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Use of a Histidine-Rich Protein 2-Based Rapid Diagnostic Test for Malaria by Health Personnel during Routine Consultation of Febrile Outpatients in a Peripheral Health Facility in Yaoundé, Cameroon

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Abstract. The role of a rapid diagnostic test (RDT) in the case management of *Plasmodium falciparum* malaria infections has not been determined in Africa. Our study was conducted during November 2007–January 2008 to assess test accuracy of an RDT in the management of febrile outpatients in a peripheral urban health facility in Cameroon. We found the overall sensitivity to be 71.4% and a specificity of 82.2%; the positive predictive value and negative predictive value were 73.8% and 80.4%, respectively. False-negative and false-positive cases represented 11.8% and 10.5% of all febrile patients. Malaria alone (31.3%) was the first cause of fever; 33.5% of fever cases were of unknown origin. Acute respiratory infections were common among children 0–2 years of age (25.5%) and decreased with age. The risk of having a clinical failure with the presumptive treatment of febrile children was seven times greater than that of the RDT-oriented management (relative risk = 6.8, 95% confidence interval = 0.88–53.4, $P = 0.03$) because of the delay of appropriate treatment of non-malarial febrile illness. Our results suggest that the RDT may be of limited utility for children greater than five years of age and adults and that diagnosis based on microscopic examination of blood smears should be recommended for these patient populations, as well as in areas of low transmission.

INTRODUCTION

Malaria is a major public health problem in Cameroon as in most sub-Saharan African countries. Malaria transmission is high in nearly all of Cameroon and the National Malaria Control Program has reported that 35–40% of mortality in public health facilities, 50% of morbidity among children less than five years of age, and 40% of annual household health expenditures are due to malaria. The persistence of malaria is related to self-medication, low adherence to treatment regimens, drug pressure with long half-life monotherapies, and drug resistance.[1,2]

In response to the widespread drug resistance and growing concerns about the use of ineffective monotherapies, the World Health Organization recommends the replacement of chloroquine, sulfadoxine-pyrimethamine, and amodiaquine with artemisinin-based combination therapies if first-line treatment failure rates are greater than 10%.[3] During 1997–2004, 25 surveys were conducted in Cameroon according to the standardized World Health Organization protocol to evaluate the therapeutic efficacy of first-line and second-line antimalarial drugs.[4] Results indicated that chloroquine is no longer effective and is associated with a failure rate greater than 25%. Sulfadoxine-pyrimethamine is associated with failure rates ranging from 8.6% to 14.1%. Amodiaquine remained effective in the entire country with a failure rate of approximately 4%.

In January 2004, the artesunate-amodiaquine (ASAQ) combination was adopted as the drug of choice for all cases of uncomplicated malaria, with artemether-lumefantrine being an alternative artemisinin-based combination therapy since 2006.[5] Until recently, presumptive treatment of fever was recommended by the National Malaria Control Program. However, currently it is no longer a satisfactory strategy because depending on malaria prevalence among febrile patients, this practice can lead to misdiagnosis and over-treatment. Presumptive treatment has poor specificity (many febrile cases will be considered as malaria although fever may be caused by other diseases), leaving non-malarial febrile patients without an appropriate treatment.[6] Over-treatment increases drug costs and may also create favorable conditions for the emergence of drug-resistant parasites.[7,8]

Accurate diagnosis is therefore important for effective management of malaria.[9] The shift from symptom-based diagnosis to parasite-based management of malaria can bring significant improvements to tropical fever management on poor malaria-endemic populations.[10] Microscopy is the gold standard and the most commonly used diagnostic laboratory tool in malaria-endemic regions. However, effective microscopic examination requires good quality equipment and reagents, technical expertise for preparation and staining of films, and identification of the parasites.[11]

Until recently, malaria rapid diagnostic tests (RDTs) have not been adopted in areas without access to microscopy. However, RDTs can be recommended for use in the absence of laboratory facilities (rural areas and highly urbanized peripheral localities) if sensitivity, specificity, and positive and negative predictive values are very high, and if the unit cost is affordable.[11] The RDTs can be performed by health staff with little previous technical training and may be an appropriate tool when combined with clinical diagnosis.[12] Studies on the use of RDTs for guiding outpatient treatment of febrile illness in some African countries have been reported.[13,14] The aim of this study was to assess the test accuracy of RDTs in the management of febrile outpatients in a peripheral urban health facility in Cameroon.

