REVIEW ARTICLE

NS1 Antigen and Laboratory Diagnosis of Dengue: A Narrative Review

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Abstract

Dengue non-structural protein 1 (NS1) is a multifunctional viral antigen that acts as a potent immunomodulator of vascular and immune pathology in dengue. It circulates at high concentrations during acute infection. NS1 promotes endothelial dysfunction through multiple complementary mechanisms, including the engagement of pattern-recognition receptors (notably TLR4), which triggers the release of proinflammatory cytokines and leads to prolonged vascular leakage. Additionally, impaired coagulation contributes to hemorrhagic manifestations. On the diagnostic front, secreted NS1 is detectable during the early stages of infection, often concurrently with viremia and before antibody seroconversion, and has been successfully utilized in capture ELISA and rapid antigen tests. This narrative review focuses on the current role of NS1 in dengue pathogenesis, as well as on accurate and timely diagnosis of dengue, which is essential for early clinical management and preventing severe disease outcomes.

Keywords: Dengue, NS1 antigen, Endothelial glycocalyx, ELISA, Rapid test

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Introduction:

Dengue virus (DENV), a mosquito-transmitted pathogen that affects humans and ultimately leads to dengue fever. It is one of the predominant causes of insect-borne viral illness and poses a major public health threat. The World Health Organization (WHO) estimates that approximately 390 million dengue infections occur annually (with a 95% confidence range of 284–528 million), of which roughly 96 million (ranging from 67–136 million) manifest clinically with varying degrees of severity.[1] In the year 2024, a staggering 14,127,435 dengue cases were recorded worldwide, which was the highest-ever total since the global dengue recording system was introduced in 2010.

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Historical records suggest dengue-like illnesses emerged as early as 1635 in Martinique and Guadeloupe, and in 1699 in Panama, though potentially even earlier accounts exist.[2–5] Bangladesh first documented dengue in the 1960s (during its period as East Pakistan), where it was termed "Dacca fever." The country witnessed the first major outbreak in 2000, with a higher case fatality rate (1.67%).[6] Dengue virus is transmitted by Aedes aegypti and Aedes albopictus, the same vectors that also transmit Chikungunya and Zika.[7] In the year 2023, Bangladesh experienced the highest cases of dengue fever (n = 321,179), and many cases remain undiagnosed.[8] The sudden onset of high fever, severe headache, joint and muscle pain, rash, and retro-orbital pain are the characteristic features of the disease.

Genomic structure of Dengue virus and its genotypes

The virus belongs to the Flavivirus family and comprises five genetically related yet different serotypes: DENV 1, 2, 3, 4, and 5.[9,10] DENV has an approximately 11-kb, single-stranded, positive-sense RNA genome that contains one open reading frame (Fig. 1).[11] This genetic material is translated into one large polyprotein and is subsequently

cleaved into three structural proteins (capsid protein – CP, envelope protein – EP, and membrane protein – MP) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[12] While structural proteins form the viral particle, non-structural proteins facilitate viral entry, genome replication, particle assembly, and disease development.[13–16] Both viral architecture and genetic variation influence dengue laboratory testing methods.

The NS1 protein is a glycosylated molecule weighing 46-55 kDa that forms dimers within cells and on cell surfaces.[17] NS1 is also released extracellularly, mainly as a hexamer, although recent work using recombinant NS1 produced in human embryonic kidney cells shows that secreted tetramers can also form.[18] In infected patients, NS1 levels in the bloodstream peak around days 3–5 after fever begins and can reach concentrations up to 50 µg/mL, which establishes NS1 as a valuable diagnostic indicator.[19] Soluble NS1 has been implicated in dengue pathogenesis through several mechanisms, which include complement activation, disruption of tight junctions and the endothelial glycocalyx in cell models, and activation of innate immune responses via TLR4 signaling.[20–22] These functions indicate a direct role for NS1 in the coagulation abnormalities, vascular leakage, and cytokine storm seen in severe dengue. Furthermore, pre-exposure of vertebrate or mosquito cells to NS1 enhances DENV replication,[23–25] because internalized NS1 can influence innate immune pathways.[26] Continuous elevation of NS1 levels in the blood has been linked to more severe disease.[27]

For the improvement of diagnostic assays and interpreting molecular epidemiology, it is important to understand the genomic organization of DENV and its diversity. With advancing sequencing capabilities, genotype identification is becoming more integrated into monitoring programs, providing critical information about viral spread, outbreak sources, and potential effects of viral evolution on diagnostic reliability.

