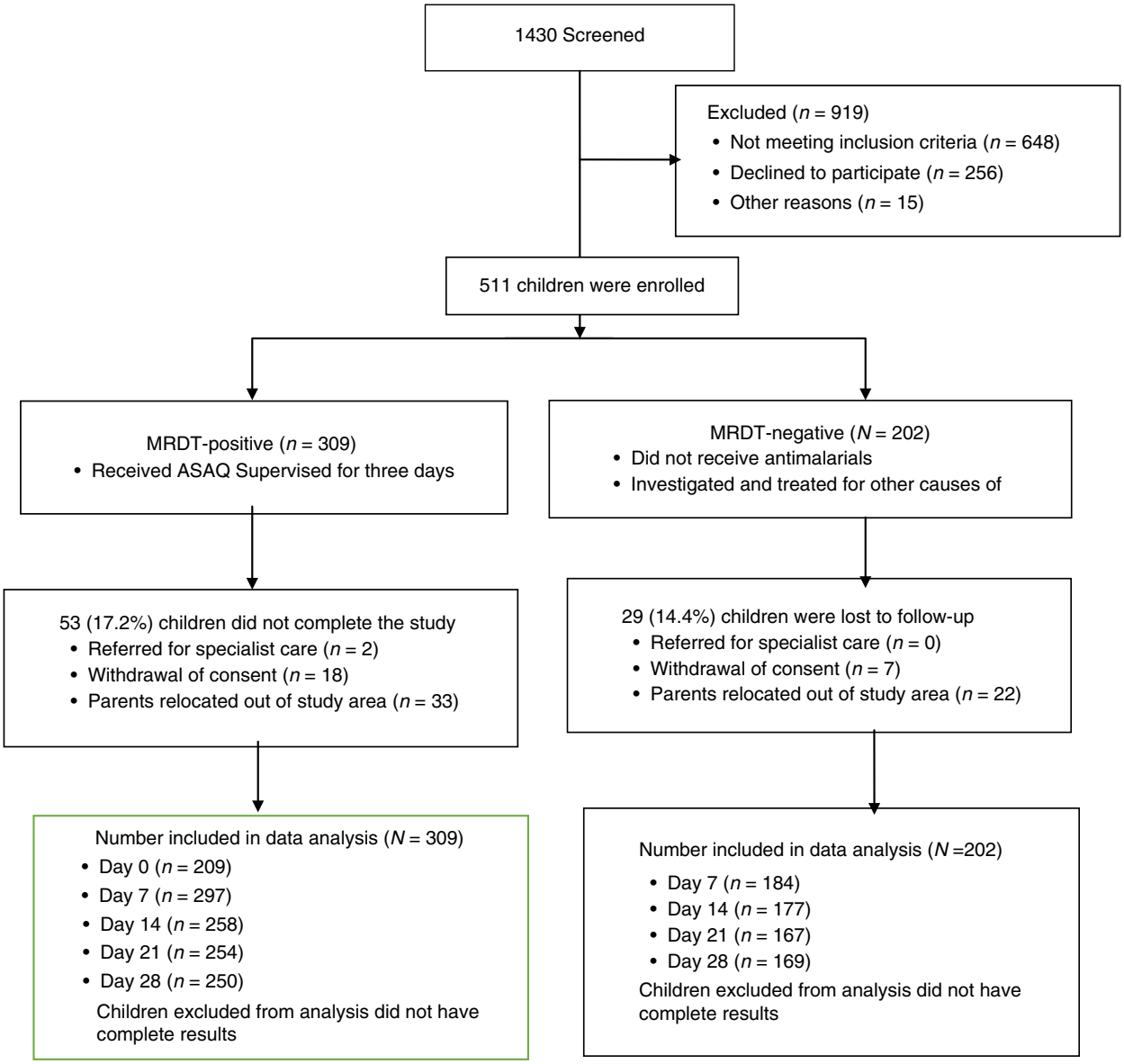

Figure 1 Study flow chart for consequences of restricting ACT treatment to malaria RDT-positive children only.