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MATERIALS AND METHODS

Patients. The study was conducted from November 2007 through January 2008 in The Father Jean Zoa Primary Health Center in Nkolndongo, Yaoundé, the capital of Cameroon. Patients ≥ 6 months of age who came to the health facility with a clinical suspicion of malaria determined by the health personnel were screened and included in the study. The diagnosis of clinical malaria was based on fever (axillary temperature > 37.5°C) or history of fever during the past 24 hours. Patients who had taken antimalarial drugs within the past seven days were not included. To be as close as possible to field conditions, febrile patients were included separately in each arm according to the nurse's clinical viewpoints (agreement or not of suspicion of malaria). (In this dispensary, as in most dispensaries in Africa, nurses make the clinical diagnosis and prescribe medication.) Clinical information for each patient was recorded. Informed consent to participate in the study was obtained from all adult participants and from parents or legal guardians of minors. The study was reviewed and approved by the Cameroonian National Ethics Committee.

Study design. In routine clinical practice in this dispensary, all febrile patients would have been treated with antimalarial drugs (alone or in association with non-antimalarial drugs in case of co-infections) based on presumptive diagnosis. For the purpose of this study, four nurse stations were available, two for pediatric consultation (patient 0–5 years of age) and two for older children and adults (≥ 6 years of age). Febrile patients satisfying the inclusion criteria and assigned to the presumptive arm were treated with a standard three-day regimen of ASAQ (one dose/day: AS, 4 mg/kg of bodyweight/day plus AQ, 10 mg/kg of bodyweight/day). Patients assigned to the RDT arm were treated based on the malaria RDT result. ASAQ was given to the patient if the RDT result was positive, but in case of negative RDT results, antibiotics and/or antipyretics, not antimalarial drugs, were prescribed depending on the presenting signs and symptoms. To minimize bias, nurses alternated between presumptive and RDT arms every week.

Rapid diagnostic test for malaria. A training session on the use of rapid diagnostic test for malaria took place two weeks before the beginning of the study. Two types of malaria RDT were used: dipsticks (kappa coefficient = 0.84) and cassettes devices (kappa coefficient = 0.92). The kappa coefficient expressed the level of concordance of 10 series of RDT results obtained by the nurses with that of an experienced technician. In practice, cassette devices seemed easier to use because it was possible to write the name or code of the patient with a pen or pencil, and whole blood and buffer solution are deposited in the same well. For dipsticks, well-labeled individual test tubes for storage were needed to prevent confusion, leading to an increased operational time during outpatient care. Based on this comparison between dipstick and cassette, a DiaSpot® malaria RDT cassette device (Acumen Diagnostics Inc., USA) was chosen. Another reason was that the DiaSpot® RDT for malaria was the most easily available malaria RDT in Yaoundé during the study period and has already been used by several primary health centers. The principle of this RDT is based on the detection of *Plasmodium falciparum* histidine-rich protein 2 in whole blood. Briefly, one drop of whole blood (approximately 10 μL) was mixed with three drops of lysis buffer on an individual cassette. The lysate was allowed to migrate, and the result was read after 10 minutes according to the manufacturer's recommendations.

Microscopic examination of Giemsa-stained blood films. A thick blood film was prepared by the nurse on day 0 and during follow-up visits and sent to the laboratory. The slides were stained with 10% Giemsa for 15 minutes and examined with a light microscope. Parasites were counted against 200 leukocytes, and parasite density was expressed as the number of asexual parasites per microliter of blood, assuming 8,000 leukocytes/μL of blood.[15] Microscopy was the gold standard. Enrolled patients were followed-up on days 3 and 7.

Endpoints and statistical analysis. Fever clearance and proportion of RDT-based cases and controls (febrile patients treated presumptively) recovering on day 7 (i.e., afebrile and negative blood smear) in each arm were the main clinical outcomes. The sensitivity, specificity, positive and negative predictive values, and impact of RDT-based and presumptive strategies were determined. Data were collected and analyzed using Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

Study population. A total of 313 patients were included in the study, 160 (51.1%) in the presumptive arm and 153 (48.9%) in the RDT arm. There were 161 (51.5%) children less than five years of age (0–2 years of age, 56 and 50 in the presumptive and 50 RDT arms, respectively, and 3–5 years of age, 30 and 25 in the presumptive and RDT arms, respectively). The remaining 152 (48.5%) were 6–10 years of age (18 in each arm) or greater than 11 years of age (56 in the presumptive arm and 60 in RDT arm). The sex ratio was 0.99.

