False-Positive Results for Rapid Diagnostic Tests for Malaria in Patients with Rheumatoid Factor

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Four different rapid diagnostic tests (RDTs) for malaria were evaluated by testing 82 healthy control patients, 89 *Plasmodium vivax*-infected patients, and 92 rheumatoid factor (RF)-positive nonmalaria patients. The false-positive rate ranged from 2.2% to 13% in RF-positive patients. High RF levels are associated with malaria RDT false positivity.

Malaria remains a major global health problem in tropical and subtropical countries, with high morbidity and mortality and extensive economic loss (1). Malaria rapid diagnostic tests (RDTs) are becoming the clinical diagnostic method of choice due to their quick results and ease of use, even by inexperienced personnel (2). However, false-positive results may be observed in patients with rheumatoid factor (RF), hepatitis C, toxoplasmosis, human African trypanosomiasis, dengue, leishmaniasis, Chagas disease, and schistosomiasis (2). Iqbal et al. (3) reported that 33 of the 35 false-positive specimens were negative when the RF was absorbed in the immunochromatographic test (ICT). The goal of this study was to use four different malaria RDTs to explore the relationship between false-positive malaria RDT results and RF.

Between April 2010 and August 2013, a total of 263 whole-blood samples with EDTA were collected from South Korean patients at the Korea University Guro Hospital, Republic of Korea. Of these 263 samples, 89 were infected with malaria, as confirmed by Giemsa-stained microscopic examination, 92 did not have malaria but did have RF, and 82 had neither malaria nor RF. Both microscopy and PCR were used to rule out malaria. Each patient provided informed consent under the protocol for human use, which was approved by the Human Use Ethical Committee, Korea University Guro Hospital.

Thick and thin blood films were prepared when blood was drawn in accordance with standard procedures. These films were stained with Giemsa and examined by trained microscopists who did not have prior knowledge of the patients' clinical history. *Plasmodium* species and the parasite density were determined. The circumsporozoite protein (*CSP*) gene of *Plasmodium vivax* was amplified by PCR using previously established methods (4). Four commercial malaria RDT kits were selected based on the multiple target antigens (Ags) detected, the BinaxNOW malaria kit (Binax Inc., Scarborough, ME, USA), the OptiMAL-IT malaria kit (Bio-Rad, Marnes la Coquette, France), the SD Bioline malaria Ag Pf/Pan rapid test (Standard Diagnostics, Inc., Yongin, South Korea), and the Humasis malaria P.f/Pan antigen test (Humasis, Anyang, South Korea). BinaxNOW detects both histidine-rich protein 2 (HRP-2), which is specific to *Plasmodium falciparum*, and aldolase, which is a pan-malarial enzyme found in the five human pathogenic *Plasmodium* species (5). OptiMAL-IT differentiates *P. falciparum*-specific lactate dehydrogenase (PfLDH) and pan-*Plasmodium* lactate dehydrogenase (pLDH) by immunological detection (6). The SD Bioline and Humasis tests target HRP-2 for *P. falciparum* and pLDH for other human malaria species (7, 8). All tests were performed according to the manufacturers' instructions. The RDT results were interpreted by multiple technicians.

RF quantitation was performed using a Seiken RF latex(X1) reagent (Denka Seiken, Tokyo, Japan) in a TBA-200FR NEO automatic analyzer (Toshiba Medical Systems Corporation, Tochigi-ken, Japan). We tested the specimens for antinuclear antibody (ANA) using the Kallestad HEp-2 cell line substrate (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions, in order to discriminate the ANA effect on false-positive malaria RDTs. We used two enzyme-linked immunosorbent assays (ELISAs) that target different malaria antigens for comparison of malaria RDTs, the SD malaria antigen Pf ELISA (Standard Diagnostics, Inc.) and the Genedia malaria antigen ELISA (Green Cross Co., Seoul, South Korea). The SD malaria antigen Pf ELISA and Genedia malaria antigen ELISA were used to detect HRP-2 and pLDH, respectively.

No malaria RDT showed false-positive results in *P. falciparum*-specific bands from the 89 patients infected with *P. vivax* and the 82 healthy controls. Of the 92 RF-positive specimens, there were 15 false positives (16.3%; 95% confidence interval, 0.1013 to 0.2517), and of the 368 (92 × 4) malaria RDT results, there were 26 false positives (7.1%; 95% confidence interval, 0.0487 to 0.1016) (Table 1). BinaxNOW had the highest false-positive rate by specimen (13%), with a rate of 9.8% for the HRP-2 and 5.4% for the aldolase bands. The SD Bioline test had the lowest false-positive rate by specimen (2.2%), with a rate of 1.1% for the HRP-2 and 1.1% for the pLDH bands. The Humasis test and the OptiMAL-IT test had a 6.5% false-positive rate by specimen. The mean RF levels were 3.2 ± 2.8 IU/ml (range, 1.0 to 14.8 IU/ml) in the healthy control patients ($n = 82$), 6.4 ± 5.2 IU/ml (range, 1.0 to 24.8 IU/ml) in the *P. vivax*-positive patients ($n = 89$), and 270.8 ± 299.2 IU/ml (range, 16.2 to 1,452.1 IU/ml) in the RF-positive patients ($n = 92$). The mean levels of RF were lowest

