DETECTION OF HISTIDINE RICH PROTEIN 2 AND PANMALARIAL ICT MALARIA 
PF/Pv TEST ANTIGENS AFTER CHLOROQUINE TREATMENT OF 
UNCOMPLICATED FALCIPARUM MALARIA DOES NOT RELIABLY PREDICT 
TREATMENT OUTCOME IN EASTERN INDONESIA

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Abstract. In regions with drug-resistant malaria, the ability to rapidly detect or predict treatment failure (TF) soon after a course of standard therapy for Plasmodium falciparum malaria would facilitate the prompt institution of second-line therapy. We thus evaluated longitudinally the ability of the ICT Malaria Pf/Pv immunochromatographic test to predict treatment outcome. Sixty-six Sumbanese Indonesians with uncomplicated falciparum malaria were treated with chloroquine and followed for 28 days by use of 1997 World Health Organization criteria for assessment of therapeutic efficacy of antimalarial drugs. The ICT Pf/Pv testing could be compared with microscopy in approximately half of the patients on each day of follow-up. Although strongly positive histidine rich protein 2 (HRP2) line intensities (equal to or greater than the control band) in convalescence were highly predictive of TF, any degree of positivity for the HRP2 and panmalarial antigens in convalescence was only moderately predictive of TF. Positive predictive values of the HRP2 and panmalarial antigens for TF were 76.9% and 87.0%, respectively, on Day 3, 82.4% and 87.5% on Day 7, and 78.9% and 78.9% on Day 14. Negative HRP2 and panmalarial antigen results in convalescence were even less predictive of an adequate clinical response, and false-negative HRP2 and panmalarial antigen test results were found in one-sixth (6 of 37) of recrudescent infections diagnosed by microscopy among patients with late treatment failure. To reliably predict treatment outcome with rapid antigen tests, further development appears necessary to improve sensitivity for viable asexual parasites while avoiding detection of both gametocytes and persistent antigen in convalescence.

INTRODUCTION

Chloroquine remains the first-line therapy for uncomplicated malaria in Indonesia; however, chloroquine efficacy is declining throughout the country, particularly in the eastern provinces.1 Because of lack of access to microscopy services, diagnosis of treatment failure (TF) is mostly based on a clinical diagnosis and is often delayed. The ability to rapidly detect or predict TF soon after a course of standard therapy for Plasmodium falciparum malaria would facilitate the prompt institution of second-line therapy.

Although currently too expensive for widespread use in malaria-endemic regions, rapid antigen tests for malaria have been shown, in general, to be sensitive and specific for the initial diagnosis of falciparum malaria.2 However, there are only limited data on the ability of antigen detection tests to detect and predict TF when used during or after the completion of therapy. Histidine rich protein 2 (HRP2) antigen is known to persist after resolution of clinical symptoms and clearance of viable asexual parasitemia,3-14 which may limit its ability to reliably predict recrudescence parasitemia. Although some studies have suggested rapid antigen detection tests may be potentially useful for monitoring the response to antimalarial therapy or predicting TF,15-18 concern has been raised by other studies that persistence of HRP2 antigen after clearance of parasitemia may lead to confusing results when used for this purpose.2,14,16 Moreover, none of the studies that have addressed this issue1-18 have used the 1997 World Health Organization (WHO) protocol for assessment of the therapeutic efficacy of antimalarial drugs.17

The ICT Malaria Pf/Pv test is based on the immunochromatographic detection of the P. falciparum-specific HRP2 antigen and a panmalarial antigen found in asexual stages of P. falciparum and P. vivax and sexual stages of P. falciparum.16-20 In our recent evaluation of this test in eastern Indonesia, we found the ICT Pf/Pv test to be very sensitive for the detection of P. falciparum but less so (75%) for detection of P. vivax.18 In contrast to HRP2, there are few data on the persistence of the panmalarial antigen after clearance of parasitemia. In a referral center study of patients treated early without convalescent gametocytemia, panmalarial antigen was reported to be rapidly cleared in parallel with clearance of asexual-stage parasites, as assessed by microscopy.18 In endemic areas, however, we found the panmalarial antigen to persist despite clearance of asexual parasitemia with treatment, with clearance of this antigen paralleling clearance of sexual-stage parasites (gametocytes).20

The purpose of this study, therefore, was to undertake serial ICT Pf/Pv testing in conjunction with the 1997 WHO protocol for assessment of efficacy of antimalarial drugs in order to evaluate longitudinally the utility of both HRP2 and panmalarial antigen detection in predicting treatment outcome after chloroquine therapy of uncomplicated falciparum malaria.

