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# Malaria among adult inpatients in two Tanzanian referral hospitals: A prospective study

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ABSTRACT

Most malaria research in sub-Saharan Africa has focused on children and pregnant women, but malaria among hospitalized adults in this region is poorly characterized. In this prospective study, we assessed the prevalence and clinical characteristics of malaria among the inpatient adults in two hospitals in Tanzania. We enrolled adults admitted with suspected malaria and performed routine thick blood smear (BS) and malaria rapid diagnostic tests (RDT). We also assessed malaria parasite clearance rates. We considered malaria status 'confirmed' or 'excluded' only in patients with two concordant tests. Malaria polymerase chain reaction (PCR) was performed in a subset of patients with discordant BS and RDT. After BS and RDT were performed on 579 adults with suspected malaria, malaria was excluded in 458/579 (79.1%) and confirmed in 16/579 (2.8%). One hundred and five out of 579 (18.1%) had discordant results. The prevalences of positive BS and positive RDT were 102/579 (17.6%) and 35/579 (6.0%), respectively, with only fair agreement (Kappa = 0.354, $p < 0.0001$). PCR results agreed with RDT in 35/35 (100%) of patients with a negative RDT but positive BS. PCR results also agreed with RDT in 9/13 (69.2%) of cases with a positive RDT but negative BS. Clinical correlates of malaria by multivariable analysis included subjective fever (OR 3.6 [1.0–12.3], $p = 0.04$), headache (OR 3.1 [1.2–8.0], $p = 0.02$) and vomiting (OR 2.7 [1.2–6.4], $p = 0.02$). Malaria parasite clearance was significantly delayed in the HIV-infected group. Our study demonstrated only fair agreement between RDT and BS malaria tests among Tanzanian adult inpatients with suspected malaria. PCR generally agreed with RDT results. HIV was associated with delayed parasite clearance in adults with malaria. We recommend the routine use of RDTs for malaria diagnosis among adults admitted to hospitals in sub-Saharan Africa.



## 1. Introduction

Malaria remains a major cause of morbidity and mortality globally (Lozano et al., 2012). About 627,000 deaths occurred in 2012 worldwide, 90% of which were in Africa (WHO, 2013). In Tanzania, more than 95% of the population is at risk of malaria infection, and malaria is diagnosed in 40% of all outpatient visits (Ministry of Health, 2006). Most malaria research in sub-Saharan Africa has focused on children and pregnant women, and little is known about malaria among adult inpatients in this region.

Studies from a variety of clinical settings in sub-Saharan Africa suggest that malaria over-diagnosis is common (A-Elgayoum et al., 2009; Chandler et al., 2008; Mwanziva et al., 2008; Nadjm et al., 2012; Reyburn et al., 2004; Zurovac et al., 2006) and that treatment for malaria is frequently given even to patients with negative test results (Abeku et al., 2008; D'Acremont et al., 2011; Harchut et al., 2013; Reyburn et al., 2007). Traditionally, routine microscopic examination of thick blood smears (BS) has been the standard for malaria diagnosis in sub-Saharan Africa, but studies from Malawi (Mundy et al., 2000) and Tanzania (Kahama-Maro et al., 2011) have reported that the poor quality of routine BS has led to massive over-diagnosis of malaria in various levels of health care facilities.

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Malaria rapid diagnostic tests (RDT) have been shown to have both high sensitivity and specificity in the outpatient setting, although results remain positive for 1 month after malaria infection (WHO, 2008), and the sensitivity is lower at lower levels of parasitemia (Beadle et al., 1994; Huong et al., 2002; Van den Broek et al., 2006). Data regarding the use of RDT among hospitalized adults in malaria-endemic regions are lacking.

In this prospective study, we enrolled adults admitted with suspected malaria to 2 referral hospitals in northwestern Tanzania over a 6 month period and performed multiple tests for malaria including BS and RDT. The objectives of this study were as follows: (1) to determine the prevalence of malaria by BS and RDT, (2) to determine the agreement between BS, RDT and malaria PCR, (3) to determine the baseline characteristics that were associated with malaria, and (4) to compare malaria parasite clearance rates between HIV-infected and uninfected patients.

## 2. Material and methods

### 2.1. Study site

This was a prospective, cross-sectional study conducted in the adult medicine wards of Bugando Medical Centre (BMC), a consultant and teaching hospital, and Sekou Toure Hospital in Mwanza, Tanzania. Bugando Medical Centre is the zonal referral hospital for the Lake Zone of northwestern Tanzania (approximately 13 million people). The Lake Zone is considered highly endemic for malaria with the malaria parasitemia prevalence of 14.8% in children between 6 and 59 months according to the most recent national survey in 2011 (TACAIDS, ZAC, NBS, 2012). The hospital has approximately 1000 total beds and roughly 4000 medical admissions per year. Sekou Toure Hospital is the regional referral hospital for the Mwanza region (approximately 3.2 million people). Sekou Toure has 375 total beds and approximately 2000 medical admissions per year. Approximately 30% and 15% of all medical admissions are suspected to have malaria at Sekou Toure and BMC, respectively. All adult medical patients admitted to both hospitals routinely undergo provider-initiated testing and counseling (PITC) for HIV, and approximately 25% are HIV-infected.