Microscopy results. Globally, 129 (41.2%) patients had a positive thick blood films, 66 (51.2%) in the presumptive arm and 63 (48.8%) in the RDT arm. The prevalence of malaria was significantly lower among children 0–2 years of age (32.1%; $P = 0.05$). In other age groups, the following malaria prevalence rates were observed: 3–5 years of age, 25 of 55 smears (41.8%); 6–10 years of age, 22 of 36 smears (61.1%), and ≥ 11 years of age, 50 of 116 smears (43.1%). The number of asexual parasites per microliter of blood ranged from 40 to 125,000. It was relatively high in children less than five years of age (geometric mean = 2,860, range = 80–125,000 for children 0–2 years of age; geometric mean = 6,440, range = 160–100,000 for children 3–5 years of age) and decreased in children 6–10 years of age (geometric mean = 1,580, range = 40–50,100) and older children (≥ 11 years of age) and adults (geometric mean = 513, range = 40–125,000).

Assessment of RDT sensitivity, specificity, and predictive values. Sensitivity and specificity were dependent on parasite density. The performance of the DiaSpot® RDT varied with age groups. When the RDT results were compared with those of microscopy, the sensitivity was 71.4% and specificity was 82.2%. The positive and negative predictive values were 73.8% and 80.4%, respectively (Table 1).

Among 153 patients included in the RDT arm, 16 had a positive RDT result with negative parasitemias (10.5% false positive), whereas 18 had a negative RDT result with positive parasitemias (11.8% false negative). Five of these false-negative cases occurred in children less than one year of age. Low parasitemia was the most probable cause of false-negative results because the mean parasite density among these 18 patients was 148 asexual parasites/μL. The heat stability of the RDTs used in this study was not evaluated.

TABLE 1
Performances of the DiaSpot® rapid diagnostic test for malaria Yaoundé, Cameroon*

Age groups, years	No.	Mean parasites/µL of blood	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	False negative (%)	False positive (%)
0–2	50	2,860	13/18 (72.2)	30/32 (93.8)	13/15 (86.7)	30/35 (85.7)	5/50 (10.0)	2/50 (4.0)
3–5	25	6,440	6/6 (100)	15/19 (78.9)	6/10 (60.0)	15/15 (100)	0/25 (0.0)	4/25 (16.0)
6–10	18	1,580	6/10 (60)	6/8 (75.0)	6/8 (75.0)	6/10 (60.0)	4/18 (22.2)	2/18 (11.1)
≥11	60	513	20/29 (69.0)	23/31 (74.2)	20/28 (71.4)	23/32 (71.9)	9/60 (15.0)	8/60 (13.3)
Total	153	1,535	45/63 (71.4)	74/90 (82.2)	45/61 (73.8)	74/92 (80.4)	18/153 (11.8)	16/153 (10.5)

* PPV = positive predictive value; NPV = negative predictive value.

Prevalence of malaria and other diseases among febrile patients. Diagnosis was based on thick blood smear results and clinical assessment of 313 febrile patients and showed that malaria, alone (31.3%) or in association with another infection (9.9%), was the primary cause of fever; 33.5% of febrile cases were of unknown origin (minor infections). Acute respiratory infections were common in children 0–2 years of age (25.5%) and decreased with age. The prevalence of malaria alone increased with age: 0–2 years of age = 21.7%, 3–5 years of age = 38.2%, 6–10 years of age = 33.3%, and ≥ 11 years of age = 36.2%.

Disease frequencies varied significantly according to treatment strategy ($P < 10^{-5}$). In the presumptive arm, fever without any clinical sign of infection (43.7%) and malaria alone (34.3%) were predominant, and acute respiratory infections represented 8.8% of the cases (Table 2). In the RDT arm, the corresponding prevalences were 22.9%, 28.1%, and 26.1%, respectively.