Two children from the MRDT-positive group required referral to a tertiary care hospital on account of severe anaemia. 70% (216/309) of children had blood slide-positive malaria at enrolment in the MRDT-positive group and 84.3% (182/216) of them completed day 28 follow-up. The day 28 adequate clinical and parasitological cure rate (ACPR) for ASAQ among these children was 78.0% (142/182). There was no case of early treatment failure. Twenty-five (13.7%) had late parasitological failure while 15 (8.2%) had late clinical failure. After PCR correction (30/40 cases were re-infection), the day 28 ACPR of the per-protocol population was 94.5%. Twenty-nine (14.4%) children who were MRDT-negative at day 0 converted to MRDT-positive before day 28. There was no significant difference in the conversion rate among children enrolled at the rural (16.5%) and urban site (11.5%) ($\rho = 0.312$).

Eighty-four (16.4%) children made at least one unscheduled visit during the 28-day follow-up period because of their caregiver's concerns about a delay in

Table 1 Socio-demographic characteristics of under 5 years old children with fever in south-west Nigeria

Variables	Total population (*N* = 511)	MRDT-positive (*N* = 309)	MRDT-negative (*N* = 202)	*ρ*-value	UOR	95% CI	AOR	95% CI
Study sites								
Rural	343 (67.1)	250 (80.9)	115 (56.9)	<0.001	3.21	2.15, 4.77	3.58	2.30, 5.57
Urban	168 (32.9)	59 (19.1)	87 (43.1)		1	–	1	–
Gender								
Male, *n* (%)	301 (58.9)	188 (60.8)	113 (55.9)	0.271	1.22	0.85, 1.75	1.16	0.78, 1.72
Female, *n* (%)	210 (41.1)	121 (39.2)	89 (44.1)		1	–	1	–
Mean age (months)	26.4 ± 15.6	29.4 ± 15.3	21.9 ± 15.0	<0.001	1.03	1.02, 1.05	1.04	1.03, 1.05
Nutritional status								
Malnourished, *n* (%)*	61 (11.9)	41 (13.3)	20 (9.9)	0.251	0.72	0.41, 1.27	0.61	0.33, 1.13
Not-Malnourished, *n* (%)	450 (88.1)	268 (86.7)	182 (90.1)		1	–	1	–
Ethnicity								
Yoruba, *n* (%)	447 (87.5)	266 (86.1)	181 (89.6)	0.500	1	–	1	–
Ibo, *n* (%)	30 (5.9)	20 (6.5)	10 (5.0)		1.36	0.62, 2.98	0.79	0.34, 1.83
Others, *n* (%)	34 (6.7)	23 (7.4)	11 (5.4)		1.42	0.68, 2.99	1	–
Number of mother's children								
1–4, *n* (%)	422 (82.6)	248 (80.3)	174 (86.1)	0.087	0.65	0.40, 1.07	0.79	0.46, 1.35
>4, *n* (%)	89 (17.4)	61 (19.7)	28 (13.9)		1	–	1	–
Social class								
High, *n* (%)	60 (11.7)	25 (8.0)	35 (17.3)	<0.001	1	–	1	–
Middle, *n* (%)	305 (59.7)	176 (57.0)	129 (63.9)		5.10	1.07, 9.37	5.91	1.07, 8.44
Low, *n* (%)	146 28.6)	108 (35.0)	38 (18.8)		11.36	2.31, 19.91	13.16	2.26, 22.23

UOR, unadjusted odds ratio; AOR, adjusted odds ratio.
*Weight-for-age Z-score < 2.

symptoms being resolved. A higher proportion of children from the MRDT-negative group made unscheduled visits than from the MRDT-positive group (23.2% *vs*. 12.0%; $\rho = 0.001$).

Causes of fever among study children

Malaria diagnosed by microscopy was the most common infection 44.4% (227/511), followed by bacterial infection 27.59% (141/511) and presumptive viral infections 32.48% (166/511). The distribution of the causes of febrile illness between day 0 and day 28 among the MRDT-positive and MRDT-ve groups is shown in Table 4.

Performance of SD Bioline™ malaria rapid diagnostic test compared with microscopy

There were ten cases of false-negative MRDT results and 93 cases of false-positive MRDT results. The geometric mean parasite density of the cases with false-negative result was 480/μL (range 76–5539 µL), while that for true positive was 8888/µL (range 40–611 600/µL). Compared with malaria microscopy results as the reference standard, the sensitivity of SD Bioline™ RDT for the diagnosis of malaria on day 0 was 95.6%. Specificity was 67.4%, however, while positive and negative predictive values were 69.9% and 95.1%, respectively. The estimated accuracy for SD Bioline™ RDT in this study was 79.8%.