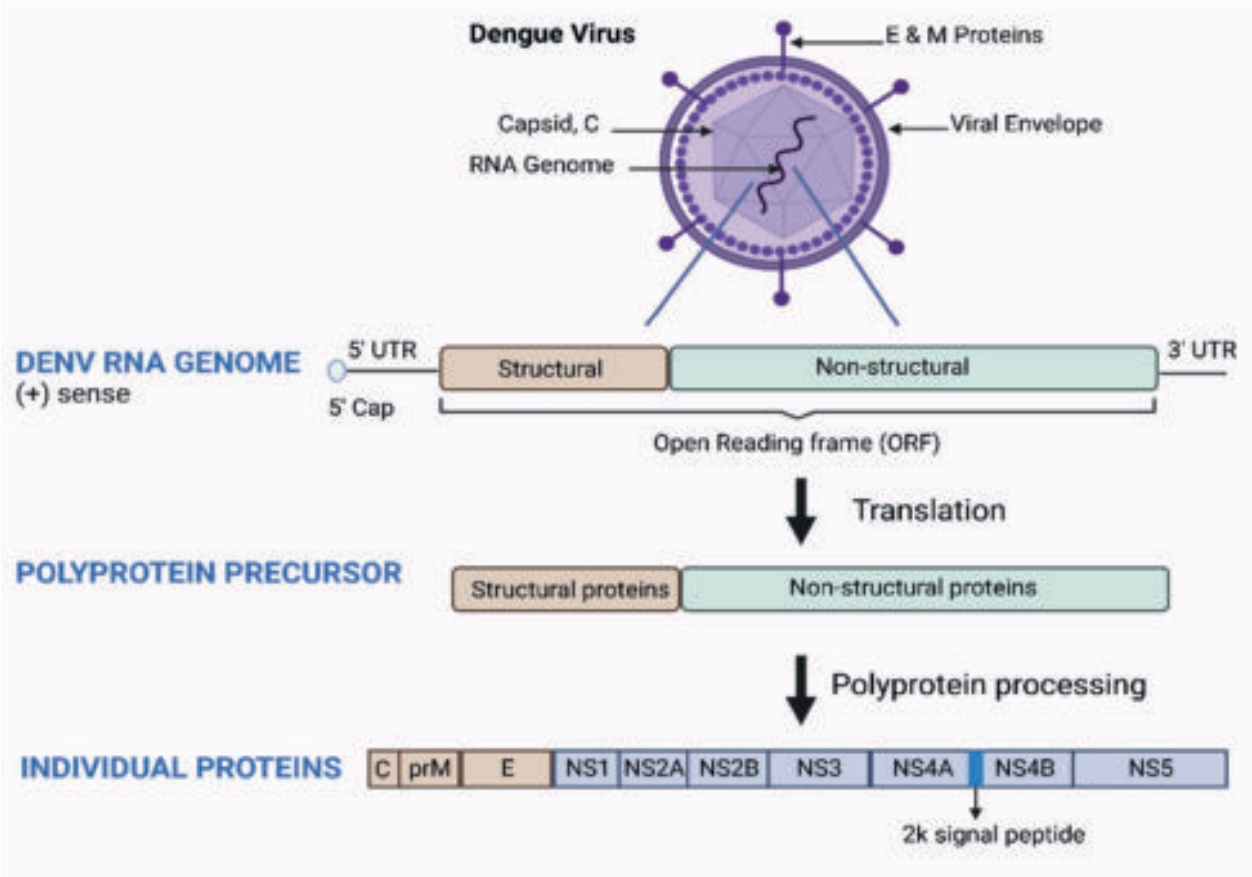

Figure 1. *An illustration of Dengue Virus (DENV) structure and genomic processing*

Dengue virus (DENV) infection occurs when infectious mosquito saliva deposits virions into the skin, where the virus infects permissive cells such as dendritic cells, tissue macrophages, and keratinocytes. Following local multiplication in these cell types, the virus spreads to nearby lymphoid tissues and enters systemic circulation; maximum viremia generally occurs before the critical phase when severe complications emerge. These initial virological processes establish conditions for both protective and harmful host responses.

