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Evaluation of a PfHRP-2 Based Rapid Diagnostic Test Versus Microscopy Method Among HIV-Positive and Unknown Serology Patients in Ouagadougou, Burkina Faso

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Abstract. We evaluated the performance of a malaria rapid diagnostic test (RDT; Malaria Quick Test®; Cypress Diagnostic) compared with the standard thick-smear microscopy method using blood samples from human immunodeficiency virus (HIV)-infected individuals and individuals of unknown HIV status collected in Ouagadougou, Burkina Faso. Our results show that 42.1% of 114 HIV-infected patients were concordantly RDT- and thick smear-positive, and 55.3% were concordantly negative. Sensitivity and specificity of the RDT test were 100.0% and 95.4%, respectively, with 5.9% false-positive results and a total agreement of 97.4%; 127 patients with unknown HIV serology were analyzed; of them, 40.9% were RDT- and thick smear-positive, and 46.4% concordantly negative. Sensitivity and specificity were 100.0% and 78.6%, respectively, with 23.5% false-positive results and a total agreement of 87.4%. Malaria Quick Test® is rapid and effective for the diagnosis of malaria and has a high sensitivity, confirming its use in general and HIV patients in particular.

INTRODUCTION

The World Health Organization (WHO) estimates that one-half of the world's population is at risk of contracting malaria, with 207 million cases estimated in 2012 and 627,000 estimated deaths, of which 90% occurred in sub-Saharan Africa.[1] In addition, in this region, nearly 22.5 million individuals are infected with human immunodeficiency virus (HIV).[2] The interaction between HIV and malaria infection is bidirectional and synergistic.[3] The geographic overlap between malaria and HIV and the rates of coinfection make the relationship between the two diseases a global health problem.[4–6] HIV impairs the immune response, which is necessary for an effective antimalarial response, resulting in more frequent and severe malaria cases and transient rises of HIV viral load during malaria episodes. This accelerates the progression of HIV infection as well as the risk of HIV transmission.[7–12] A key for effective management of malaria is early and accurate diagnosis in both endemic areas and imported cases in non-endemic countries.[12] Because of the global impact of malaria, there has been an increase in the development of diagnostic strategies. The WHO recommendations have also been updated, and the WHO now suggests that all cases of suspected malaria must be confirmed with a diagnostic test before receiving treatment.[13] This underlines the importance and need for simple, low cost, and rapid diagnostic tools, such as rapid diagnostic tests (RDTs). Accurate diagnosis for malaria is necessary to reduce and prevent morbidity and mortality while differentiating malarial from non-malarial fevers, thus avoiding the unnecessary use of antimalarial drugs.[14,15] This is particularly important in HIV-positive patients, where febrile illnesses other than malaria are common.

The use of antigen-detecting RDTs is part of such strategy aimed at expanding access to malaria etiologic diagnosis in areas where good quality microscopy (Giemsa; the reference method) cannot be performed.[16]

Giemsa microscopy and RDTs represent the two diagnostic tools most likely to have the largest impact on malaria control today. These two methods together, each with strengths and limitations, represent the best hope for accurate diagnosis as a key component of successful malaria control.[17]

Good performance of an RDT has already been reported among HIV-negative people but not yet properly investigated among HIV-positive individuals.[18,19]

In this study, we evaluated the performance of a commercially available *P. falciparum* histidine-rich protein type-2 (*Pf*HRP-2) -based RDT compared with the gold standard microscopy method using samples from HIV individuals and individuals of unknown HIV serology collected in Ouagadougou, Burkina Faso.

MATERIALS AND METHODS

Study setting. This study was carried out from August to December of 2011 during and right after the rainy season, when the peak of malaria incidence, mostly caused by *Plasmodium falciparum*, is usually observed. The study was performed at three health centers in Ouagadougou, the capital town situated on the central plateau of Burkina Faso, an endemic area for malaria. The most recent official data on malaria and HIV prevalence in Ouagadougou (from the Health and Demographic Survey [HDS] carried out in 2010) show that malaria prevalence among children ages 6–59 months is 18%, whereas HIV prevalence in the age range between 15 and 49 years is 0.8% in male and 1.2% in female.[20] The three centers included in the study were Saint Camille Medical Center (SCMC; a large mother and child center providing care for HIV/acquired immunodeficiency syndrome [AIDS] patients), the Center for Biomolecular Research Pietro Annigoni, Saint Camille (CERBA; providing care for HIV/AIDS patients), and the outpatient clinic Centre d'Accueil Notre Dame de Fatima (CANDAF; open to all typologies of people needing care).

