Two Cases of False-Positive Dengue Non-Structural Protein 1 (NS1) Antigen in Patients with Hematological Malignancies and a Review of the Literature on the Use of NS1 for the Detection of Dengue Infection

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INTRODUCTION

Dengue fever is a self-limiting systemic mosquito-borne viral illness characterized by fever, myalgia, rash, leucopenia, and thrombocytopenia. Its clinical presentation and associated clinical laboratory investigations could mimic other viral infections, including acute human immunodeficiency virus (HIV) infection, and non-viral diseases, like severe gram-negative sepsis, systemic inflammatory disorders, and hematological malignancies.

Commercial kits for the rapid in vitro diagnosis of dengue infection have been developed and adopted as corroborative evidence for the early laboratory confirmation of the disease in endemic regions. The reported sensitivity of the non-structural protein 1 (NS1) antigen tests ranges between 48.5% and 58.6%, and the specificity ranges between 92.5% and 99.4%. The combined sensitivity of the dengue NS1 antigen and immunoglobulin M (IgM) antibody test increases to 89.9–92.9%, with a specificity of 75.0–88.8%.

The use of such tests on patients with a low pre-test probability could result in an increase in false-positive results, leading to dilemmas in clinical assessment and delays in initiating appropriate management for the individual patient.

We report two cases of false-positive NS1 antigen detection in patients with underlying hematological disorders treated at Tan Tock Seng Hospital (TTSH) and review the literature for causes of false-positive dengue NS1.

CASE REPORT

Case 1. A 53-year-old female patient with a past medical history of diabetes mellitus and hypertension presented with a 1-month history of fever associated with chills, night sweats, and lethargy. In addition, she had also lost 2 kg over 1 month. She had no ill contacts and no significant travel history leading up to her hospitalization.

On admission, she was febrile, with a temperature of 38.9°C. Examination findings were unremarkable. On the day of admission, laboratory investigations showed pancytopenia (hemoglobin = 9.7 g/dL, white blood count = 2.5 × 10^9/L, and platelets = 101 × 10^9/L). Her liver enzymes were also elevated (alanine transaminase [ALT] = 75 U/L and aspartate aminotransferase [AST] = 156 U/L). Dengue NS1 antigen was detected on the commercially available rapid dengue diagnostic kit SD Bioline Dengue Duo Combo Device (Standard Diagnostic Inc., Korea). Dengue IgM and IgG antibodies were not detected in the same sample of blood. HIV screen and blood cultures done on admission were negative.

The history of prolonged fever was atypical for dengue; the admission diagnosis of dengue was reviewed, and the patient was further tested. Over the next 5 days, the patient remained persistently febrile. There was progressive pancytopenia in association with disseminated intravascular coagulation (prothrombin time [PT] = 18.6 seconds, thrombin clotting time [TCT] = 40.0 seconds, fibrinogen = 0.7 g/L, and D-dimer > 4.00 µg/mL) and elevated serum ferritin levels (> 1,500 µg/L). Splenomegaly was shown on computer tomography of the abdomen. An urgent bone marrow evaluation was arranged on the fifth day of admission, which later confirmed the diagnosis of an aggressive natural killer (NK) cell lymphoma. Unfortunately, she deteriorated rapidly and died on the fifth day of hospitalization soon after the diagnosis of a aggressive lymphoma was made. The NS1-positive blood specimen, which was stored at 4°C, was retrieved for additional investigations; a reverse transcriptase polymerase chain reaction (RT-PCR) done showed that dengue RNA was not detected using a protocol developed locally and the Centers for Disease Control (CDC) DENV Real-Time RT-PCR assay. The dengue RT-PCR test was run as a batch test (Table 1).

Case 2. A 68-year-old female patient presented with a 3-day history of fever, chills, and myalgia on a background of hypertension and dyslipidemia and a recent diagnosis of myeloproliferative disorder transforming into acute myeloid leukemia (AML). She had not been started on systemic chemotherapy.

On admission, her temperature was 40.0°C, and she was normotensive, with a blood pressure of 141/56 mmHg. Apart from pallor, physical examination was otherwise unremarkable.