Discussion

Our results show that restricting ACT treatment to MRDT-positive children only does not result in significant adverse health outcomes. In addition, the risk of developing microscopy-positive malaria within 28 days was remarkably higher among MRDT-positive children than MRDT-negative children. The burden of malaria was higher among children in the rural study site, where it was the cause of fever in 52.9% of cases vs. 23.3% in urban study site. The MRDT-positive children came from a relatively lower socio-economic class than the MRDT-negative group. These findings are consistent with previous studies from Nigeria, which suggested that children from urban and wealthier households are less likely to report fever attributable to malaria [25–28]. Our data also showed that children in the MRDT-positive group were relatively older than the MRDT-negative group.

Table 2 Risk of developing clinical malaria (both recrudescence and new infections) within 28 days post-treatment among MRDT-positive or negative febrile children

	Microscopy results*		Unadjusted odds ratio (OR)			Adjusted odds ratio (AOR)†		
Post-treatment days MRDT-positive or -ve group	MP negative*n* (%)	MP positive*n* (%)	OR	95% CI	*ρ*-value	AOR	95% CI	*ρ*-value
Day 1–7								
Day 0 MRDT-ve	182 (98.9)	2 (1.1)	–	–	0.083	–	–	0.993
Day 0 MRDT-positive‡	275 (100.0)	0 (0.0)						
Day 1–14								
Day 0 MRDT-ve	173 (96.6)	6 (3.4)	0.22	0.05, 1.13	0.068	0.21	0.04, 1.18	0.077
Day 0 MRDT-positive‡	257 (99.2)	2 (0.8)						
Day 1–21								
Day 0 MRDT-ve	161 (94.7)	9 (5.3)	1.94	0.88, 4.26	0.095	1.87	0.81, 4.35	0.145
Day 0 MRDT-positive	231 (90.2)	25 (9.8)						
Day 1–28								
Day 0 MRDT-ve	170 (92.4)	14 (7.6)	2.76	1.48, 5.16	0.001	2.74	1.40, 5.37	0.003
Day 0 MRDT-positive	220 (81.5)	50 (18.5)						

MRDT-negative, Negative MRDT result; MRDT-negative, Positive MRDT result.
*Malaria parasite by microscopy on days 7, 14, 21 or any time within 28 days of treatment with Artesunate–amodiaquine for malaria.
†Variables included in logistic regression model: 'day 0 MRDT results', site, age and socio-economic status.
‡Fisher's exact test reported.

Table 3 Risk of anaemia among febrile under 5 years old children in Ibadan, Nigeria

Characteristic	MRDT-Positive on day 0	MRDT-Negative on day 0	*ρ*-value	UOR (95% CI)	AOR (95% CI)*
Anaemia day 0	125/309 (40.5%)	26/202 (12.9%)	<0.001	4.59 (2.87, 7.36)	5.37 (3.20, 9.02)
Anaemia day 28	29/231 (12.6%)	5/160 (3.1%)	0.001	2.33 (1.07, 5.05)	2.35 (1.02, 5.38)

Anaemia was defined as haematocrit less than 30%.
*Variables included in logistic regression model: 'day 0 MRDT results' are as follows: study site, age and socio-economic status.

Table 4 Common causes/sites of infection identified in under 5 years old febrile children in south-west Nigeria

Infectious agents	MRDT-positive on day 0	MRDT-negative on day 0
Malaria parasitaemia (by microscopy)	217/309 (69.9%)	10/202 (4.5%)
Bacterial infections		
UTI (Urine Culture)	50/247 (20.2%)	29/145 (20.2%)
Bacteraemia (Blood culture)	7/297 (2.4%)	5/196 (2.6%)
Skin infection	14 (4.5%)	23 (11.4%)
Otitis media	8 (2.6%)	5 (2.5%)
Measles	1 (0.3%)	4 (2.0%)
Probable viraemia	56 (18.1%)	105 (52%)

Possible viral infection, urinary tract infection, sepsis of skin, bacteraemia and otitis media were the common causes of the non-febrile illness among both groups.

This disparity in age among RDT-positive and RDT-negative children is consistent with the findings from south-west Nigeria [4] and Ghana [29]. A high proportion of children in the MRDT-positive group were from middle to lower socio-economic classes from the rural study site. Although children acquire immunity to malaria from repeated exposure and episodes of malaria attacks [30], it has also been reported that children from wealthier backgrounds were less likely to have malaria [31].

