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False-Positive Nonstructural Protein 1 Antigen in a Patient with Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia: A Case Report with Literature Review

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Patient:	Male, 52-year-old
Final Diagnosis:	Philadelphia chromosome-positive acute lymphoblastic leukemia
Symptoms:	Fever • waxing and waning of gum bleeding • weight loss • mucocutaneous bleeding
Medication:	—
Clinical Procedure:	—
Specialty:	Hematology
Objective:	Mistake in diagnosis
Background:	A rapid investigation of dengue viral infection is needed for physicians who manage patients with suspected dengue infection. The nonstructural protein 1 (NS1) test kit is commonly used to diagnose patients with acute febrile illness in dengue-endemic countries, although this test kit can yield false-positive results. The Dengue NS1 test kit mostly relies on cross-reaction among febrile illness patients with other viral infections rather than malignancies.
Case Report:	A 52-year-old male patient presented with 3 days of fever, intermittent gum bleeding, weight loss, and mucocutaneous bleeding. He was transferred to a second hospital with acute febrile illness. Both dengue NS1 antigen test kits were positive from the 2 hospitals where he was previously treated. Fever and cytopenia persisted, and then the dengue RT-PCR test was performed to establish the cause of illness. A peripheral blood smear was reviewed and showed blast cells. A bone marrow examination was done to test for the compatibility of lymphoblastic leukemia. The flow cytometry test showed B cells ALL with Philadelphia-positive chromosome. Finally, the result of the dengue RT-PCR test was negative.
Conclusions:	Our patient presented with fever and viral-like illness, but he was finally diagnosed with Ph+ ALL. We demonstrated the first case of false-positive dengue NS1 antigen in a Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) patient. Moreover, we reviewed the literature to gather information on false-positive results using the dengue NS1 test kit. The dengue NS1 test kit is useful and produces reliable clinical findings, especially in patients with hematological malignancies.
Keywords:	Dengue • False Positive Reactions • Philadelphia Chromosome • Precursor Cell Lymphoblastic Leukemia-Lymphoma • Viral Nonstructural Proteins
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Background

Dengue is one of the tropical infectious diseases transmitted by mosquitoes. Its clinical presentation is an acute febrile illness with nonspecific organ involvement. Symptoms include fever, rash, arthralgia, and myalgia. Apart from the nonspecific symptoms, laboratory parameters (eg, leucopenia, increased atypical lymphocyte cells, and thrombocytopenia) can be used to assess the viral etiology [1]. The combination of clinical presentations and laboratory parameters may help physicians to distinguish between dengue infection and other conditions (eg, human immunodeficiency virus, septicemia, an autoimmune disorder, and hematological malignancies) [2,3]. The nonstructural protein 1 (NS1) antigen is a reliable diagnostic test kit for dengue infection, which showed sensitivity and specificity of 48.5-58.6% and 92.5-99.4%, respectively, and the positive predictive value was 88.5-99.3% [4-6]. The NS1 antigen structure is composed of a 46-55 kDa enigmatic protein. The NS1 antigen test for the diagnosis of dengue infection is more promising and widely accepted than the immunoglobulin M (IgM) test [7]. If the test result is positive, the patient is most likely diagnosed with dengue infection. The limitation of the NS1 antigen test is that it can produce either a false-negative result (the result depends on the day of testing) or a false-positive result (including cross-reactivity with other *Flaviviruses* or other non-infectious conditions) [2,8,9]. Limited data are available on false-positive NS1 antigen test results among patients with hematologic conditions. Only 2 nonspecific febrile hematologic patients were reported in the literature and were first diagnosed with dengue infection [9]. The limitation of the delay in diagnosing the cases was documented only in patients with acute myeloid leukemia (AML) and natural killer (NK) cell lymphoma. Some questions of false-positive NS1 antigen test result detection exist for other types of hematologic malignancies. Therefore, we report the first case of a false-positive dengue NS1 antigen test result in a patient with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) and review the literature for the possibility of testing for cross-reactivity. This patient gave written informed consent for publication of the case and any accompanying documents.