MATERIALS AND METHODS

Time and study site. The evaluation was carried out from February to May 1998 in Radamata Health Centre, Laratama subdistrict, West Sumba district, Nusa Tenggara Timur province, Indonesia, a hypoendemic area for malaria (parasite rate of 5.1% in children aged 0–9 years).18. Informed consent was obtained by all adult study subjects and from a parent or guardian for minors. The study was approved by the ethics committee of the National Institute of Health Research and Development, Indonesian Ministry of Health, Jakarta,
Malaria in the presence of parasitemia with the same species from Day 4 to Day 28 for late TF: danger signs or severe ing the follow-up period (the first 3 days for early TF, and was defined as the development of one of the following during the follow-up days indicated. An additional 10% of patients on Day 7 had false-positive ICT Pf/Pv readings of *P. falciparum* in vivo criteria. Patients were treated with a total of orally administered chloroquine 25 mg base per kilogram of body weight over 3 days.

**Study design and data collection.** This was a part of a broader study evaluating the diagnosis and treatment of malaria in areas of different endemicity in eastern Indonesia. The study was performed in parallel with 28-day in vivo assessment of therapeutic efficacy of chloroquine. Microscopy by an expert microscopist was used as the gold standard.

Clinical evaluation, temperature, parasitemia, and ICT antigen testing were performed on Days 0, 1, 2, 3, 7, 14, and 28 and on any day patients became unwell. Therapeutic outcome was classified as TF or adequate clinical response by WHO criteria for assessment of therapeutic efficacy of antimalarial drugs for uncomplicated *P. falciparum* malaria, 43 patients (65%) were considered to have failed to respond to treatment, with 77% of TFs occurring on or after Day 14. Because of a temporary interruption in supply of ICT tests to the field site, 27, 33, 32, 29, 37, and 34 patients could be evaluated by both microscopy and ICT Pf/Pv on Days 1, 2, 3, 7, 14, and 28, respectively.

**RESULTS**

Of the 66 chloroquine-treated patients with uncomplicated *falciparum* malaria, 43 patients (65%) were considered to have failed to respond to treatment, with 77% of TFs occurring on or after Day 14. A thick film was considered negative if no parasites were seen in 100 high-power fields. All discordant slides were cross-checked by an experienced referral center microscopist in Darwin (M. D.), with at least 200 high-power fields being checked before a slide was considered negative. Ten percent of discordant slides were also cross-checked.

The ICT Pf/Pv (ICT Malaria Pf/Pv test kits; Amrad ICT, Sydney, Australia; MLO2 lot 041388) were identical to marketed kits (Garcia M, personal communication). Testing on each day was performed according to manufacturer’s instructions by trained health care workers. In addition, as previously described, the intensity of each of the HRP2 and panmalarial antigen lines was compared with the intensity of the test kit’s control line and classified as absent, faint, intermediate, and equal to or greater than the control line intensity.

**Data analysis.** Data were analysed by Epi Info 6 (CDC, Atlanta, GA). The denominator used on each day of follow-up was the number of patients for whom an ICT Pf/Pv test result was available. Based on prediction of ultimate outcome, ICT Pf/Pv test results on each day were classified as true positive (TP), false positive (FP), true negative (TN) and false negative (FN). Positive predictive value—the proportion of positive ICT Pf/Pv test readings that truly reflected TF on or after each day of follow-up—was calculated as TP/(TP + FP). Negative predictive value, the proportion of the test’s negative readings that truly predicted an ultimate adequate clinical response, was calculated as TN/(TN + FN).

**RESULTS**

Of the 66 chloroquine-treated patients with uncomplicated *falciparum* malaria, 43 patients (65%) were considered to have failed to respond to treatment, with 77% of TFs occurring on or after Day 14. Because of a temporary interruption in supply of ICT tests to the field site, 27, 33, 32, 29, 37, and 34 patients could be evaluated by both microscopy and ICT Pf/Pv on Days 1, 2, 3, 7, 14, and 28, respectively.

ICT Pf/Pv test positivity on each day of follow-up was higher than microscopic positivity for asexual parasites on all days except Day 28 (Figure 1a). Conversely, among those ultimately having an adequate clinical response, only a minority had negative ICT Pf/Pv results before Day 7 (Figure 1b).
Positive predictive values (95% confidence intervals) of microscopy and each of the ICT Pf/Pv immunochromatographic test antigens on each day of follow-up in predicting adequate clinical response

