Nonstructural Protein NS1: Giving a New Structure to Dengue Diagnosis

In a recent article (6), Wang et al. reported an extensive evaluation of NS1 antigen and compared it with real-time PCR, an IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), and a hemagglutination inhibition assay for diagnosis of dengue virus infection. The authors reported MAC-ELISA positivity for 46 (92%) out of 50 samples positive for dengue virus by real-time PCR (RT-PCR). However, the literature suggests (5) that viremia in dengue lasts for less than 5 days and that the IgM antibody response takes 5 to 10 days to develop in cases of primary dengue virus infection and 4 to 5 days in cases of secondary dengue virus infection (2, 5). Hence, at a particular time postinfection, it is unlikely that the results for both of these tests will be positive. The authors report that they were able to detect viral RNA up to 8 days postinfection. However, this does not explain the overlap period, as it does not explain why RT-PCR positivity was observed in only 1 out of 100 dengue virus IgM-positive samples. It is important to know in how many cases the virus genome could be detected until day 8 out of the 50 samples that were positive by real-time PCR. It would also be valuable to know whether the same patient’s blood was drawn for 8 consecutive days and checked for viremia and, if so, whether the virus was detectable.

The authors mention that they were able to detect NS1 antigen up to 14 days from the onset of fever. Previous studies (1, 3) have reported that NS1 antigen is positive for 9 days postinfection in cases of primary dengue and up to 5 days postinfection in cases of secondary dengue.

We have also evaluated and compared NS1 with IgM for diagnosis of acute dengue virus infection in 87 patients. NS1 antigen had a sensitivity of 71 to 100% when used for patients who had a fever for 3 days. We suggest that NS1 antigen should be considered the test of choice for patients presenting with a history of fever of up to 3 days of duration. On day 4, a combination of MAC-ELISA and an NS1 antigen test would increase the sensitivity of diagnosis. We found that detection of NS1 antigen was not useful beyond day 4 of fever. We also compared both of these diagnostic modalities with conventional RT-PCR for 40 samples from patients with acute dengue. The results for the NS1 test were positive for an additional 15 samples which were negative by conventional RT-PCR (4). It is important to know that NS1 testing is also available as a strip test and also as a dengue duo test (NS1/IgM/IgG), which has further increased the sensitivity of detection.

REFERENCES


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Authors’ Reply

In our study, the IgM antibody response was observed to develop on day 2 after the onset of illness. The rapidity with which IgM develops differs considerably among patients. Although most patients had detectable IgM antibody by day 5 of illness, some patients developed IgM on day 3 or 4 after the onset of illness, whereas others may develop IgM only 7 to 8 days after the onset. Most of our cases were secondary dengue cases. Also, we note that in secondary cases the overwhelming IgG response can block detection of IgM. We therefore cannot agree that at a particular time postinfection it is unlikely that both RT-PCR and MAC-ELISA will give positive results. We have also shown this in our previous publications indicating that viral RNAs were detected by a real-time assay in about 70% of our IgM-positive samples (3, 6). In our current study, 46 (92%) out of the 50 confirmed RT-PCR-positive samples tested positive by MAC-ELISA (Table 1). However, for the overall 320 study subjects, 88 (54.3%) out of the 162 RT-PCR positive samples tested positive by MAC-ELISA (Table 1).

The proper handling and storage of specimens affect the sensitivity of RT-PCR and virus isolation. In addition, the level of viremia differs greatly, depending on the time after onset, the antibody titers, and/or the strain of the infecting virus. Although viremia is usually detectable for an average of 4 to 5 days, in our study, the virus genome could be detected until day 8. As for doing daily blood collection, it is not feasible to draw blood for 8 consecutive days, as the blood collected was obtained from outpatient or hospitalized patients from our dengue surveillance study.

Previous studies by Alcon et al. (1), Dussart et al. (2), and Kumarasamy et al. (4) have reported that the NS1 antigen was detectable from the first day after the onset of the fever up to day 9. Although our study has shown that NS1 antigen was detectable 14 days after onset of the fever, this is in agreement with findings from Xu et al., whose data suggested that the NS1 antigen, limited to dengue virus serotype 1, was detectable until day 18 after the onset of the symptoms (5).
In our opinion, all evaluation studies should follow standard criteria, including correct sample grouping in accordance with the gold standard and a minimum number of samples in each group. It should be understood that different populations respond differently immunologically. The world is now quite heterogeneous, and therefore, blanket inferences cannot apply. In our opinion, several of the studies cited include too few samples to be valid. The minimum number that we would suggest is 250 to 300 samples per evaluation. Even then, only suggestions can be given. To determine what tests are appropriate for a certain population, one must take into account the endemicity in the regions, the population at risk, their genetic background, and their living conditions. All of this is going to affect the immunological response.

**REFERENCES**


**TABLE 1.** Samples positive for both RT-PCR and MAC-ELISA on days 1 through 8 after the onset of illness

<table>
<thead>
<tr>
<th>Day after the onset of illness</th>
<th>Total</th>
<th>Positive by MAC-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of confirmed RT-PCR-positive samples from the current study</td>
<td>No. of RT-PCR-positive samples from the overall 320 study subjects</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4</td>
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<tr>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>46</td>
</tr>
</tbody>
</table>

In our opinion, all evaluation studies should follow standard criteria, including correct sample grouping in accordance with the gold standard and a minimum number of samples in each group. It should be understood that different populations respond differently immunologically. The world is now quite heterogeneous, and therefore, blanket inferences cannot apply. In our opinion, several of the studies cited include too few samples to be valid. The minimum number that we would suggest is 250 to 300 samples per evaluation. Even then, only suggestions can be given. To determine what tests are appropriate for a certain population, one must take into account the endemicity in the regions, the population at risk, their genetic background, and their living conditions. All of this is going to affect the immunological response.

**REFERENCES**


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