Case Report

A 52-year-old male patient presented with a 3-day history of fever, rigor, myalgia, and intermittent gum bleeding. He lost 1 kg of weight during the illness. He had no known sick contacts and had no significant travel history before hospitalization. He denied comorbidities and previous dengue infection. During hospitalization in Malaysia, he had a high-grade fever (temperature, 39°C), and was normotensive with a blood pressure of 132/60 mmHg. Apart from pallor, the results of the physical

examination were normal. His basic laboratory tests showed leucopenia (white blood count with 3600 cells/mm³), thrombocytopenia (platelets count=13 000 cells/mm³), and hemoglobin 12.4 g/dL. Evaluation of his liver enzymes revealed transaminitis (aspartate aminotransferase, 42 U/L; alanine transaminase, 274 U/L). The result of the dengue NS1 antigen test was positive through the use of the rapid dengue diagnosis kit SD BIOLINE Dengue Duo Combo test (Standard Diagnostics, Inc., South Korea), and the combined dengue IgM and immunoglobulin (IgG) antibodies tests were negative in the same specimen. A rapid HIV test and blood cultures for bacteria were performed, which showed negative results. The history of acute febrile illness and positive dengue NS1 antigen test positively led him to be diagnosed with dengue infection. The patient continued to have high-grade fever, continuous gum bleeding, and ecchymosis in both arms on the fifth day. One unit of platelet concentrate was transfused to improve his bleeding condition and raise his platelet level. He was classified as dengue hemorrhagic fever grade III with progressive disseminated intravascular coagulation (prothrombin time, 13.4 s; activated partial thromboplastin time, 12.4 s). He was referred to the Hospital for Tropical Diseases, Bangkok, Thailand and was tested using a commercially available rapid dengue diagnostic kit (Panbio, Standard Diagnostics, Inc., South Korea) in the second week. The dengue NS1 antigen and dengue IgG antibody results were positive, but the dengue IgM antibody test result was negative. Peripheral blood smears were reviewed and lymphoblastic leukemia cells (70% of blast cells) with low red blood cells and platelet count (Figure 1A-1C) were found. An urgent bone marrow study was performed to confirm the diagnosis of ALL (Figure 1D, 1E). Flow cytometry analysis of bone marrow showed 62.7% blasts and revealed an increased abnormal population in CD19, PAX5, CD10, CD34, HLA-DR, and PAX5; and negative for CD7, CD3, Cytoplasmic CD3, and MPO. Aberrant expression of CD33 was noted. These immunophenotype results were compatible with B cell ALL and the bone marrow showed a Philadelphia-positive chromosome. Finally, his test for dengue using a reverse transcriptase polymerase chain reaction (RT-PCR) showed a negative result. Chemotherapy was administered with a specific chemotherapy protocol for B cell ALL.

Discussion

This patient presented with viral-like fever and thrombocytopenia. The most common symptoms of dengue are fever and cytopenia, especially thrombocytopenia. In addition to assessing the clinical presentation and physical signs, we used the commercial screening laboratory test SD BIOLINE Dengue Duo test due to its high sensitivity and specificity [8]. The reliability of the dengue NS1 antigen test is ~90% [2,5,6]. This patient was previously repeatedly tested with the dengue NS1 test kit at 2