<table>
<thead>
<tr>
<th>Day</th>
<th>Microscopy positive for asexual parasites</th>
<th>Microscopy positive for gametocytes but not asexual parasites</th>
<th>HRP2 antigen</th>
<th>Panmalarial antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>65.8 (48.6–80.3)</td>
<td>None</td>
<td>67.6 (50.1–81.4)</td>
<td>69.4 (51.7–83.1)</td>
</tr>
<tr>
<td>1</td>
<td>87.5 (67.6–97.3)</td>
<td>100 (2.5–100)</td>
<td>88.0 (67.7–96.8)</td>
<td>91.3 (70.5–98.5)</td>
</tr>
<tr>
<td>2</td>
<td>100 (83.1–100)</td>
<td>57.1 (18.4–90.1)</td>
<td>79.3 (59.7–91.3)</td>
<td>87.5 (66.5–96.7)</td>
</tr>
<tr>
<td>3</td>
<td>83.3 (35.9–99.6)</td>
<td>72.2 (46.5–90.3)</td>
<td>76.9 (55.9–90.2)</td>
<td>87.0 (65.3–96.9)</td>
</tr>
<tr>
<td>7</td>
<td>100 (47.8–100)</td>
<td>71.4 (41.9–91.6)</td>
<td>82.4 (55.8–95.3)</td>
<td>87.5 (60.4–97.8)</td>
</tr>
<tr>
<td>14</td>
<td>100 (73.5–100)</td>
<td>64.3 (35.1–87.2)</td>
<td>78.9 (53.9–93.0)</td>
<td>78.9 (53.9–93.0)</td>
</tr>
<tr>
<td>28</td>
<td>100 (79.1–100)</td>
<td>None</td>
<td>93.3 (66.0–99.7)</td>
<td>100 (69.9–100)</td>
</tr>
</tbody>
</table>

A WHO report has confirmed the need for studies to assess the potential role of rapid diagnostic tests in the detection of TF. One potential application of such tests is to detect persistent or recrudescing parasitemia that would reliably predict TF. Results of this study suggest that posttreatment testing for the HRP2 and panmalarial antigens with the ICT Pf/Pv test are only moderately predictive of TF with its current largely qualitative format. Moreover, negativity for either of these antigens in convalescence was even less predictive of an adequate clinical response.

**Table 2**

Negative predictive value (95% confidence intervals) of microscopy and each of the ICT Pf/Pv immunochromatographic test antigens on each day of follow-up in predicting ultimate adequate clinical response

<table>
<thead>
<tr>
<th>Day</th>
<th>Microscopy negative for asexual parasites</th>
<th>Microscopy negative for gametocytes but not asexual parasites</th>
<th>HRP2</th>
<th>Panmalarial antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 (15.8–100)</td>
<td>66.7 (9.4–99.1)</td>
<td>100 (19.8–100)</td>
<td>75.0 (21.9–98.7)</td>
</tr>
<tr>
<td>2</td>
<td>83.3 (35.9–99.6)</td>
<td>61.5 (35.6–86.1)</td>
<td>50.0 (9.2–90.8)</td>
<td>55.6 (22.7–84.7)</td>
</tr>
<tr>
<td>3</td>
<td>50.0 (15.7–84.3)</td>
<td>34.6 (17.2–55.7)</td>
<td>66.7 (24.1–94.0)</td>
<td>77.8 (40.2–96.1)</td>
</tr>
<tr>
<td>7</td>
<td>50.0 (18.7–81.3)</td>
<td>37.5 (18.5–95.4)</td>
<td>50.0 (22.3–77.7)</td>
<td>53.8 (26.1–79.6)</td>
</tr>
<tr>
<td>14</td>
<td>81.8 (48.2–97.7)</td>
<td>56.0 (34.9–75.6)</td>
<td>55.6 (31.3–77.6)</td>
<td>55.6 (31.3–77.6)</td>
</tr>
<tr>
<td>28</td>
<td>100 (79.4–100)</td>
<td>100 (81.5–100)</td>
<td>89.5 (65.5–98.2)</td>
<td>81.8 (59.0–94.0)</td>
</tr>
</tbody>
</table>
positive predictive values for TF with the ICT Pf/Pv test being highest in those line intensities with either antigen being equal to or greater than the control. This suggests that overall predictive accuracy may be improved by modifying current rapid diagnostic test formats to provide more quantitative data, particularly if these are used in conjunction with clinical assessment. 2

