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Performance of a prototype malaria rapid diagnostic test versus thick film microscopy among HIV-positive subjects in rural Rakai, Uganda

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Abstract

In this study, we report the performance of a prototype malaria rapid diagnostic test, Malaria F-test (MFT), compared with thick blood films from HIV-positive Ugandans undergoing malaria testing. In total, 21/154 samples (13.6%) were concordantly positive by both thick film and MFT and 129/154 samples (83.8%) were concordantly negative; 1 sample (0.6%) was thick film-positive but MFT-negative and 3 samples (1.9%) were thick film-negative but MFT-positive. The sensitivity of MFT was 95.5% (95% CI 77.2–99.9%) compared with thick film microscopy and the specificity was 97.7% (95% CI 93.5–99.5%). MFT was simple, rapid and effective for detection of *Plasmodium falciparum* among HIV-positive subjects in a rural, malaria-endemic African setting.

Keywords

Malaria; HIV/AIDS; Diagnosis; Rapid diagnostic test; Africa; Uganda

1. Introduction

There is an urgent need for accurate, inexpensive and simple malaria rapid diagnostic tests (RDT) for use in resource-poor settings. Recent studies have reported the performance of a wide range of malaria RDTs among HIV-uninfected individuals.¹ However, the performance

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Authors' contributions: LAM and JK conceived and designed the study with SJR, TCQ and RHG; SA implemented the study in the laboratory under the guidance of JPS; JBB managed the data and study records; LAM, KN and VS performed the statistical analysis; RHG supervised all other study authors; LAM and JPS drafted the manuscript. All authors read and approved the final manuscript. LAM is guarantor of the paper.

Conflicts of interest: None declared.

Ethical approval: The Rakai Community Cohort Study has received institutional approval from Western Institutional Review Board and Uganda Virus Research Institute, Entebbe, Uganda.

of these assays among HIV-infected individuals has been less well demonstrated. Here we report the performance of a prototype malaria RDT [Malaria F-test (MFT)] on samples from HIV-positive rural Ugandans.

2. Materials and methods

2.1. Study setting

This study was performed in Rakai, Uganda, which is meso-hyperendemic for *Plasmodium falciparum* and where 41.5% of outpatient visits to clinics and hospitals involve suspected malaria.² Participants were identified between 1 November 2006 and 18 December 2007 from the HIV treatment cohort and the maternal HIV pregnancy outcomes study of the Rakai Community Cohort Study. Baseline insecticide-treated net (ITN) usage in this region of Uganda is low; only 13% of households had a net in 2004–2005.³ However, participants in this study had received nets from the HIV treatment programme. Participants also received co-trimoxazole prophylaxis (Cosmos Limited Corporation, Nairobi, Kenya) regardless of T-cell lymphocyte count as per Uganda Ministry of Health guidelines as well as antiretroviral therapy (ART) if their T-cell lymphocyte count was <250 cells/mm³ or they had WHO stage IV disease. Women in the pregnancy outcomes study whose T-cell lymphocyte counts were ≥ 250 cells/mm³ had received peripartum antiretroviral medications for prevention of mother-to-child transmission of HIV.

All participants provided consent to have their records and samples used for research.

2.2. Point-of-care activities

Each HIV treatment cohort participant underwent monthly evaluation for signs or symptoms consistent with malaria (including fever, arthralgia, myalgia, headache, fatigue and others) and was enrolled if the clinician suspected malaria. In addition, all postpartum women from the pregnancy outcomes study were tested, regardless of symptoms. Participants underwent aseptic venipuncture for collection of 3–5 ml of EDTA-anticoagulated whole blood. Tubes were inverted >10 times, placed in vertical tube racks in cool boxes (approximate temperature 10 °C) and transported over land within 4–6 h to a central laboratory where thick film microscopy and the prototype malaria RDT were performed within 24 h of sample collection.