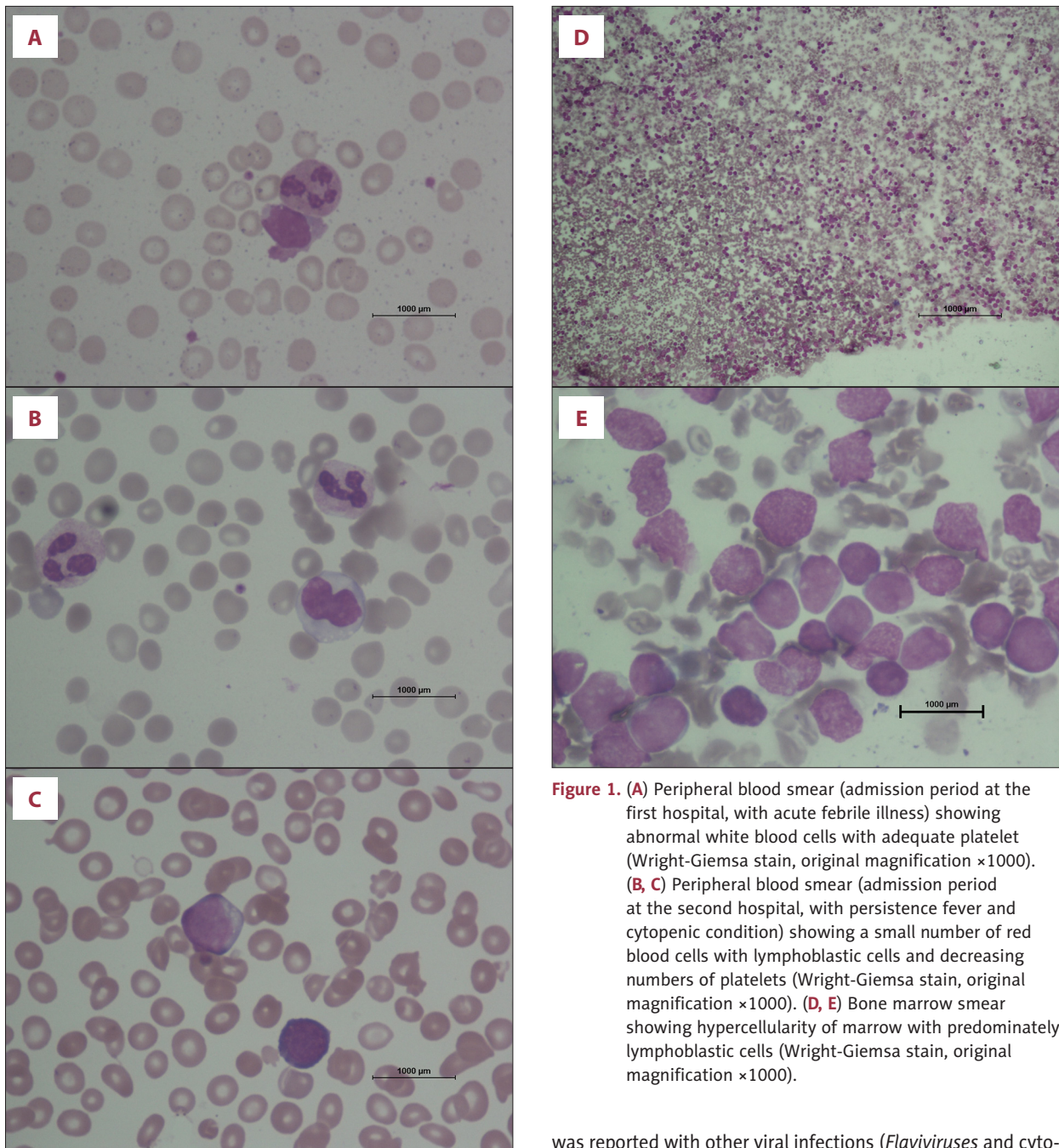


Figure 1. (A) Peripheral blood smear (admission period at the first hospital, with acute febrile illness) showing abnormal white blood cells with adequate platelet (Wright-Giemsa stain, original magnification $\times 1000$). (B, C) Peripheral blood smear (admission period at the second hospital, with persistence fever and cytopenic condition) showing a small number of red blood cells with lymphoblastic cells and decreasing numbers of platelets (Wright-Giemsa stain, original magnification $\times 1000$). (D, E) Bone marrow smear showing hypercellularity of marrow with predominately lymphoblastic cells (Wright-Giemsa stain, original magnification $\times 1000$).

different hospitals. Two dengue NS1 test kit results were positive, but a peripheral blood smear showed a young lymphoid series. Thus, we questioned the result of the dengue NS1 test kit in this patient with febrile illness. The limitations of the test have been continuously reported in situations such as immune dysfunction, but some patients with hematological malignancies have cross-reactivity with the dengue NS1 antigen. Diagnosis must be carefully performed when there is a false-positive dengue NS1 antigen test result in a lymphoblastic leukemia patient. Cross-reactivity of the dengue NS1 antigen test

was reported with other viral infections (*Flaviviruses* and cytomegalovirus), and the dengue NS1 antigen test shows a negative result among patients with secondary dengue infection (dengue serotypes 2 and 4) [10,11]. Dengue infection should be carefully diagnosed when using the dengue NS1 test, especially in hematologic patients. The literature contains reports of 2 hematologic patients presenting with fever and false-positive results using the NS1 test SD BIOLINE Dengue Duo test [9]. Both of these patients with hematologic malignancies were diagnosed with AML and aggressive NK cell lymphoma [9]. Our case presented with acute febrile illness with a bleeding disorder, which is typical for a dengue infection (Table 1). The dengue

Table 1. Clinical parameters of 3 hematologic malignancy patients with a false-positive dengue NS1 test result.

