One of the most pronounced problems in controlling the morbidity and mortality caused by malaria is limited access to effective diagnosis and treatment in areas where malaria is endemic. Clinical diagnosis of malaria still relies upon identification of a malaria parasite in Giemsa-stained blood smear of the peripheral blood. Recently, rapid nonmicroscopic tests for the detection of Plasmodium falciparum infection have been introduced to overcome problems associated with time constraint and low sensitivity in diagnosing malaria infections with a low level of parasitemia by microscopy. These rapid tests are based on the detection of antigen(s) released from the parasitized erythrocytes. Two of the tests, ParaSight F (Becton Dickinson, Paramus, N.J.) and the ICT Malaria Pf (ICT Diagnostics, Australia), detect P. falciparum histidine-rich protein 2 (PfHRP-2) and detect P. falciparum infection only (4, 6, 14). The third test, OptiMAL (Flow, Inc., Portland, Ore.), detects a different malarial antigen, Plasmodium-specific lactate dehydrogenase (pLDH), and can be used to detect infections with any of the Plasmodium spp. infecting humans (5). The ParaSight F and the ICT Malaria Pf tests have been evaluated at various epidemiological settings and are claimed to have a constant specificity and sensitivity of around 80% relative to standard microscopy of thick film (12). According to one study done in the Trujillo area of northern Honduras during a malaria outbreak, the OptiMAL test demonstrated sensitivities of 94 and 88% and specificities of 100 and 99%, respectively, when compared to traditional blood films for the detection of Plasmodium vivax and for P. falciparum malaria (11). However, there are certain limitations to these rapid tests, including the decrease in sensitivity (<70%) in parasitemias of <50/μL and the occurrence of false-positive reactions in patients due to persistence of antigen for up to 28 days after asexual parasite clearance by antimalarial therapy from the peripheral blood (7, 13, 16; B. Mishra, J. C. Santanaray, A. Kumar, and B. R. Mirdha, Letter, J. Clin. Microbiol. 37:1233, 1999).

This study was conducted to further extend recent studies in which false-positive reactions were observed when the ParaSight test was used in rheumatoid factor-positive patients (2, 3, 9). We investigated groups of patients without malaria, but with infections that are generally associated with rheumatoid factor (RF) and/or with antinuclear antibodies (ANAs). These patients belonged to groups with different immunological disorders or had different parasitic infections.

**MATERIALS AND METHODS**

**Patients.** A total of 225 patients from various disease groups were included in the study. All of the patients included in this study were negative for malaria on microscopy of Giemsa-stained blood film. One hundred thirty-three of the patients were positive for RF and/or for ANAs. The selection of the following groups of patients was made in accordance with the relative increased prevalence of these infections in our population. Informed consent was obtained from all patients. This study was approved by the Ethical Committee of the Faculty of Medicine, Kuwait University, Kuwait. The groups of patients included in the study were patients with rheumatoid arthritis, patients with viral infections, and patients with chronic parasitic infections. The rheumatoid arthritis group was divided into 50 patients with positive RF only, 25 patients with positive RF and ANAs, and 25 patients with positive ANAs only. The viral infection group was made up of 25 patients with chronic hepatitis C. The chronic parasitic infection group was made up of 50 patients with schistosomiasis, 25 patients with Toxoplasma gondii infection, and 25 patients with Echinococcus granulosus infection (hydatid disease).

**Selection of immunocapture diagnostic assays.** The following two immunocapture diagnostic assays were used to detect malaria-specific antigens in patients: a PfHRP-2-based assay (ICT Malaria Pf), which detects only P. falciparum infection, and a pLDH-based test (OptiMAL), which can detect infections with any of the Plasmodium spp. infecting humans. All specimens were tested with the ICT assay, but only those found positive by the ICT assay were then tested with the OptiMAL assay.

**Malaria diagnosis with the pLDH-based assay (OptiMAL).** The OptiMAL test was performed as described earlier by following the manufacturer’s instructions (11). Briefly, 1 drop of whole blood was mixed with 2 drops of lysis buffer A, which disrupts the erythrocytes and releases the pLDH, and the specimen was
allowed to migrate to the top of the OptiMAL strip. After 8 min, the OptiMAL strip was cleared by adding 2 drops of buffer B. A negative control sample was included with each batch tested. A monospecific antibody that recognizes only P. falciparum is present in the bottom reaction zone. A second pan-specific antibody that recognizes the pLDH isoforms of P. vivax is present immediately above this zone. A third reaction zone, containing a pan-specific monoclonal antibody, is present at the top of the test strip, which serves as a positive control for the assay. Malaria diagnosis with the PfHRP-2 antigen-based assay (ICT Malaria). The ICT assay was performed as described earlier by following the manufacturer’s instructions. Briefly, 10 μl of whole blood is added to the test strip, where lysis occurs, and any PfHRP-2 antigen present binds to the colloidal gold-labelled antibody in addition of buffer to the strip, the blood and labelled antibody migrate up the test strip, crossing the second antibody line. In a positive sample, PfHRP-2 complexed with the gold-labelled antibody is captured by the antibody on the membrane, and a pink line forms. In a negative sample, a pink line does not appear.