An important finding from this study is that the odds of clinical malaria was significantly higher in the MRDT-positive than MRDT-negative group by day 28, even after adjusting for study site, age and socio-economic background. This finding correlates with the report from Ghana [29], which showed that fewer children who tested negative to RDT and did not receive ACT developed clinical malaria within a short period compared with the children who were MRDT-positive and received

ACT in a high-transmission setting [29]. This finding suggests that the children who were MRDT-positive at enrolment have some unfavourable factors that predispose them to recurrent malaria infection. These factors include relative poverty and environmental factors that increase the risk of malaria infection. The fact that all 30 cases of parasite recurrence that were characterised by PCR were due to re-infection supports high transmission and special pre-disposition. The MRDT-negative to MRDT-positive conversion rate of 9.4% over a 28-day period appears low compared with 18.5% found in a cohort of children in Ghana [29], which, like south-west Nigeria, has malaria transmission all year round.

The prevalence of anaemia among MRDT-positive group (37.9%) in this study is comparable to that observed among children with uncomplicated malaria in Ibadan, Nigeria [32]. The finding of anaemia is not surprising, as previous studies [32–34] have demonstrated that children who have malaria are at greater risk of developing anaemia, and this was apparent even after controlling for parasitaemia and nutritional status.

The PCR uncorrected ACPR of 78% for ASAQ among the per-protocol population in this study is lower than that reported from an urban hospital setting in the same area in 2007–2008 [34]. This can be explained by very high malaria transmission in the rural study site and the possible development of tolerance to ASAQ over time. The day 28 ACPR observed in this study is lower than that reported by several studies in sub-Saharan Africa [35–38]. However, the ACPR observed in this study needs to be interpreted with caution because this is not a clinical efficacy study of ASAQ, and it did not have strict inclusion/exclusion criteria, such as specific parasite density cut off or absence of any co-morbidity. It is of note though that the PCR corrected day 28 ACPR for ASAQ was high (94.5%). Given the high level of re-infection within 28 days post-treatment in the study area, a long-acting antimalarial combination such as Dihydroartemisinin plus piperaquine needs to be considered as first-line treatment for malaria in high-transmission areas [39].

Overall, the performance of SD Bioline™ malaria Ag-*Pf* kit used in this study compares well with recent findings reported from screening of malaria patients at Bangui, Central African Republic and Nigeria [40,41]. The sensitivity of the MRDT was 95.6% and the false-negative results did not result in any adverse clinical outcomes in this study. However, in a routine healthcare delivery system without robust follow-up, one should be concerned about the consequences of false-negative MRDT results. All cases of malaria that occurred during the follow-up period in this study were detected early and offered an appropriate treatment. The procedures set up in our study to ensure adequate follow-up of the children may not be replicated in routine clinical practice. As some children with a false-negative malaria diagnosis may report late to the health facility, and thereby run the risk of developing severe malaria, restricting ACT to MRDT-positive children may still pose some danger in routine healthcare delivery systems. The recent developments of ultrasensitive MRDTs would be safer than the currently available MRDTs [42].

The estimated sample size of 959 was not achieved, and this could be a limitation of this study. However, with 7.6% of MRDT-negative group becoming microscopy positive between days 1–28 and the odds of MRDT-positive group that received an ACT becoming microscopy-positive malaria during this period was >2, we would require only 175 children in each group to achieve a power of 80%. Thus, the sample size achieved is sufficient to reach a valid conclusion regarding the risk of malaria during the 28 days post-treatment period.

In conclusion, offering antimalarial treatment to MRDT-positive children only in this high-transmission setting did not increase risk of developing malaria during the 28 days post-treatment period among MRDT-negative children. However, the risk of re-infection within 28 days was significantly higher among MRDT-positive children, even though they were treated with ASAQ. A longer-acting ACT may be needed as the first-line drug of choice for treating uncomplicated malaria in high-transmission settings to prevent re-infections.

Acknowledgement

We are grateful to all the children and their parents/guardians for participating in this study. We also thank the members of staff of St Mary's Catholic Hospital & Oluyole PHC as well as our research staff and entire study team for making this project a success. The study was made possible by a research grant from Malaria Consortium under the Support for National Malaria Programme (SuNMaP Nigeria) funded by UKaid through DFID.