Her admission hemoglobin was 5.8 g/dL, white blood cell count was 43.4 × 10^9/L with 3% blasts, and platelet count was 140 × 10^9/L. Liver enzymes were not available at the point of admission. The dengue NS1 antigen was positive on the Dengue Duo kit by SD Bioline and negative for dengue IgM.
and IgG antibodies on the same assay. HIV screen and blood cultures done on admission were negative (Table 1).

Within 24 hours of admission, the patient developed progressive respiratory distress, was found to be unresponsive, and went into cardiopulmonary arrest, with a down time of 10 minutes. She was successfully resuscitated and initially managed for severe dengue in the intensive care unit.

To follow up on the positive Dengue Duo test, the dengue RT-PCR performed on the fourth day of her febrile illness was negative. On close scrutiny, her cardiopulmonary arrest was mostly likely secondary to type 2 myocardial infarction precipitated by pneumonia and anemia. Her serum procalcitonin was 2.37 μg/L in association with bilateral infiltrates on chest radiograph. Troponin I was elevated (38.24 ng/mL), and her ejection fraction on cardio echogram was depressed at 35% with impaired systolic and diastolic function.

This patient had progressive AML, with 94% of blast cells seen on serial full blood examination. She had also remained persistently febrile throughout the course of her admission, with recurrent episodes of culture-negative pneumonia. A repeat Dengue Duo test performed 3 weeks later was negative for the NS1 antigen and dengue IgM and IgG antibodies, failing to show seroconversion. She died on day 22 of hospitalization from complications of AML.

**COMMENT**

In this report, both patients had presented with fever and thrombocytopenia. Singapore experienced the largest dengue epidemic in 2013. It has become common practice for most patients with fever and thrombocytopenia to be screened for dengue by the primary care or emergency physician using the commercially available SD Bioline Dengue Duo test. Although the NS1 test in the SD Bioline Dengue Duo kit has high specificity, we proceeded to evaluate these patients for possible false-positive NS1 results because of their atypical presentation.

For case 1, prolonged fever of 1 month would be extremely atypical for dengue. In the right clinical context, the dengue NS1 test is best performed during the first 7 days of illness, and serologic testing is best done after the febrile phase of illness. Using dengue RT-PCR as a comparator, the specificity of the NS1 on the Dengue Duo test supplied by Standard Diagnostics is 98.4%. In the literature, the rates of false-positive dengue NS1 range between 0.5% and 2.0%. Nonetheless, in the appropriate clinical context, the detection of dengue NS1 antigen is a highly specific and useful for

### Table 1

<table>
<thead>
<tr>
<th>Laboratory investigations</th>
<th>Reference range</th>
<th>Patient 1 (day 30 of febrile illness)</th>
<th>Patient 2 (day 3 of febrile illness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.0–15.0</td>
<td>9.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Blood leukocyte count (×10^9/L)</td>
<td>3.6–9.3</td>
<td>2.5</td>
<td>43.4</td>
</tr>
<tr>
<td>Platelets (×10^9/L)</td>
<td>170–420</td>
<td>101</td>
<td>140</td>
</tr>
<tr>
<td>Blast cells (%)</td>
<td>Not seen</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>17–63</td>
<td>75</td>
<td>Not available</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>15–41</td>
<td>156</td>
<td>Not available</td>
</tr>
<tr>
<td>Dengue Duo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dengue NS1</td>
<td>Positive</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Dengue IgM</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Dengue IgG</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Dengue RT-PCR</td>
<td>Not detected</td>
<td>Not detected*</td>
<td></td>
</tr>
</tbody>
</table>

* Dengue RT-PCR was performed on day 4 of febrile illness for patient 2.
the diagnosis of dengue. It can also be rapidly detected in about 20 minutes by commercially available rapid diagnostic test kits (e.g., SD Bioline Dengue Duo).20 In a recent prospective cohort study in Singapore, the SD Bioline Dengue Duo was shown to fulfill World Health Organization-assured criteria for point-of-care testing and enhances dengue diagnosis in endemic setting.2,4

CONCLUSIONS

Although the NS1 test is highly specific, we report two cases of false-positive NS1 tests in patients with hematological malignancies. The indiscriminate use of the NS1 test strip in clinical settings with low pre-test probability is not advised. All laboratory diagnostic test results have to be interpreted in the appropriate clinical context. We suggest the inclusion of serum samples from hematological patients to validate the interference or cross-reactivity between NS1 tests and hematological illnesses.

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