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TABLE 1 Characteristics associated with 15 false-positive results produced by malaria RDT kits and malaria ELISAs

No. of RDTs used	Sample no.	Gender[a]	Age (yr)	IgM RF[b] (IU/ml)	BinaxNOW malaria HRP-2	BinaxNOW malaria Aldolase	OptiMAL-IT PfLDH	OptiMAL-IT pLDH	SD Bioline malaria Ag Pf/Pan rapid test HRP-2	SD Bioline malaria Ag Pf/Pan rapid test LDH	Humasis malaria Pf/Pan antigen test HRP-2	Humasis malaria Pf/Pan antigen test LDH	Absorbance value of SD Bioline malaria antigen ELISA for HRP-2 detection[d] (cutoff, 0.1575)	Absorbance value of Genedia malaria antigen ELISA for pLDH detection[d] (cutoff, 0.12)	ANA[e]	Diagnosis[f]
1 (RF: 348 ± 277.7 IU/ml)	1	F	36	113.9	+ (weak)	—	—	—	—	—	—	—	**0.326**	0.085	+	Rheumatoid arthritis
	2	F	73	117.4	—	—	—	—	+ (weak)	—	—	—	**0.268**	**0.171**	+	HTN DM
	3	M	54	156.1	+ (weak)	—	—	—	+ (weak)	—	—	—	**0.371**	0.079	+	Rheumatoid arthritis
	4	F	20	208.9	+ (weak)	—	—	—	—	—	—	—	0.134	0.069	—	Arthritis
	5	M	56	338.8	+	+	—	—	—	—	—	—	**0.553**	**0.265**	—	Asthma
	6	M	44	410.1	+ (weak)	—	—	—	—	—	—	—	0.136	0.098	—	Health check-up
	7	F	18	500.3	—	—	+ (weak)	+ (weak)	—	—	—	—	**0.694**	**0.136**	+	Rheumatoid arthritis
	8	M	62	938.5	—	—	—	—	—	—	+ (weak)	+ (weak)	**0.237**	**0.178**	+	Rheumatoid arthritis
2 (RF: 713.4 ± 608.8 IU/ml)	9	M	45	341.8	+ (weak)	—	—	—	+ (weak)	—	+ (weak)	—	**0.583**	**0.251**	—	Health check-up
	10	F	71	382.5	+ (weak)	—	—	—	+ (weak)	—	+ (weak)	—	**0.34**	0.059	—	Bursitis
	11	F	43	1,416.0	+ (weak)	+ (weak)	+ (weak)	+ (weak)	—	—	—	—	**0.606**	0.098	+	HBV
3 (RF: 1147.5 ± 292.0 IU/ml)	12	F	33	817.6	+ (weak)	+ (weak)	+ (weak)	+	—	+ (weak)	—	—	**0.585**	0.103	—	HBV
	13	F	53	995.9	+	+ (weak)	+ (weak)	+ (weak)	—	—	+ (weak)	+ (weak)	**0.246**	0.077	+	Rheumatoid arthritis
	14	M	47	1,324.2	—	+	+ (weak)	+ (weak)	—	—	+	—	**0.352**	**0.125**	+	Rheumatoid arthritis
	15	M	55	1,452.1	—	+	+ (weak)	+ (weak)	—	—	+	+	**0.169**	**0.126**	+	Arthrosis