Sampling method. Patients enrolled in the study were HIV-positive adults and children and adults and children with unknown HIV status identified according to signs or symptoms consistent with malaria (including fever, arthralgia, headache, fatigue, and asthenia).

Patients having been treated with antimalarial drugs during the previous 15 days were not eligible for this study.

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Two groups of patients were sampled: (1) HIV-positive patients (mostly women as a reflection of the sex ratio of CERBA and CMSC patients) and (2) individuals with unknown HIV serology (principally represented by children). Burkina Faso is an area of high malaria endemicity, and symptomatic cases are predominantly among children who have not yet reached an efficient level of immunity against the parasite.

Laboratory tests. Venous blood was collected by standard venipuncture into a collection tube containing ethylenediaminetetraacetic acid (EDTA), or capillary blood was obtained by puncture of a finger; 5 μL blood was immediately tested for malaria using the Malaria Quick Test® (Cypress Diagnostic, Langdorp, Belgium) according to the manufacturer's instructions. When the test could not be performed immediately, the blood was stored for up to 3 days at 2–8°C. The Malaria Quick Test® is a rapid and qualitative antigen-capture assay detecting the presence of the soluble *P. falciparum* histidine-rich protein 2 (PfHRP-2), which is present in and released from *P. falciparum*-infected red blood cells. The presence of both control and temple lines shows that the *P. falciparum* antigen is present, and the test can be considered positive.[21]

In case the control band did not appear on the first test kit, the test was considered null, and the use of a second kit (identical to that previously used and belonging to the same lot) was allowed.

Thick and thin smears were prepared from EDTA anticoagulated fresh blood or blood from a finger prick, stained with Giemsa solution, and microscopically evaluated according to WHO guidelines.[22,23] Examination of the smears was performed by three different laboratory technicians who were unaware of the subject's identity or the RDT test results. Results of blood slides were compared, and another additional reading was performed in case of positive–negative disagreement. The positivity of the thick smear was defined by the presence of one or more confirmed asexual malaria parasites, and the density was calculated according to WHO guidelines. Finally, the correlation between the results of RDT and microscopy was calculated, and the total agreement (*K* value) was interpreted according to Altman.[24]

Data processing and analysis. Data were collected and elaborated with Excel (Windows 7) and SAS (SAS V8; SAS Institute Inc., Cary, NC). The study sample was divided in two groups: HIV-positive patients and patients with unknown HIV serology. Both groups of individuals were analyzed according to four variables: age, sex, RDT results, and thick smear results.

Informed consent and ethical aspects. The Ethics Committees of CERBA, CANDAF, and SCMC approved this study; each patient at the moment of recruitment was informed about the characteristics and course of the study and asked for an informed consent.

A detailed information sheet was prepared in French and read to patients; if necessary, a translation into the local language was assured by local staff. In the case of minor patients, consent for inclusion in the study was signed by parents or those who accompanied children at the moment of examination.

RESULTS

In total, 114 HIV-positive patients with suspected malaria were included in the study: 23 of 114 (20.2%) were male, and 91 of 114 (79.8%) were female. The mean age of the individuals was 35 years old (range = 3–63 years old). As shown in Table 1, 48 of 114 (42.1%) samples were both RDT- and thick smear-positive, and 63 of 114 (55.3%) were concordantly negative. None of the samples were found to be thick film-positive and RDT-negative, whereas 3 of 51 (5.9%) samples were found to be thick film-negative and RDT-positive.

TABLE 1
Performance of the RDT versus thick film microscopy among HIV-positive participants and participants with unknown serology

	Thick smear		
RDT	Positive	Negative	Total
HIV-positive participants			
Positive	48 (94.1%)	3 (5.9%)	51 (100.0%)
Negative	0 (0.0%)	63 (100.0%)	63 (100.0%)
Total	48 (42.1%)	66 (57.9%)	114 (100.0%)
Unknown serology participants			
Positive	52 (76.5)	16 (23.5%)	68 (100.0%)
Negative	0 (0.0%)	59 (100.0%)	59 (100.0%)
Total	52 (40.9%)	75 (59.1%)	127 (100.0%)

Sensitivity and specificity of the RDT test were 100.0% (95% confidence interval [95% CI] = 95.9–100.0%) and 95.5% (95% CI = 89.4–98.3%), respectively, with 5.9% false-positive results. The total agreement found in the HIV-positive population between RDT and microscopy was very good (*K* = 97.4%) based on the *K* value.