References

1. WHO. World malaria report 2013. World Health Organization: Geneva, Switzerland; 2014.
2. National Population Commission (NPC) and ICF International 2014. Nigeria Demographic and Health Survey 2013. National Population Commission; 2014.
3. Chandler CIR, Jones C, Boniface G, Juma K, Reyburn H, Whitty CJM. Guidelines and mindlines: why do clinical staff over-diagnose malaria in Tanzania? A qualitative study. *Malar J* 2008: **7**: 53–56.

4. Oladosu OO, Oyibo WA. Overdiagnosis and overtreatment of malaria in children that presented with fever in Lagos, Nigeria. *ISRN Infect Dis* 2013: **2013**: 1–6.
5. Masanja MI, McMorrow M, Kahigwa E, Kachur SP, McElroy PD. Health workers' use of malaria rapid diagnostic tests (RDTS) to guide clinical decision making in rural dispensaries, Tanzania. *Am J Trop Med Hyg* 2010: **83**: 1238–1241.
6. WHO. *Guideline for the Treatment of Malaria*, 3rd edn. World Health Organization: Geneva; 2015.
7. WHO. Monitoring antimalarial drug resistance. Rep a WHO Consult Geneva, Switz 3-5 December 2001; WHO/CDS/CSR/EPH/2002.17 WHO/CDS/RBM/2002.39; 2002.
8. WHO. World Malaria Report 2015. World Health Organization: Geneva, Switzerland; 2015: 243.
9. WHO. *Guidelines for the Treatment of Malaria*. World Health Organization: Geneva, Switzerland; 2010.
10. FMOH. *National Guidelines for Diagnosis and Treatment of Malaria*. Federal Ministry of Health, National Malaria and Vector Control Division: Abuja, Nigeria; 2011.
11. Ansah EK, Narh-Bana S, Epokor M *et al*. Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: a randomised controlled trial in Ghana. *BMJ* 2010: **340**: 635.
12. Abeku TA, Kristan M, Jones C *et al*. Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. *Malar J* 2008: **7**: 202.
13. Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ, Dorsey G. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *J Infect Dis* 2008: **197**: 510–518.
14. Koita OA, Doumbo OK, Ouattara A *et al*. False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. *Am J Trop Med Hyg* 2012: **86**: 194–198.
15. Lee JH, Jang JW, Cho CH *et al*. False-positive results for rapid diagnostic tests for malaria in patients with rheumatoid factor. *J Clin Microbiol* 2014: **52**: 3784–3787.
16. Luchavez J, Baker J, Alcantara S *et al*. Laboratory demonstration of a prozone-like effect in HRP2-detecting malaria rapid diagnostic tests: implications for clinical management. *Malar J* 2011: **10**: 286.
17. Amoah LE, Abankwa J, Oppong A. Plasmodium falciparum histidine rich protein-2 diversity and the implications for PfHRP 2: based malaria rapid diagnostic tests in Ghana. *Malar J* 2016: **15**: 101.
18. Bjorkman A, Martensson A. Risks and benefits of targeted malaria treatment based on rapid diagnostic test results. *Clin Infect Dis* 2010: **51**: 512–514.
19. Faucher JF, Makoutode P, Abiou G *et al*. Can treatment of malaria be restricted to parasitologically confirmed malaria? A school-based study in Benin in children with and without fever. *Malar J* 2010: **21**: 104.
20. WHO. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg* 2000: **94**(Suppl 1): S1–S90.
21. Falade CO, Ogundele AO, Yusuf BO, Ademowo OG, Ladipo SM. High efficacy of two artemisinin-based combinations (artemether- lumefantrine and artesunate plus amodiaquine) for acute uncomplicated malaria in Ibadan, Nigeria. *Trop Med Int Heal* 2008: **13**: 635–643.
22. Sorlin P, Mansoor I, Dagyaran C, Struelens MJ. Comparison of resin-containing BACTEC(TM) plus Aerobic/F* medium with conventional methods for culture of normally sterile body fluids. *J Med Microbiol* 2000: **49**: 787–791.
23. Lauritsen J, Bruus M. EpiData (version 3). A comprehensive tool for validated entry and documentation of data Odense: EpiData Association (2003–2008).
24. Oyedeji G. Socio-economic and cultural background of hospitalized children in Ilesha, Nigeria. *Niger J Paediatr* 1985: **12**: 111–118.
25. National Population Commission of Nigeria. Nigeria Demographic and Health Survey 2013. National Population Commission; 2014.
26. Novignon J, Nonvignon J. Socioeconomic status and the prevalence of fever in children under age five: evidence from four sub-Saharan African countries. *BMC Res Notes* 2012: **5**: 380.
27. Yusuf OB, Adeoye BW, Oladepo OO, Peters DH, Bishai D. Poverty and fever vulnerability in Nigeria: a multilevel analysis. *Malar J*. 2010: **9**: 235.
28. Gayawan E, Arogundade ED, Adebayo SB. A bayesian multinomial modeling of spatial pattern of co-morbidity of malaria and non-malarial febrile illness among young children in Nigeria. *Trans R Soc Trop Med Hyg* 2014: **108**: 415–424.
29. Baiden F, Webster J, Tivura M, Delimini R, Berko Y, Amenga-Etego S *et al*. Accuracy of rapid tests for malaria and treatment outcomes for malaria and non-malaria cases among under-five children in rural, Ghana. *PLoS ONE* 2012: **7**: e34073.
30. Baird JK. Age-dependent characteristics of protection v. susceptibility to *Plasmodium falciparum*. *Ann Trop Med Parasitol* 1998; **92**: 367–390.
31. National Malaria Elimination Programme (NMEP). National Population Commission (NPopC), National Bureau of Statistics (NBS), and ICF International. 2016. Nigeria Malaria Indicator Survey 2015. Abuja, Nigeria, and Rockville, Maryland, USA: NMEP, NPopC, and ICF International.
32. Sowunmi A, Gbotosho GO, Happi CT, Fateye BA. Factors contributing to anaemia after uncomplicated *Plasmodium falciparum* malaria in children. *Acta Trop* 2010: **113**: 155–161.
33. Price RN, Simpson JA, Nosten F *et al*. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg* 2001: **65**: 614–622.
34. Falade CO, Dada-Adegbola HO, Ogunkunle OO, Oguike MC, Nash O, Ademowo OG. Evaluation of the comparative efficacy and safety of artemether-lumefantrine, artesunate-amodiaquine and artesunate-amodiaquine-chlorpheniramine (Artemoclo™) for the treatment of acute uncomplicated

