

DIAGNOSIS OF MALARIA IN NON-ENDEMIC COUNTRIES BY THE PARASIGHT-F® TEST

MARIE-PIERRE BRENIER-PINCHART, CLAUDINE PINEL, ANNE CROISSONNIER, JEAN-PAUL BRION, ODILE FAURE,
DENISE PONARD, AND PIERRE AMBROISE-THOMAS

Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire, Grenoble, France; Clinique Médicale et des Maladies Infectieuses, Centre Hospitalier Universitaire, Grenoble, France; Laboratoire d'Autoimmunopathologie, Etablissement de transfusion sanguine de l'Isère et de Savoie, La Tronche, France

Abstract. QBC®, examination of thin blood smears, and Parasight-F® were performed for every case of malaria suspected between May 1997 and December 1998. Data from 310 patients were reported. Fifty had malaria infection diagnosed by QBC® and thin blood film, among whom 39 had *Plasmodium falciparum* infection. Three of these 39 were negative with the Parasight-F® test. Eleven patients had a positive ParaSight-F® test but microscopic diagnosis methods were negative. Interpretation of these 11 positive ParaSight-F® results is proposed to depend on clinical criteria.

INTRODUCTION

In non-endemic areas, diagnosis of imported malaria on the basis of microscopic examination remains the major diagnostic method. However, interpretation of thick and thin blood smears requires trained microscopists and, for the QBC® method (Becton Dickinson, Meylan, France), specific and relatively expensive equipment (fluorescence microscope and specific kits). Newer methods based on detection in blood of a soluble glycoprotein specific to *Plasmodium falciparum*, the histidine rich protein-II (HRP-II), have been developed.¹⁻³ These commercially available tests do not require specific equipment and are rapid and simple to perform. Their sensitivity and specificity are near 90% but vary in different studies.^{1,2,4,5} To estimate the impact of the ParaSight-F® test (Becton Dickinson) on malaria diagnosis in a clinical laboratory, we retrospectively analyzed the discrepancy between ParaSight-F® test results and conventional diagnostic methods according to the clinical course and final clinical diagnosis of patients in Grenoble, France.

MATERIALS AND METHODS

For each case of suspected malaria seen between May 1997 and December 1998, at Grenoble University Hospital, QBC®, examination of thin blood smear, and ParaSight-F® test were performed. Species identification and intensity of parasitemia were determined on thin blood smears. Parasitemia was expressed as the percentage of infected erythrocytes. Data from 310 patients were analyzed. These febrile patients were French travelers returning from malarial endemic areas or immigrants from endemic areas, mainly Africa. Clinical information was collected. Rheumatoid factor and anti-nuclear antibody were measured when discordance was observed between QBC®/thin blood smear and antigen detection by the ParaSight-F® test. Sensitivity, specificity and predictive values were calculated using microscopy as the reference method.

RESULTS

Fifty of 310 patients had malaria infection according to positive parasite detection by QBC® and thin blood film examination. Thirty-eight had *P. falciparum* (35 positive with

ParaSight-F®), one mixed infection with *P. falciparum* and *Plasmodium ovale* (positive with ParaSight-F®) and 11 with other species (seven *P. ovale* and four *P. vivax*; all were negative with ParaSight-F®).

Accordingly, antigen detection resulted in three false-negative results. One of these patients had only sexual forms of *P. falciparum*, and the other two had *P. falciparum* trophozoites with parasitemia < 0.01%. During the follow-up of one of these two cases, the ParaSight-F® test became positive two days after diagnosis and treatment with halofantrine whereas the microscopic examination became negative.

For 11 patients, the ParaSight-F® was positive and the QBC® and thin smear were negative. These results are summarized in Table 1. They show that: 1) for three patients, positive ParaSight-F® test was present when the subjects were empirically treated with anti-malarial drugs for several days before diagnosis (cases 3 to 5); 2) for six patients, the interpretation of the ParaSight-F® test was equivocal and it was not possible to determine if this corresponded to true malaria infection (cases 6 to 11). Another diagnosis was made in five of these patients and only one was treated despite the absence of positive antigenemia in repeated blood samples. Three of these patients had previously received chemoprophylaxis with chloroquine and proguanil association or mefloquine during their stay in endemic areas. And 3) Two positive ParaSight-F® tests were false-positive. The first patient had not traveled in a malarial endemic area (the West Indies) and the rheumatoid factor level was high. The final diagnosis for the second patient was a primary cytomegalovirus infection. The ParaSight-F® test was not consistently positive in this group.

Overall, the sensitivity and specificity of the ParaSight-F® test were 92.3% and 95.9% compared with QBC® and microscopy, respectively. The positive and negative predictive values were 76.5% and 98.8%, respectively. If the three cases with persistent antigenemia were not considered false-positive, despite negative microscopy, the sensitivity and specificity of the ParaSight-F® test were 92.8% and 97%. The positive and negative predictive values were 82.9% and 98.8%.

