Perspectives

Is the Dengue NS1 Antigen test a credible indicator for clinical decision-making?

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Abstract

A valid, credible diagnostic test result is essential for adequate case management and to decrease the events of misdiagnosis of dengue infection. Of the available diagnostic tests, the NS1 rapid antigen detection immune-chromatographic strip test (ICT) is the most frequently used test for diagnosing dengue infection in resource-poor settings. A preliminary study was conducted in a Teaching Hospital in Sri Lanka to compare the NS1 rapid antigen test resultsperformed in the local private laboratories with the RT-PCR test in a group of patients with 'probable dengue'. The sensitivity, specificity, positive predictive value and negative predictive value of the NS1 rapid antigen test compared to RT PCR were 41.6%, 63.2%, 20.83% and 82.3%, respectively. These values are much lower than the previous literature questioning the credibility of NS1 antigen detection ICT results. Thus, clinical decisions would ideally be based on clinical features and the combination of laboratory tests, and the regulatory requirement of local laboratory testing was noted.

Keywords: Dengue, NS1ICT, RT-PCR, diagnosis, credibility

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Introduction

Dengue fever is a vector-borne disease common in tropical and subtropical regions. It is estimated that at least 10% of dengue fever cases progress to severe forms of the disease that may even cause fatality[1]. Being an acute febrile illness, clinical features and preliminary laboratory investigation results of dengue fever are similar to those of other febrile illnesses in the same geographical areas. Therefore, a valid diagnostic test result is of utmost importance for adequate case management and decreasing misdiagnosis events. Four laboratory methods are available for diagnosing dengue: detection of Non Structural antigen 1 (NS1) test, specific IgM/IgG antibodies in paired sera or detection of viral RNA through PCR-based methods, and viral isolation in culture. Ideally, combining at least two of these tests would better diagnose dengue[2].

Viral isolation is not practical in early diagnosis. Detection of dengue-specific IgM/IgG antibodies can be used for diagnosis, but utility in the acute phase is limited as seroconversion takes days[3]. Therefore, the detection of viral RNA or NS1 antigen is widely used in diagnosis in the early phase of illness. Molecular methods such as

RT-PCR and other PCR-based techniques provide results within 6-8 hours, but they are expensive and unavailable in local hospitals. Due to the ease of conducting, rapidity of results, and ability to perform with minimum facilities and training, the NS1 rapid antigen detection by immune-chromatographic strip test (ICT) is the most frequently used test for diagnosing dengue infection in resource-poor settings. Furthermore, NS1 test is less costly compared to other methods. For instance, a PCR test costs six-seven times the cost of an NS1 rapid antigen test.

The antigen, NS1, is a glycoprotein belonging to Flaviviruses, which is shared among viruses of the same family; dengue, Japanese encephalitis, yellow fever, and tick-borne encephalitis [1]. The specificity of the NS1-based dengue diagnostic tests is reported to lie within 86 -100 %, according to the literature[3]. The sensitivity has been reported to have higher variability, that is, 37-98.9%[2]. This variability may be partly explained by the fact that sensitivity decreases with time after the onset of fever and in secondary infections[4].

A preliminary study was done in a Teaching Hospital in Anuradhapura District, North Central Province (NCP), Sri Lanka, which has provided evidence suggesting that the NS1 test results are not a credible indicator for the diagnosis of dengue infection. In Sri Lanka, NS1 detection ICT is widely available in private sector laboratories, including small-scale laboratories, at a high cost. This study which was conducted upon the approval of the Ethics Review Committee, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2016/089), has compared the NS1 rapid antigen test results performed in the local private laboratories with the RT-PCR test results in a group of patients with 'probable dengue' diagnosed according to the 2009 WHO dengue classification [1]. A venous blood sample was collected from each patient at the time of admission to the hospital and tested for dengue RT-PCR at PCR laboratory, Faculty of Veterinary Medicine and Animal Sciences, University of Peradeniya. We followed the protocol published by Santiago et al. 2009[5]. A total of 202 probable dengue patients were included and there were 191 patientsts tested with NS1 rapid antigen test within 02 days of hospital admission in local laboratories. Usually, a blood sample collected in a plain bottle is accepted by the local laboratories for NS1 testing. Of them,72 had positive NS1 rapid antigen test results. Table 1 compares the RT- PCR test results against dengue NS1 rapid antigen test results.

Table 1: Comparison of the RT PCR test results against the dengue NS1 Rapid Antigen Test

	PCR result		Total
	PCR Positive	PCR Negative	
NS1 Positive	15	57	72
NS1 Negative	21	98	119
Total	36	155	191

PCR = Polymerase Chain Reaction, NS1 = Non-structural antigen rapid immune-chromatographic strip test

The sensitivity of the available NS1 rapid antigen test results compared to RT PCR was 41.6%

The specificity of the NS1 rapid antigen test results compared to RT PCR was 63.2%. The positive predictive value (PPV)and negative predictive value(NPV) of NS1 rapid antigen test results compared to RT PCR were 20.83% and 82.3%, respectively. Apart from the NPV of 82.3%, all other indicators of diagnostic accuracy were much lower compared to the previous literature [6].The authors believe that the discrepancy arises due to the loss of credibility of lab results generated by small-scale private labs. Use of non-registered low-quality test kits that are inexpensively available in the local market, lack of quality control(QC) measures and supervision can increase the technical errors in testing, reading, and interpretation.

Dimech et al. (2020) demonstrated that using an evidence-based QC program is critical for detecting anti-Hepatitis C virus antibodies[7]. The same theory may apply to dengue, though no studies on the importance of QC for dengue diagnostic tests have been conducted.

The observed discrepancy may lead to a controversy in a situation where NS1 results have a critical role to play in the diagnosis of dengue especially when the diagnosis is questionable due to marginal changes in the other parameters. The clinicians who rely on these test results have no control over the quality control aspects of the laboratories unless the NS1 testing is performed in the

hospital laboratory. In a poor resource setting, not all patients get the chance to get their NS1 antigen tested at the government hospital laboratory. In the actual situation, the clinicians have to make decisions based on a test result despite the place where the test was performed. Our results indicate that relying on one NS1 may lead to the misdiagnosis of dengue patients.

However, not doing the RT-PCR on the same sample is a limitation of this study. We could not use the same blood sample used for the NS1 antigen test to test the PCR because the NS1 had been performed before the recruitment. Instead, we excluded samples obtained after 48 hours from the NS1 test from the analysis to avoid potential bias due to genetic material loss over time.

We would like to draw the attention of the authorities to take necessary measures to assure the quality of laboratory tests performed by the privately owned laboratories in local setups. Further, the clinicians actively involved in managing patients must make clinical decisions based on clinical features and a combination of laboratory tests as much as possible.

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Authors' Contributions

SSG had the initial concept and conducted the sample collection, laboratory analysis, statistical analysis, manuscript draft and manuscript editing. SAMK contributed to the designing of the study, supervised the clinical data collection / statistical analysis and reviewed the final draft of the manuscript. JR and RM assisted during the designing of the study and supervised the laboratory procedures. SAMK, JR, and RM contributed to the final version of the manuscript by reviewing and editing it.

Conflicts of Interest/ Competing Interests

The authors of this article have no conflicts of interest with the content of the article.

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