[a] F, female; M, male.
[b] RF, rheumatoid factor.
[c] HRP-2, histidine-rich protein 2; PfLDH, *P. falciparum* lactate dehydrogenase; pLDH, pan-*Plasmodium* lactate dehydrogenase; +, positive; −, negative.
[d] Enzyme-linked immunosorbent assay (ELISA)-positive results greater than the cutoff value are noted in bold type.
[e] RF levels in the antinuclear antibody (ANA)-positive group were not significantly different from those of the ANA-negative group (Mann-Whitney U test, $P = 0.346$).
[f] HTN, hypertension; DM, diabetes mellitus; HBV, hepatitis B virus infection.
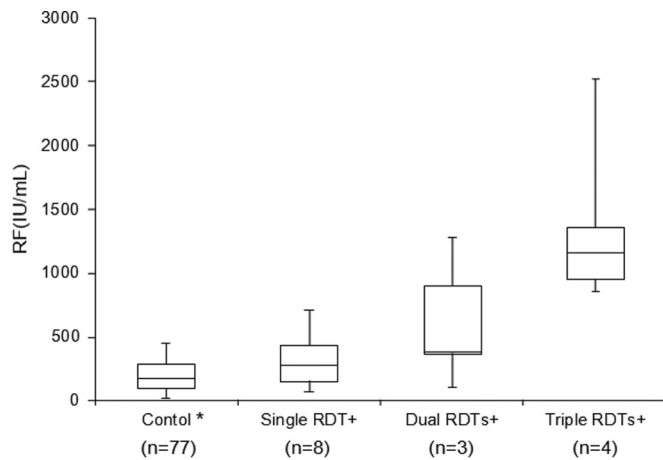
FIG 1 Rheumatoid factor (RF) levels according to number of reactive malaria rapid diagnostic tests (RDTs). The control group shows RF levels for negative RDT results. The upper and lower boxes show interquartile RF values, the inner lines indicate median RF values, and the whiskers illustrate the standard deviation around the mean.

in the samples with a single positive malaria RDT result (348 ± 277.7 IU/ml) and highest in the cases with three positive RDT results ($1,147.5 \pm 292.0$ IU/ml) (Fig. 1). The HRP-2-based SD ELISA had a false-positive rate of 67.4% (62/92) in RF-positive specimens, with a mean RF level of 332.1 ± 308.8 IU/ml (range, 101.7 to 1,452.1 IU/ml), while the pLDH-based Genedia ELISA had a false-positive rate of 33.7%, with a mean RF level of 288.3 ± 179.1 IU/ml (range, 101.7 to 938.5 IU/ml). Of the 15 cases of false positives in the RF-positive group, the antinuclear antibody was present in 9 of the serum samples (60%).

Many types of malaria RDTs are available, and the World Health Organization (WHO) and Foundation for Innovative New Diagnostics (FIND) have carried out detailed performance assessments of these tests (9–12). The exact mechanism behind the reaction of RF with malaria RDTs has not been fully elucidated. However, one possible explanation for the false positives observed in malaria RDTs is that there is a reaction between RF and specific antibodies on the malaria RDT strips (13–15). RF is an autoantibody directed against antigenic determinants on the fragment crystallizable (Fc) region of immunoglobulin G (IgG) molecules; thus, RFs can bind to the trapping antibody (colloidal gold-labeled antibody). This antibody complex is easily detected by capturing the antibody on the strip, resulting in a false positive (14). This study systematically investigated the pattern of false positives in malaria RDTs. The composition of the target antigen varies between malaria RDTs. HRP-2-based RDTs are more sensitive than aldolase- or PfLDH-based RDTs for *P. falciparum*, whereas pLDH-based RDTs are more sensitive than aldolase-based RDTs for *P. vivax* (2, 10). Nevertheless, specificity is also important for selecting malaria RDTs, and our study suggests new points for consideration for malaria RDTs to reduce false-positive results. In addition, the prevalence of rheumatoid arthritis (RA) is about 1% in Caucasians but ranges from 0.1% in rural Africans to 5% in indigenous peoples of North America (16, 17). This difference could be related to the structural characteristics of RF. Soltys et al. (18) proposed that some RFs may not bind to certain types of IgG or that not all IgG can be bound by RFs. The highest false-positive rate was for the anti-HRP-2 band, which is specific to *P. falcipa-*

rum. This finding was also observed in malaria ELISA results, with a false-positive rate of 67.4% and 33.7% for HRP-2 and pLDH-based malaria Ag ELISA, respectively. Suspected malaria cases that are HRP-2 positive without microscopy should undergo confirmation tests, including malaria RDTs that target other malaria antigens such as pLDH and aldolase. Compared to the pLDH or aldolase band, the HRP-2 band has a higher rate of false positives at lower levels of RF. Malaria RDT false positives tend to increase with increased RF levels. These findings were identified in all four types of malaria RDTs. Of the 62 false-positive HRP-2 ELISA results, 31 were positive for antinuclear antibody (ANA), and 32 were negative for ANA. Of the 31 false-positive pLDH ELISA results, 12 were ANA positive, and 19 were ANA negative. RF levels in the ANA-positive group were not significantly different from those in the ANA-negative group. There was no clear false-positive pattern according to ANA presence or absence. High RF levels may be able to produce false-positive malaria RDT results regardless of ANA presence. High RF levels tend to be associated with false positives in malaria RDTs. We should carefully interpret malaria RDTs and consider clinical situations and laboratory, and especially microscopic confirmation tests in cases of suspected false-positive results for malaria RDT.

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