DISCUSSION

This retrospective analysis adds to our understanding of the ParaSight-F® test in non-endemic areas. In spite of the

TABLE 1
Description of the 11 patients with positive ParaSight-F® and negative microscopic examination

Patient no. (returning from)	Prophylaxis	Treatment before diagnosis	Positivity of ParaSight-F® on different blood samples	Final clinical diagnosis	RF/AA	Interpretation
1. (West Indies)	No	No	1/1	Pyelonephritis	280/Neg	Certain false-positivity
2. (Vietnam)	ND	No	2/4	CMV primary infection	Neg/Neg	Probable false-positivity
3. (Ivory Coast)	No	Mefloquine, 24 hours before	1/1	Malaria	ND	Persistence of antigenemia
4. (Benin)	Paludrine + chloroquine	Sulfadoxine + pyrimethamine 15 days before	1/1	Malaria	ND	Persistence of antigenemia
5. (Senegal)	Paludrine + chloroquine	Probably	1/1	Probable malaria	ND	Probable persistence of Ag
6. (Vietnam)	Mefloquine	No	1/3	Fever of unknown origin pre- sumptive treatment by halo- fantrine	Neg/Neg	Undetermined efficacy of prophylaxis?
7. (Benin)	Paludrine + chloroquine	No	1/1	Schistosomiasis	40/Neg	Undetermined efficacy of prophylaxis?
8. (Tanzania)	Mefloquine	No	1/1	Apyretic, macular eruption vi- ral infection	ND	Undetermined efficacy of prophylaxis?
9. (Mali)	ND	No	1/1	Peritonitis, appendectomy	ND	Undetermined
10. (Ivory Coast)	ND	No	1/1	Hepatic encephalopathy, delir- ium tremens	ND	Undetermined
11. (Antecedent of malaria, Guinea)	ND	No	1/1	Hepatic encephalopathy, de- mentia	ND	Undetermined

RF = rheumatoid factor (N < 30 IU/ml); AA, antinuclear antibody; Ag = antigenemia; ND = not done; CMV = cytomegalovirus.

simplicity of the test, it yielded false-negative and false-positive results. Fourteen of 310 cases had a discordance between the ParaSight-F® test and conventional diagnostic procedures.

The low level or absence of HRP-II secretion by sexual forms may explain the negative results in one case.¹⁻⁴ For the other two false-negative results, low parasitemia (< 0.01%, ~300-500 parasites/ μ l) may explain failure to detect antigen. Sensitivity of the HRP-2-based test is > 90% when there are at least 60-100 parasites/ μ l, and the threshold of detection is 40-60 parasites/ μ l.^{3,6} However some authors reported false-negative results with high parasitemia (0.1%, ~5000 parasites/ μ l).⁷

Compared with microscopic diagnosis, the ParaSight-F® test was falsely positive in 11 patients. Among these, the persistence of antigenemia following empirical treatment can explain three cases of discordance. Indeed, antigenemia may remain positive 3-28 days after disappearance of circulating parasites.⁸ This test should improve the retrospective diagnosis of malaria after presumptive treatment or self-medication.

Several authors have reported false-positive results with the ParaSight-F® test.^{2,9} Rheumatoid factor seems to be responsible for false-positive results in most cases⁹⁻¹¹ but other situations such as phlebitis and hepatitis have been described.² In the current study, one false-positive may be related to rheumatoid factor. This could be due to a non-specific reaction of rheumatoid factor with the capture IgG monoclonal antibody. Rheumatoid factor does not seem to lead to false-positive results with ICT Malaria Pf® (ICT Diagnostics, Sydney, Australia).¹¹ The capture monoclonal antibody in this test is IgM, to which rheumatoid factor does not bind.¹¹ Variability of ParaSight-F® test results was observed for two patients without rheumatoid factor. Cross-reactivity with proteins rich in histidine normally present in serum may contribute in these cases.² We also observed a false-positive result in a patient who presented with primary cytomegalovirus infection.

In conclusion, this study underlines the difficulty of interpreting the results of the ParaSight-F® test when there is discordance with microscopic examination. Despite its simplicity and rapidity, the ParaSight-F® should not replace microscopy for the diagnosis of malaria. A false-positive ParaSight-F® test can lead to incorrect diagnosis of malaria and exclude consideration of other serious diseases.

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Authors' addresses: Marie-Pierre Brenier-Pinchart, Claudine Pinel, Anne Croissonnier, Odile Faure, and Pierre Ambroise-Thomas, Service de Parasitologie-Myologie, Centre Hospitalier Universitaire de Grenoble, B. P. 217, 38043 Grenoble Cedex 09, France. Jean-Paul Brion, Clinique Médicale et des Maladies Infectieuses, Centre Hospitalier Universitaire de Grenoble, B. P. 217, 38043 Grenoble Cedex 09, France. Denise Ponard, Laboratoire d'Autoimmunopathologie, Etablissement de transfusion sanguine de l'Isère et de Savoie, BP 35, 38701 La Tronche Cedex, France.

Reprint requests: Marie-Pierre Brenier-Pinchart, Service de Parasitologie-Myologie, Centre Hospitalier Universitaire de Grenoble, B. P. 217, 38043 Grenoble Cedex 09, France.

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