Impact of diagnostic strategies on treatment adequacy on day 0. Among 160 patients treated presumptively, 94 had a negative parasitemia, which indicated that there was a misuse of antimalarial treatment in 61.6% of presumably non-immune children (0–5 years of age) and in 55.4% of presumably immune patients (≥ 6 years of age). Conversely, use of the DiaSpot® RDT reduced antimalarial drug misuse to 8% and 12.8%, respectively (Figure 1). Thus, RDT-oriented treatment in febrile children less than five years of age living in Yaoundé reduced the number of antimalarial drug overtreatments seven-fold, compared with case management based on presumptive clinical diagnosis.

Impact of diagnostic strategies on clinical outcomes on day 7. Of 313 patients, 297 (94.9%) were followed-up until day 7. Among 16 patients lost to follow-up, 11 whose clinical status improved on day 3 refused to continue the study, four children (0–5 years of age) were referred to the district hospital because of clinical signs of danger between days 1 and 6, and one 25-year-old man was excluded because of poor compliance caused by amodiaquine-induced pruritus. Among 297 patients, 285 (96%) had a satisfactory response on day 7 with no fever and negative blood smear. The difference between the recovery rates of age groups (0–2 years of age = 94.0%, 3–5 years of age = 94.4%, 6–10 years of age = 97.1% and ≥ 11 years of age = 98.1%) was not statistically significant ($P = 0.42$).

Among presumably immune patients ≥ 6 years of age, the recovery rate was 100% (all 68 patients) when treated presumptively and 96% (72 of 75 patients) when treated according to the RDT-oriented strategy ($P = 0.24$). In presumably non-immune persons (0–5 years of age), the use of a malaria RDT increased the recovery rate on day 7 by 8.6%, i.e., 75 of 83 (90.4%) in the presumptive group versus 70 of 71 (98.6%) in the RDT group. Thus, the risk of having a clinical failure with the presumptive strategy was seven times greater than that of the RDT-oriented fever management of these children (relative risk = 6.8, 95% confidence interval = 0.88–53.4, $P = 0.03$).

TABLE 2
Prevalence of malaria and other diseases among 313 patients, Yaoundé, Cameroon

Diagnosis (cause of fever)	Presumptive arm (%) (n = 160)	Rapid diagnostic test arm (%) (n = 153)
Malaria alone	34.3	28.1
Malaria and another infection	6.9	13.1
Acute respiratory infections	8.8	26.1
Otitis, sore throat, rhinitis	4.4	0.7
Diarrhea	0.6	1.3
Skin eruptions	0.0	0.7
Acute respiratory infections plus otitis or diarrhea	1.3	7.1
Fever of unknown origin	43.7	22.9

DISCUSSION

The study evaluated the accuracy of DiaSpot® cassettes and showed the frequencies of co-morbidities and the impact of diagnostic strategies on antimalarial drug use and clinical outcomes. There are no previously published data on the use of DiaSpot® malaria tests in Cameroon or elsewhere. Nevertheless, in a previous study using a histidine-rich protein 2–based RDT in Yaoundé in 1999, the ICT® malaria Pf test (ICT Diagnostics, Brookvale, New South Wales, Australia) showed a sensitivity of 98% and specificity of 88.8%.[16]

The present study highlights the lack of sensitivity of malaria RDTs when used among patients with low parasitemias because most cases with false-negative RDT results had a parasite density less than 500 asexual parasites/µL of blood.[17,18] The performances of the DiaSpot® malaria RDT seemed to be higher when used for febrile children less than

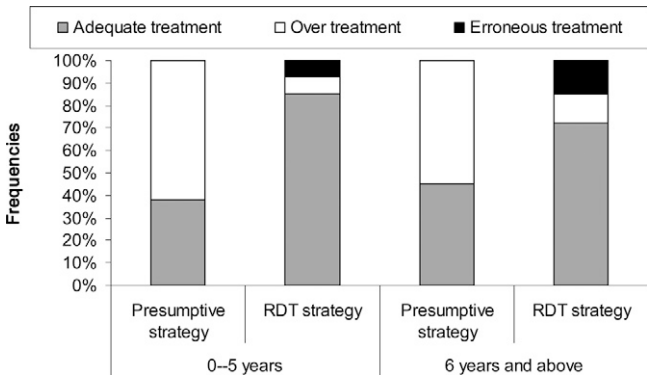

FIGURE 1. Diagnostic strategies and treatment adequacy of suspected malaria patients, Yaoundé, Cameroon.