malaria in Nigerian children. *Med Princ Pract* 2014: **23**: 204–211.
35. Abuaku B, Duah N, Quaye L *et al.* Therapeutic efficacy of artesunate-amodiaquine and artemether-lumefantrine combinations in the treatment of uncomplicated malaria in two ecological zones in Ghana. *Malar J* 2016: **15**: 6.
36. Singana BP, Bogreau H, Matondo BD *et al.* Malaria burden and anti-malarial drug efficacy in Owando, northern Congo. *Malar J* 2016: **15**: 6.
37. Yavo W, Konaté A, Kassi FK *et al.* Efficacy and safety of artesunate-amodiaquine versus artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in sentinel sites across Côte d'Ivoire. *Malar Res Treat* 2015: **2015**: 1–8.
38. Schramm B, Valeh P, Baudin E *et al.* Efficacy of artesunate-amodiaquine and artemether-lumefantrine fixed-dose combinations for the treatment of uncomplicated *Plasmodium falciparum* malaria among children aged six to 59 months in Nimba County, Liberia: an open-label randomized non-inferiority. *Malar J* 2013: **12**: 251.
39. Cairns ME, Walker PGT, Okell LC *et al.* Seasonality in malaria transmission: implications for case-management with long-acting artemisinin combination therapy in sub-Saharan Africa. *Malar J* 2015: **14**: 321.
40. Djallé D, Gody JC, Moyen JM *et al.* Performance of Paracheck™-Pf, SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan for diagnosis of falciparum malaria in the Central African Republic. *BMC Infect Dis* 2014: **14**: 109.
41. Ajumobi O, Sabitu K, Nguku P *et al.* Performance of an HRP-2 rapid diagnostic test in Nigerian children less than 5 years of age. *Am J Trop Med Hyg* 2015: **92**: 828–833.
42. Das S, Jang IK, Barney B *et al.* Performance of a high-sensitivity rapid diagnostic test for *Plasmodium falciparum* malaria in asymptomatic individuals from Uganda and Myanmar and naive human challenge infections. *Am J Trop Med Hyg* 2017: **97**: 1540–1550.

Corresponding Author **Catherine Falade**, Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria. Tel.: +234 803 326 4593; E-mail: lillyfunke@yahoo.com