### 2.2. Study population

Eligible patients included all those over 18 years of age admitted to the medical wards of Bugando Medical Centre and Sekou Toure Hospital who were suspected to have malaria by a clinician. All adults admitted with suspected malaria who provided informed consent for study participation and consented to undergo PITC were enrolled.

### 2.3. Study protocol

Demographic and clinical information was collected from each patient (or a close relative if the patient was unable to communicate) using a structured questionnaire. Medical history such as use of anti-malarials during the course of the present illness was also obtained. For those who were known to be HIV-infected, additional items from the medical chart including anti-retroviral therapy (ART) status and duration and cotrimoxazole prophylaxis usage were recorded. All patients also underwent a standard physical examination, and the findings were recorded.

A routine thick blood smear for malaria was taken from all patients on admission using the hospital's standard procedure, which is in accordance with World Health Organization (WHO) guidelines (WHO, 2006). The initial BS was interpreted by on-duty hospital laboratory technicians, who have 2-year diploma-level training in laboratory technology and at least 1 year of experience. At the time of this study, there was no quality assurance (QA) program for malaria microscopy in place at our hospitals.

For patients with positive BS at the time of admission, repeat thick blood smears were taken at 24-h intervals from the time of treatment initiation on days 1, 2, and 3 in order to determine parasite clearance times. These slides were interpreted by an independent laboratory technician who holds a bachelor's degree in laboratory science and technology and has >5 years of experience as a microscopist in the field of parasitiology. For quality assurance, 10% of these slides underwent a second reading by another independent, experienced laboratory technician. In the case of discrepancy, a third experienced technician was involved as a tiebreaker. All microscopists were blinded to HIV status of the patients.

RDT for malaria were also performed on all patients within 24 hours of admission using the RDT (SD BIOLINE *Malaria Antigen P.f./pan* test, Standard Diagnostics, Inc., Kyonggi-do, Korea) since this is among the RDTs recommended by the WHO and Tanzanian Ministry of Health (WHO, 2012a). RDT is specific for the detection of an antigen called histidine-rich protein-2 (HRP-II) that is specific to *Plasmodium falciparum* as well as plasmodium lactate dehydrogenase (pLDH) antigen, which is an enzyme that is common to all malaria species.

Blood samples were frozen at −20 °C for later testing of samples with discrepant BS/RDT results by PCR for *P. falciparum*. PCR testing was performed at the Tanzanian National Institute for Medical Research (NIMR). Briefly, DNA was isolated from 200 μl blood with QIAamp Blood Kit spin columns using the manufacturer's recommendations. In each sample, a fixed amount of Phocin Herpes virus 1 (PhHV-1) was added within the isolation lysis buffer to serve as an internal control. *Plasmodium*-specific PCR primers, *P. falciparum*-specific XSprobe (Biolegio, Nijmegen, the Netherlands), and primers and detection probe for PhHV-1 were used as described previously (Kaisar et al., 2013). An amplification reaction of each DNA sample was performed in a volume of 25 μl with PCR buffer (HotstarTaq mastermix; Qiagen, Valencia, CA, USA), 5 mM $MgCl_2$, 12.5 pmol of each *Plasmodium*-specific primer and 15 pmol of each PhHV-1-specific primer, 2.5 pmol of each *P. falciparum*-specific MGB Taqman probe and PhHV-1-specific Cy5 double-labeled detection probe (Biolegio, Nijmegen, the Netherlands), and 5 μl of the DNA sample. Amplification consisted of 15 min at 95 °C followed by 50 cycles of 15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C. Amplification, detection and data analysis were performed with the Rotor gene real-time system (Qiagen).

We considered malaria status to be confirmed positive or negative in any patient for whom at least 2 tests for malaria (BS, RDT, or PCR) agreed. Patients who had 2 discordant tests but who did not receive a third confirmatory test were excluded from further analysis.

HIV tests were performed on all patients according to the Tanzanian national guidelines for PITC (NACP, 2007). We excluded from the study those who did not consent for HIV test.

All patients were managed according to the hospital protocol. All results were provided promptly to the clinician, and treatment decisions were left to the clinician involved in the patient's care, including the decision of when to discontinue malaria treatment. Our research team (a researcher and two nurses) followed up closely on all patients to ensure that prescribed medications were issued in a correct and timely manner. Patients were followed until discharge, and discharge conditions were noted.

### 2.4. Data analysis

Data was analyzed using STATA software version 11 (College Station, Texas, USA). The primary outcome was the prevalence