five years of age, but were low in the general population. The RDT limitations for a parasite density less than 5,000 asexual parasites/μL of blood have recently been reported in asymptomatic children in Cameroon. The Hexagone® RDT (IND Diagnostic Inc., Delat, British Columbia, Canada) showed a sensitivity of 85.3% and a specificity of 95.5%.[19] The reasons why RDTs were not highly accurate in the present study are not known. Possible causes include poor heat stability of RDTs greater than 25°C (the ambient temperature is often greater than 25°C in Yaoundé) and batch-to-batch variation in RDT quality.[20]

The frequency of false-negative RDT results in this study showed that there is some concern that the benefits of parasitologic confirmation when using malaria RDT for guiding the management of febrile outpatients must also be taken into account because of the potentially fatal risk of not treating malaria-infected children with false-negative RDT results.[6,21] While awaiting the development of more sensitive RDTs, microscopy-based diagnosis should be improved, especially in areas of Cameroon with low transmission of malaria, where concomitant occurrence of febrile illnesses is frequent.[22]

Acute respiratory infections and fever of unknown origin were the most common sources of fever in patients with negative blood smears in Yaoundé. Pneumonia has been identified as one of the six causes that account for 73% of the annual deaths in children less than five years of age.[23] Among nine children less than five years of age who were still febrile and/or had clinical symptoms on day 7 and did not have malaria (negative blood smear), 8 (88.9%) were in the presumptive arm. In treating febrile patients with this strategy, there is a high risk of insufficient care for non-malarial diseases (late diagnosis and late treatment). Conversely, under field conditions, clinical diagnosis should be improved in both arms for better management of co-infections and patients with negative RDT results (e.g., respiratory rate count for acute respiratory infections). In doing so, nurses could refrain from systematic prescription of antibiotics because many febrile cases may be of viral origin.

The impact of the RDT-based strategy on the clinical outcome and adequacy of the treatment of febrile patients has been evaluated in a previous study.[14] In Tanzania, introduction of RDT for case management resulted in an additional 9.4% of patients being correctly treated.[24] In Kenya, the use of RDT improved malaria treatment with 61% less over-treatments but 8% more under-treatments.[25]

The proportion of diseases (especially acute respiratory infections) in each arm confirms our observations in the field. We found that four nurses had a tendency to favor the recruitment of more patients with apparent clinical symptoms when they were assigned to the RDT arm. They tended to accept more febrile patients with no clinical manifestations when they were assigned to the presumptive group. Conversely, we observed that among children less than five years of age, although the RDT result was negative, the nurse still prescribed antimalarial drugs without justification. Thus, for them, a negative RDT does not necessarily mean abstention from prescribing an antimalarial drug. Deviations in the nurses' adherence to test results have also been reported in Tanzania.[13] This behavior reflects the actual practice of health personnel, and it would be difficult to prevent it if health practitioners are not involved in the implementation policy. To improve case management of fever and prevent antimalarial drug misuse through the RDT strategy, the practitioners' behavior must be taken into account. Clinicians may pay limited attention to negative test results. Substantial number of cases of potentially fatal febrile illness that can be treated with affordable antibiotics could be missed.[26] There is also a need to review the malaria RDT-oriented management under the strategy of integrated management of childhood illness for better treatment of non-malarial diseases and patients with negative RDT results.[27] Conversely, this study also showed that the RDT-based diagnosis may be of limited use for children greater than five years of age and adults.[28] Our results highlight the need that such studies should be designed according to operational conditions.

The clinical advantages of RDTs include early diagnosis and rapid treatment of children with positive (malarial) and negative (non-malarial) RDT results, and a positive impact on health outcomes. There was a seven-fold increase in risk of treatment failure in the presumptive arm because non-malarial diseases are less likely to be treated or their treatment is delayed under this strategy. The performances of the DiaSpot®-based strategy are only valid for the management of febrile patients in Yaoundé, Cameroon using this batch of cassettes within the study period. Broad recommendations require adjustments to specific local context. Further studies should be conducted in a larger sample with more sensitive malaria RDTs. Microscopic diagnosis should be recommended in areas of low transmission of malaria although it is currently not a feasible option in Africa.

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