In total, 127 individuals had unknown serological status for HIV, of whom 57 (44.9%) were male and 70 (55.1%) were female. The mean age was 12 years old, with a range of 3 months to 46 years. As shown in Table 1, 52 of 127 (40.9%) samples were both RDT- and thick smear-positive, and 59 of 127 (46.4%) samples were concordantly negative. None of the samples were found to be thick film-positive and RDT-negative, whereas 16 of 68 (23.5%) samples were found to be thick film-negative and RDT-positive.

Sensitivity and specificity of the RDT in this population were 100.0% (95% CI = 96.3–100.0%) and 78.7% (95% CI = 70.3–85.2%), respectively, with 23.5% false-positive results. In the population with unknown serology, the total agreement was good (*K* = 75.12%) between the two tests compared.

DISCUSSION

In this study, we evaluated the accuracy of one *Pf*HRP-2–based RDT in HIV-positive patients and patients with unknown HIV serology compared with thick smears results.

Both groups of individuals were also analyzed according to their age and sex, but data obtained did not show significant results. Our HIV-positive population was composed of individuals followed in medical centers providing free care and examinations for HIV/AIDS patients.

The population with an unknown HIV serology came from a primary healthcare service (CANDAF), where patients with different medical complaints are treated: these patients do not have free access to treatments or examinations. Another important challenge for this group of patients was collecting the information related to antimalarial drugs taken before the RDT test.

The different characteristics of these populations have affected the specificity of the test, which is reflected by the different values obtained. For example, specificity of the HIV-positive population was 95.5%, whereas for the population with unknown HIV serology, a specificity of 78.7% was

obtained. There was also a difference in false-positive results between the two groups; 5.9% and 23.5% were found for the HIV-positive population and the population of unknown HIV serology, respectively. This result may likely be related to the fact that patients from CANDAF were mainly children under 5 years old frequently accompanied by parents or relatives who were unable to recall the previous intake of antimalarial drugs, which has already been reported.[25] In addition, the cost of care has a significant relevance on the decision to consult a healthcare center, and according to these patients, before consulting a doctor, they often try to take drugs or use traditional medicine at home and do not inform the health staff during the interview. However, patients enrolled in the HIV program could easily access the health facilities and obtain free tests and drugs without taking self-treatment as the first choice.

The high rate of false-positive results is an important aspect that should be taken in account, because it might cause over-treatment of malaria or failure to treat non-malaria–related fever cases properly. Providing a correct diagnosis of malaria may eventually limit the unnecessary use of antimalarial drugs.

The observed sensitivity of the test is 100.0% for both populations, which is in good agreement with other studies on HIV-positive populations and HIV-uninfected individuals.[15,18,26,27] Given that, in HIV-positive patients, the risk of malaria misdiagnosis is high because of febrile illness or other infection that may mimic malaria, our findings show that most HIV patients with presumptive malaria infection were appropriately diagnosed for *P. falciparum* using the RDT in examination.[28] This shows the high sensitivity of the RDTs, compared with the reference microscopy method. Furthermore, the 100% sensitivity of the test in the HIV-positive group, the low number of false-positive cases, and the absence of false-negative cases suggest that HIV does not interfere or corrupt the performance of the test used in this study, confirming the good performance of RDTs in general and HIV patients in particular.[13,18,29,30]

Despite some of the limitations associated with the Malaria Quick Test®, such as the inability to detect mixed infection or infection caused by plasmodia other than *P. falciparum*, it is rapid (less than 20 minutes), it is easy to perform, and it does not need particular laboratory facilities or health worker training. All of these characteristics make it useful in resource-limited countries. Another limitation of the study was the relatively short period (15 days) that we used as inclusion criterion in the cohort; although it is known that the average time that the *Pf* HRP-2 remains positive is about 2 weeks, it is also known that this protein can take more than 1 month to be cleared. Therefore, the short period defined could explain the false-positive results reported.[19,31,32]

Nevertheless, considering the results obtained from this evaluation, which are in agreement with other studies, RDTs might have an important role for the diagnosis of malaria and in general, the management of febrile illnesses of all typologies in individuals, including HIV-positive patients.

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