2.3. Laboratory activities

Thick films were prepared from EDTA-anticoagulated fresh blood, Giemsa stained and then microscopically evaluated according to WHO guidelines;⁴ microscopic fields containing 500 white blood cells were reviewed prior to declaring films negative. Positive thick film results were defined by the presence of one or more confirmed malaria parasite.

MFT (BD Diagnostics, Sparks, MD, USA) is a prototype rapid chromatographic immunoassay for qualitative detection of histidine-rich protein-2 (HRP2), a *P. falciparum*-specific antigen. MFT kits expire after 6 months and do not require refrigeration; results are interpreted 15 min after inoculation of blood onto the test strip membrane. MFT was performed on the same blood samples used for thick films, by trained laboratory technicians who were blinded to each subject's identity and in most instances to each sample's thick film result (except in approximately 25% of cases, when only one laboratory technician was available to perform both assays). Appearance of the control band signified that the blood sample had migrated along the test strip membrane correctly; use of a second test kit was allowed if the control band did not appear on the first test kit. Positive MFT results were defined by the presence of a visible band on the test strip membrane, indicating the presence of HRP2 from *P. falciparum* in the blood sample.

Clinicians were contacted regarding all positive samples. Treatment with artemether/lumefantrine (Coartem; Novartis, Basel, Switzerland) and appropriate follow-up were provided according to Uganda Ministry of Health guidelines. Data were entered using FoxPro and analysis was performed with STATA software (Stata Statistical Software release 10.0; StataCorp., College Station, TX, USA).

3. Results

In total, 119/154 participants (77.3%) were female and 37/154 (24.0%) were in the pregnancy outcomes study. The median age was 30 years (range 2–62 years, with three participants ≤ 18 years of age). The median T-cell lymphocyte count was 346 cells/mm³ (interquartile range 213–589 cells/mm³). Control bands indicating valid tests were obtained for all MFT-tested samples, and no non-falciparum infections were detected by microscopy. Because the study was performed on a non-population-based sample, negative and positive predictive values are not reported. Results are detailed in Table 1.

4. Discussion

A high sensitivity and specificity of the MFT relative to thick films was observed, but confidence intervals were wide because there were few cases of malaria among these patients receiving HIV care. The low rates of malaria in these HIV-infected patients corroborate findings that malaria is markedly reduced among African HIV patients receiving co-trimoxazole, ITNs and ART.⁵ Although complete blinding of laboratory technicians would have decreased the risk of bias, given the straightforward interpretation (e.g. colorimetric band present or absent) of this rapid test, biased interpretation is unlikely. The three apparent false-positive MFT results may represent true false-positives, but might instead indicate the presence of residual antigenaemia following clearance of parasitaemia, which is known to occur with HRP2.⁶ Alternatively, the negative thick film results in these cases may reflect the imperfect sensitivity of thick films, especially at low parasite density, or may represent instances in which parasites became degraded in samples during transportation from the field site to the central laboratory.

The one sample that was MFT-negative but thick film-positive also tested positive on a different malaria RDT kit (Binax NOW Rapid Malaria Test; Inverness Medical Innovations Inc., Waltham, MA, USA), indicating a likely failure in the sensitivity of MFT. However, overall the prototype malaria RDT was simple and rapid to perform, valid relative to conventional microscopy and had excellent, although imperfect, performance characteristics in HIV-positive rural African subjects. When performed at the point of care, malaria RDTs have the potential to improve greatly the clinical management of febrile illness for HIV patients in resource-limited, malaria-endemic settings.

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Table 1

Performance of the Malaria F-test (MFT) versus thick film microscopy for detection of *Plasmodium falciparum* among HIV-positive participants in rural Uganda

	Positive thick film	Negative thick film	Total
Positive MFT	21	3	24
Negative MFT	1	129	130
Total	22	132	154

	Value	Exact binomial 95% CI
Sensitivity	95.5%	77.2–99.9%
Specificity	97.7%	93.5–99.5%