Role of NS1 in Dengue Pathogenesis

NS1 uses multiple ways in dengue pathogenesis by engaging several host pathways that culminate in vascular dysfunction. Interaction of NS1 with Toll-like receptor 4 (TLR4) stimulates innate immune cells to release pro-inflammatory cytokines and contributes to endothelial injury by disrupting the glycocalyx and promoting apoptosis. Simultaneously, NS1 stimulates mast cells and platelets, triggering the release of vasoactive substances including histamine, leukotrienes, and platelet-activating factor, which leads to inflammation and plasma leakage. This protein also activates complement activation by increasing C5a production, which worsens endothelial permeability. Due to these pathogenic effects, NS1 is an important immunological target. Neutralizing monoclonal antibodies like 2B7 and 1G5.3 have shown the capacity to prevent NS1-induced endothelial injury, which restricts vascular leakage, decreases viremia, and enhances survival in experimental systems, emphasizing the therapeutic promise of targeting NS1-mediated pathways.

Role of NS1 antigen in vascular leakage

NS1 plays a key immunomodulatory role in dengue by influencing both innate and adaptive immune pathways. As a part of the innate immune system, soluble NS1 engages pattern recognition receptors, such as TLR4, which drives the release of pro-inflammatory cytokines and chemokines from monocytes, macrophages, and dendritic cells.[28] NS1 also compromises the endothelial barrier by disrupting the glycocalyx,[29] activating complement (particularly via enhanced C5a generation), and stimulating mast cells and platelets to release vasoactive mediators.[30] These combined effects enhance inflammation and contribute directly to the vascular leakage that leads to severe dengue. NS1 has been proven to stimulate the release of macrophage migration inhibitory factor (MIF), which participates in NS1-driven autophagy in endothelial cells.[31] Experimental models showed that DENV-infected cells also produce MIF, which leads to increased endothelial permeability.[32] Supporting these findings, Mif-/- mice develop markedly less severe disease in a model of dengue, underscoring MIF's role in driving pathology.[33] Clinical trials further observe this association as circulating MIF levels are elevated in dengue patients and are significantly higher in individuals with fatal DHF compared with survivors or patients with uncomplicated dengue fever.[34]

Role of NS1 antigen in coagulopathy and thrombocytopenia

Apart from vascular leakage, NS1 can also exacerbate severe dengue by interfering with coagulation pathways. Complexes of NS1 with thrombin have been detected in patient serum, and NS1 binding to prothrombin has been shown to impair its activation, resulting in prolonged aPTT.[35] Although the contribution of the NS1 antigen to thrombocytopenia remains unclear, evidence from TLR4-mediated platelet activation suggests a potential mechanism. Lipopolysaccharide (LPS) activates and aggregates platelets through TLR4/MyD88 signaling, and because NS1 can similarly engage TLR4, it may drive platelet activation and aggregation, which can accelerate platelet destruction during infection.[36] Collectively, emerging evidence indicates that NS1 acts as a central mediator of dengue pathology, contributing not only to plasma leakage but also to coagulopathy and hemorrhagic manifestations.

Laboratory Diagnosis

Diagnostic biomarkers for dengue target either the virus itself through viral culture, detection of viral RNA, or other direct methods, such as the secreted NS1 antigen, or the host's immune response, measured by dengue-specific IgM and IgG antibodies. The temporal appearance and persistence of these markers in primary and secondary infections are illustrated in Figure 2. In the following, we briefly review both established and emerging methods used to detect these diagnostic indicators.