The results of this and a companion study20 support the consensus that persistence of HRP2 antigenemia after clearance of asexual stages results in false-positive findings in convalescence and contributes to the suboptimal accuracy of HRP2 tests in predicting treatment outcome.2 There are only limited data on posttreatment persistence of HRP2 antigenemia with the immunoglobulin (Ig) M monoclonal antibody to HRP2 used in the ICT Pf and Pf/Pv tests,16,18,20 but these suggest rates of persistence at least as high as those found with the IgG HRP2 monoclonal antibody used in the ParaSight-F test.8–3,10,13,14 In the Sumbanese population described above, we found persistence of HRP2 in 29 and 10% of those negative for asexual parasites by microscopy on Days 7 and 14, respectively.20 The causes of posttreatment persistence of HRP2 with both the ICT test and ParaSight-F tests are unclear; they may vary with drug treatment used and geographic region.2 Potential causes include persistent viable asexual parasitemia below the detection limit of microscopy, delayed clearance of circulating antigen (free or in antigen-antibody complexes),21 and rheumatoid factor (although this is much less commonly a cause of false positivity with the ICT IgM monoclonal for HRP2 than with the IgG monoclonal antibody used in the ParaSight-F test22). We recently found an association between HRP2 antigenemia and gametocytemia in patients negative for asexual stages on Day 7 of follow-up,20 suggesting that contrary to earlier thought,22 the presence of HRP2 in both early- and late-stage gametocytes24 may also contribute to false-positive ICT Pf HRP2 test results in convalescence.20

Our results suggest that in addition to the predictive in-accuracies generated by false-positive test results, false-negative test results also diminished test accuracy in diagnosing TFs in convalescence. One-sixth of the late TFs occurring on or after Day 7 were HRP2 and panmalarial antigen negative on the first day in which microscopic positivity for asexual parasites first diagnosed them as late TFs. Although the ICT Pf/Pv test usually detects relatively low levels of parasitemia in patients presenting with their initial clinical illness,18 this inability to reliably detect similarly low parasitemia early in recrudescence may reflect a shorter duration of recrudescent infection relative to the initial clinical illness. This supports an earlier observation in Cambodian children that the ability to accurately diagnose low parasitemia with antigen tests may depend on not only the number of parasites, but also the length of the infection (and the level of antigen accumulating in plasma).11

The ability of the panmalarial antigen to predict treatment outcome has not previously been reported. In a recent referral center study, early effective treatment resulted in the absence of posttreatment gametocytemia and the disappearance of the panmalarial antigen in parallel with the decline of asexual parasitemia after treatment, raising the notion of the possible utility of panmalarial antigen testing in monitoring response to antimalarial therapy.15 Our findings in eastern Indonesia, however, show that in malaria-endemic regions, the panmalarial antigen persists at higher frequencies after treatment than HRP220 and that positive results are only moderately predictive of TF. Reasons for this include longer duration of infection in endemic areas and high frequencies of posttreatment gametocytemia in convalescence, with panmalarial antigen positivity after treatment being closely associated with gametocytemia. The panmalarial antigen may prove to be useful in predicting TF in settings where posttreatment gametocytemia is rare—for example, in nonimmune travelers with short duration of illness and in patients treated with artemisinin derivatives.21 However, as with HRP2 testing, negativity for the panmalarial antigen in convalescence did not reliably predict an adequate clinical response, and false-negative results with the panmalarial antigen were also found in one-sixth of recrudescent infections. An alternative rapid antigen test for malaria, OptiMAL (Flow Inc., Portland, OR), has also been evaluated for antigen persistence after treatment. The OptiMAL test detects Plasmodium-specific LDH (pLDH), with one monoclonal antibody detecting P. falciparum-specific pLDH and the other detecting panmalarial pLDH.24 Levels of pLDH have been shown to decline in parallel with clearance of asexual parasitemia; and investigators in these referral center studies have suggested that this lack of antigen persistence after treatment may make this test useful in predicting TF.27,29 However, as with the panmalarial antibody of the ICT Pf/Pv test, the pLDH monoclonal antibodies of the OptiMAL test detect not only asexual parasites, but also gametocytes.26,30,31 This suggests that in contrast to referral center findings reported to date, and as suggested by a preliminary African study,30 it is possible that the high rates of posttreatment gametocytemia found after chloroquine or sulfadoxine-pyrimethamine in primary health centers in malaria-endemic areas may lead to gametocyte-associated persistence of pLDH despite clearance of asexual parasites, and as with the ICT Pf/Pv test, it may result in an unreliable ability to predict treatment outcome.22 Studies of the ability of pLDH clearance to predict treatment outcome in primary health centers in endemic areas are required before pLDH rapid antigen tests can be advocated as prognostic tools in these settings.22

In conclusion, although positive ICT Pf/Pv antigen results in convalescence were moderately predictive of TF, neither HRP2 or panmalarial antigen detection reliably predicted treatment outcome after chloroquine treatment of falciparum malaria in eastern Indonesia. Although improvements in quantitation of current antigens may improve predictive ability, on the basis of this and other studies, the use of alternative antigens with more rapid clearance and greater sensitivity and specificity for viable asexual (but not sexual) parasites is likely to be required before rapid antigen detection tests can be used to reliably predict malaria TF.
REFERENCES