IFA. The indirect immunofluorescence assay (IFA) was performed on glutar-aldehyde-fixed monolayers of F32 P. falciparum-infected erythrocytes (F32 strain kindly provided by Klaive Bezin, Department of Immunology, Stockholm University, Stockholm, Sweden) as described previously (8). The visualization of bound malaria-specific antibodies was performed with biotinylated goat anti-human immunoglobulin G (IgG) and fluorescein-conjugated avidin.

Absorption of RF with IgG-coupled Sepharose. The human IgG was coupled to cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) (0.1 mg of IgG per ml of packed gel) according to the instructions of the manufacturer. Three hundred microliters of RF-positive serum was mixed with 500 μl of the coupled Sepharose and incubated at room temperature for 30 min. The mixture was centrifuged, and absorbed sera were collected from the supernatant. The adsorbed RF was eluted from the Sepharose with 0.1 M glycine HCl (pH 3.5) and neutralized with 1 M Tris (pH 9.0).

RESULTS AND DISCUSSION

A total of 255 patients were included in this study. All of the patients included in this study were negative for malaria parasites on microscopy. The patients belonged to various groups classified according to infections and immunological disorders that are generally associated with RF. The patients belonged to the following disease groups: rheumatoid arthritis, chronic hepatitis C, and chronic parasitic infections (toxoplasmosis, schistosomiasis, or hydatid disease). All three of the parasitic infections included in the study (schistosomiasis, hydatid disease, and toxoplasma infections) are relatively common in Kuwait. More than 30% of the population, including immigrants, have high titers of antibodies to one or more of these infections. Although the majority of them are asymptomatic, they have high titers of antibodies due to persistence of parasites, larvae or eggs in the body. Patients with various complications are also frequently encountered. We only included those patients who were negative for malaria parasites on thick blood film microscopy and who gave no history of malaria or visit to a country where malaria is endemic in the last 2 to 3 years.

Forty-two patients had both RF and ANAs, 91 patients had only RF, and 38 had only ANAs. The patients were screened with the ICT assay to detect PfHRP-2 antigen. Twenty-seven of the 100 (27%) patients from the rheumatoid arthritis group gave a positive reaction with the ICT assay; 6 of these 27 were positive for both RF and ANAs, 18 of them had RF only, and 3 had ANAs only (Table 1). Eight of the 25 patients with chronic hepatitis C (32%) were positive by the ICT assay; 6 of the 8 patients had high titers of RF, and two were positive for both RF and ANAs (Table 1). Of the patients with parasitic infections, all 25 patients with the Echinococcus granulosus infection (hydatid disease) were negative by the ICT assay (Table 1); however, 2 of the 50 (4%) patients with schistosomiasis and 2 of the 25 (8%) patients with toxoplasmosis gave a positive reaction with the ICT assay (Table 1). Both the ICT-positive toxoplasma patients and one of the schistosomiasis patients had RF.

Although only 35 of the 133 patients with RF gave a false-positive reaction with the ICT assay, of the 39 ICT-positive patients, 35 (90%) had high titers of RF. We observed a relatively smaller proportion of false-positive reactions (26%) with the ICT assay among our RF-positive patients compared to the results of earlier studies that reported up to 80% false-positive reactivity with the ParaSight test (2, 3, 9). This discrepancy could be due to differences in the tests used, because the specificities of the monoclonal antibody used in the ParaSight test may be different from that used in the ICT assay. In addition, in the earlier study (3), only 19 RF-positive sera were tested, whereas in this study, we tested 133 RF-positive specimens which were obtained from patients with various infections.

In order to further evaluate the false-positive results with the ICT assay, the ICT-positive specimens were tested with the OptiMAL test, a recently introduced nonmicroscopic test for the detection of malaria infection. The OptiMAL test is a pLDH-based test that, unlike the ICT test, can diagnose all species of Plasmodium infecting humans by detecting various isoforms of pLDH specific for various species. Only 4 of the 39 ICT-positive specimens gave a positive reaction with the OptiMAL test; 3 of these 4 specimens gave a positive reaction for P. falciparum, and one was positive for both P. falciparum and P. vivax infection. None of the four OptiMAL-positive specimens were from rheumatoid arthritis patients who had high titers of RF, and the fourth specimen was from a chronic hepatitis C patient and was positive for RF and ANAs (Table 2).