Clinical parameters	Our case	Patient 1	Patient 2
Duration of fever (days)	3	30	3
Additional symptoms	Myalgia, weight loss	Unremarkable	Myalgia
Comorbidity disease(s)	No	Hypertension, diabetes mellitus	Hypertension, dyslipidemia
Physical examination			
Body temperature (oC)	39.0	38.9	40.0
Organomegaly	Hepatosplenomegaly	No	No
Lymphadenopathy	Yes	No	No
CBC			
Hemoglobin (g/dL)	9.6	9.7	5.8
White blood cells count (cells/mm ³)	3300	2,500	43,400
Blast cells (%)	70	N/A	3
Platelets count (cells/mm ³)	7000	101,000	140,000
LFT			
AST (U/L)	102	156	N/A
ALT (U/L)	141	75	N/A
Detection of dengue			
Dengue NS1	Positive	Positive	Positive
Dengue IgM	Negative	Negative	Negative
Dengue IgG	Positive	Negative	Negative
Dengue RT-PCR (performed date)	Not detected (5 th day)	Not detected	Not detected (4 th day)
Type of hematologic malignancy	B cells – acute lymphoblastic leukemia (Ph+)	Aggressive natural killer cell lymphoma	Acute myeloid leukemia

CBC – complete blood count; LFT – liver function test; AST – aspartate aminotransferase; ALT – alanine aminotransferase; N/A – not available.

NS1 antigen test results were positive in the first few days of hospitalization. Lastly, these 2 patients developed pancytopenia and were tested again with dengue RT-PCR for comparison. The result of the dengue RT-PCR was negative. A bone marrow examination was done to test for the compatibility of lymphoblastic leukemia. The flow cytometry test showed B cells ALL with Philadelphia-positive chromosome.

A false-positive dengue NS1 antigen test result can occur with other viral infections, but there are few reports on hematologic malignancy patients. Only 2 patients showed myeloid leukemia and lymphoma [9]. However, a false-positive dengue NS1 antigen test result has not been reported in a lymphoid leukemia patient. The false-positive dengue NS1 antigen test result may be due to a cross-activity with dengue-specific protein.

The dengue NS1 antigen is a specific enigmatic protein with a small molecular weight ranging from 46 to 55 kDa [12]. Cells in patients with hematologic malignancies rapidly proliferate and can produce a small-molecular-weight protein [13], and this protein may bind in a way similar to that in the dengue NS1 antigen test [9]. Hematologic malignancies can produce a large number of nonspecific proteins which can coat the NS1 antigen [3,14]. It is hypothesized that this is similar to false-positive results with the dengue test kit in patients with positive rheumatoid factor. Moreover, the Philadelphia chromosome may affect the enigmatic protein [15,16]. Nevertheless, physicians may be unable to distinguish between atypical lymphocyte and leukemic cells [17]. Our case showed only leukemic cells in the peripheral blood smear, which can also be rapidly diagnosed as a hematologic malignancy rather than a dengue

infection. Clinical parameters and correct blood smear interpretation should be used to differentiate between dengue infection and conditions of hematologic malignancies. Thus, a peripheral blood smear was suitably used to fulfill the World Health Organization's criteria for bedside testing and proper diagnosis in endemic areas for hematologic patients.

Conclusions

We report the first case of a false-positive NS1 test result in a Ph+ ALL patient. The dengue NS1 antigen test was repeated and persistently showing a false-positive result using diagnostic test kits from different companies. Use of appropriate clinical parameters is more useful than use of the highly specific NS1 test, especially in patients with hematologic malignancies. In

dengue-endemic countries, hematologic patients with febrile illnesses may develop a cytopenia condition which overlaps dengue infection. Further research is needed to elucidate the mechanism involved in false-positive rapid dengue test results in febrile patients with hematologic malignancies.

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Conflict of Interest

None.

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