

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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Abstract. Early diagnosis of dengue has been made easier in recent years owing to the advancement in diagnostic technologies. The rapid non-structural protein 1 (NS1) test strip is widely used in many developed and developing regions at risk of dengue. Despite the relatively high specificity of this test, we recently encountered two cases of false-positive dengue NS1 antigen in patients with underlying hematological malignancies. We reviewed the literature for causes of false-positive dengue NS1.

INTRODUCTION

Dengue fever is a self-limiting systemic mosquito-borne viral illness characterized by fever, myalgia, rash, leucopenia, and thrombocytopenia.^{1,2} 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.^{3,4} 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%.⁵

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 NS1 antigen.

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 = $2.5 \times 10^9/L$, and platelets = $101 \times 10^9/L$). 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 an 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 locally⁶ and the Centers for Disease Control (CDC) DENV Real-Time RT-PCR assay.⁷ 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 $43.4 \times 10^9/L$ with 3% blasts, and platelet count was $140 \times 10^9/L$. 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

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TABLE 1
Laboratory investigations of two patients performed on the days of their admissions

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

*Dengue RT-PCR was performed on day 4 of febrile illness for patient 2.

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 $\mu\text{g/L}$ 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 2013.⁸ 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,^{3,4,7} 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.^{1,2} Using dengue RT-PCR as a comparator, the specificity of the NS1 on the Dengue Duo test supplied by Standard Diagnostics is 98.4%.⁹ In the literature, the rates of false-positive dengue NS1 range between 0.5% and 2.0%.^{3,10–12}

There were 9,072 SD Bioline Dengue Duo tests performed at the TTSH in 2013. In a separate study performed at the

TTSH campus with the same laboratory facilities between 2011 and 2012, the specificity of NS1 in the SD Bioline Dengue Duo test was 98%, similar to that reported by the manufacturers.^{4,9} In addition, the Dengue Duo kits were stored and run according to the manufacturer's instructions in a College of American Pathologists-accredited laboratory. Quality control tests for each batch of kits were also performed. It is unlikely that NS1 results were aberrant because of bad kits or poor methodology.

False-negative NS1 tests have been reported in dengue serotypes 2 and 4 infections.^{13,14} NS1 tests are also reported to be less sensitive in secondary dengue.^{3,12,15} Little is known about the causes of false-positive NS1 tests, except for possible cross-reactivity with other flaviviruses and possibly, cytomegalovirus (CMV).^{11,16} The two patients were not exposed to other flaviviruses, but they were not tested for CMV.

There were two prospective studies that identified false-positive NS1 in patients with febrile illnesses who were evaluated with the NS1 tests using the SD Bioline Dengue Duo kit in Cambodia and the NS1 antigen enzyme-linked immunosorbent assay (ELISA; Platelia; Bio-Rad Laboratories) in Vietnam.^{3,10} The causes of these false-positive results are unknown. In both of our patients, their dengue NS1 bands were reported to be weak, similar to the false-positive dengue NS1 results reported in Cambodia.³

False-positive dengue NS1 tests have not been reported in hematological malignancies. Although there are case reports of dengue causing hemophagocytosis,^{17,18} the diagnosis of dengue cannot explain the first patient's clinical progress and account for the hemophagocytosis. She had a negative dengue RT-PCR test and histologically proven aggressive NK cell lymphoma on bone marrow examination that contributed to her illness trajectory and eventual demise. In the second patient with AML, her repeat Dengue Duo test was negative for both dengue NS1 and dengue IgM and IgG antibodies. This makes an admission diagnosis of dengue rather unlikely.

The causality of the positive dengue NS1 tests in these two hematological patients cannot be identified at present. Dengue NS1 antigen is an enigmatic protein that has a molecular weight of 46–55 kDa. Its structure and function have still remained somewhat elusive.¹⁹ One postulation for the false-positive dengue NS1 antigen is that malignant cells undergoing rapid apoptosis in patients with hematological malignancies may release intracellular proteins that bear homology to the dengue NS1 antigen.

Nonetheless, in the appropriate clinical context, the detection of dengue NS1 antigen is a highly specific and useful for

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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