In order to confirm the role of RF in giving false-positive results, the ICT-positive patients were tested with an additional negative control test (Sight test (2, 3, 9)). This disallowed the ICT-positive patients to be classified as RF cross-reactive. The ICT-positive patients were tested with the Sight test, and all patients were negative for malaria on microscopy. All ICT-positive patients were found negative for malaria antibodies by IFA. The sight test is a nonmicroscopic test for the detection of malaria infection. The Sight test is performed on thick blood film microscopy, and all ICT-positive patients were negative for malaria antibodies by IFA.

| Table 1. Performance of the ICT assay on groups of patients
<table>
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<td>Patient group</td>
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<td>Schistosomiasis</td>
<td>Toxoplasmosis</td>
<td>Hydatid disease</td>
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<td>-----------------</td>
<td>------------------</td>
<td>------------------</td>
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<tr>
<td>RF</td>
<td>18/50</td>
<td>6/17</td>
<td>1/13</td>
<td>2/9</td>
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<tr>
<td>ANA</td>
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<td>0/2</td>
<td>0/4</td>
<td>0/5</td>
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<tr>
<td>RF + ANAs</td>
<td>6/25</td>
<td>2/5</td>
<td>0/7</td>
<td>0/3</td>
<td>0/2</td>
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<td>Negative for RF or ANAs</td>
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<td>1/28</td>
<td>0/9</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27/100</td>
<td>8/25</td>
<td>2/50</td>
<td>2/25</td>
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</tbody>
</table>

*All patients were negative for malaria on microscopy.

a All ICT-positive specimens were found negative for malaria antibodies by IFA.

| Table 2. Detailed evaluation of all ICT-positive patients
<table>
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<td>Patient group (no. of patients)</td>
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<td>ICT (postabsorption)</td>
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<td>------------------</td>
<td>------------------</td>
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<td>Rheumatoid arthritis</td>
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<td>RF only (18)</td>
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<td>ANAs only (3)</td>
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<tr>
<td>Chronic hepatitis C (8)</td>
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<tr>
<td>Schistosomiasis (2)</td>
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</tr>
<tr>
<td>Toxoplasmosis (2)</td>
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</table>

*All of the ICT-positive patients tested negative for malaria antibodies by IFA.

a The absorption of RF was determined as described in Materials and Methods. All of the absorbed sera tested negative for RF.
reactions with the ICT assay, all 35 false-positive specimens that had RF, including the 4 specimens positive according to OptiMAL, were retested after adsorption of RF onto the IgG-coupled activated CNBr-Sepharose 4B columns. Thirty-three of the 35 absorbed sera became negative after adsorption; however, the 2 ICT postabsorbed positive samples remained positive with OptiMAL. To further confirm the reactivity of RF, the adsorbed RFs were eluted from some of the columns with glycine HCl (pH 3.5) and retested with the ICT assay: 8 of the 10 eluted fractions gave positive reactions with the ICT assay. It is suggested that the RFs bind to the trapping antibody (colloidal gold-labelled antibody), and the complex is then detected by the detecting antibody on the strip, giving a false-positive reaction.

It remains to be investigated how RFs from some of the patients provoke a false-positive reaction. Generally the RFs exhibit considerable immunochemical heterogeneity (15), and thus only RFs from patients that have high affinity for the trapping antibody bind, which may explain the 26% false-positive reactions seen with the ICT assay. RFs are autoantibodies directed against antigenic determinants on the Fc fragment of IgG molecules. They are often associated with rheumatoid arthritis and are also present in a variety of other rheumatic disorders, viral infections (hepatitis and human immunodeficiency virus infection), chronic bacterial infections (leprosy and tuberculosis), parasitic infections, and other hyperglobulinemic states. In addition, RFs are present in 5% of healthy individuals, and the frequency increases with age (10). In the tropics and developing countries, healthy persons have been found to have increased seroprevalence of RF, possibly due to the effect of chronic infections (1).

New rapid nonmicroscopic methods for the diagnosis of malaria that complement or support microscopy of blood films would be of great use in the diagnosis and treatment of patients with malaria and in epidemiological studies. However, they must be evaluated thoroughly for their sensitivity and specificity at various epidemiological settings before they are put into practice. The detection of false-positive reactions with the PHiHRP-2 antigen-based assay (ParaSight F or ICT) should be taken into consideration when interpreting a positive result, especially because RFs occur in a variety of disorders and are more commonly found in tropical countries, where there is an increased incidence of chronic parasitic infections and where malaria is endemic. Until we can exclude false-positive reactions, the examination of a thick blood film is essential for the diagnosis of P. falciparum malaria or detection of treatment failure.

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REFERENCES