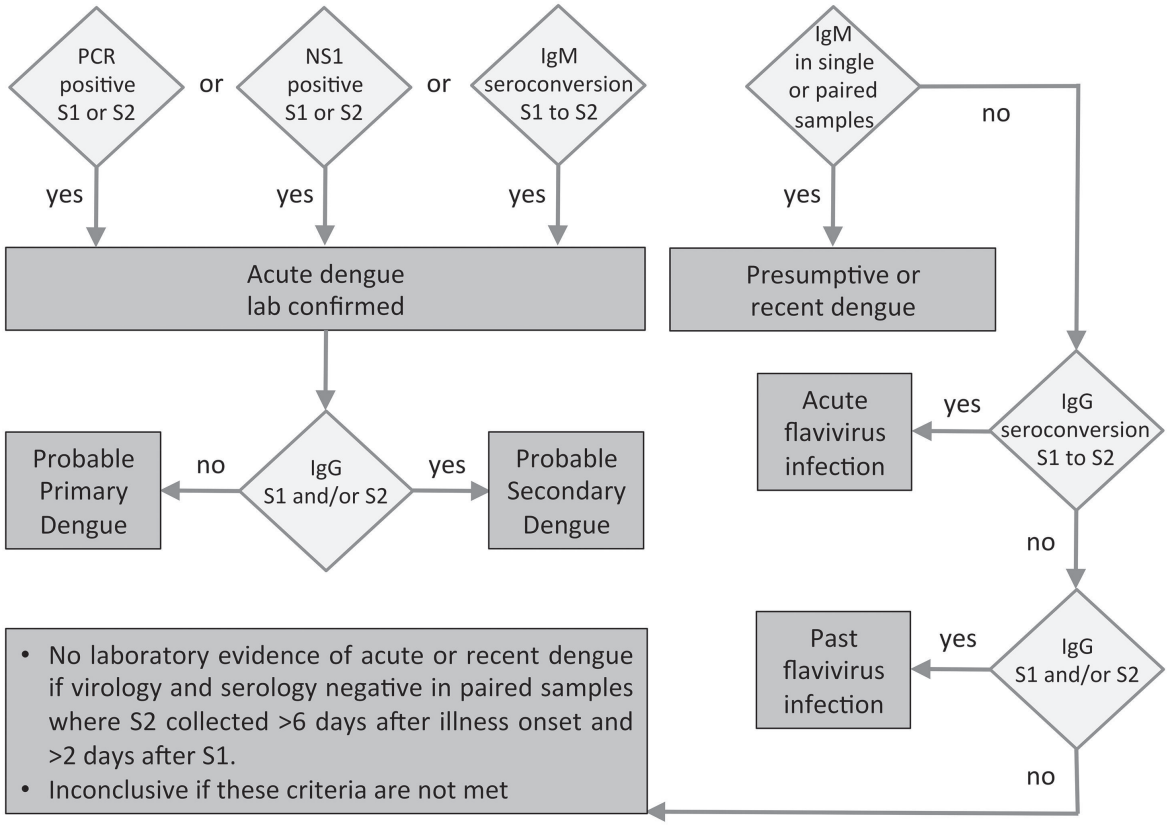

Figure 2: Diagnostic algorithm for laboratory diagnosis of dengue virus infection.

PCR or NS1 positivity in either acute (S1) or convalescent (S2) clinical samples confirms current dengue infection. Seroconversion of S1 to S2, as measured by immunoglobulin M (IgM) ELISA, confirms acute dengue infection. The presence of immunoglobulin G (IgG) in acute laboratory-confirmed dengue indicates a probable secondary dengue infection. The presence of IgM in a single sample indicates a presumptive or recent dengue infection. While negative IgM with IgG seroconversion between S1 and S2 indicates an acute flavivirus infection, the presence of IgG in S1 and S2 indicates a past flavivirus infection.

Virus Isolation

Historically, virus isolation has served as the standard method for confirming dengue virus (DENV) infection. Over time, however, it has been largely superseded by reverse-transcription polymerase chain reaction (RT-PCR) and, more recently, by NS1 antigen capture ELISAs, which offer faster diagnostic turnaround.[37] In this approach, patient samples are inoculated into various mosquito-derived cell lines (such as AP61, Tra-284, AP64, C6/36, and CLA-1) or mammalian cell lines (including LLCMK2, Vero, and BHK-21), and in some protocols, into live mosquitoes.[38] The highest isolation success rates are obtained from blood collected during the febrile phase, particularly within the first 5 days after symptom onset. Isolation becomes more challenging in secondary infections because early, robust anamnestic antibody responses generate cross-reactive immune complexes that bind circulating virus and reduce recoverable viral titers.[39] Although virus isolation provides definitive confirmation of DENV, it is labor-intensive and slow, often requiring several days to weeks to complete.[40]

RT-PCR

For the successful diagnosing of DENV infection RT-PCR Molecular methods such as RT-PCR and nucleic acid hybridization have been used. During the acute phase of disease, PCR-based methods are helpful for early diagnosis of DENV. The viral RNA can be detected from the onset of illness and is sensitive, specific, fast, less complicated, and cheaper than virus isolation methods, which is a major advantage of the PCR-based technique.[41] But this

technique requires a laboratory with specialized equipment and trained staff to perform the analysis. These are not always an option in resource-poor remote settings where dengue is endemic. Furthermore, despite the availability of commercial kits, the bulk of reported RT-PCR methods are developed in-house and also lack center-to-center standardization.[42] Non-PCR-based methods that mimic in vitro nucleic acid amplification, such as isothermal amplification (eg, single-tube reverse transcription–loop-mediated isothermal amplification), have shown high levels of sensitivity and specificity when used alongside other diagnostic methods.[43]

NS1 antigen capture-based method

Non-structural protein 1 (NS1) serves as an excellent diagnostic target because it is actively secreted by DENV-infected cells, which leads to substantial circulating levels in the blood. NS1 can be detected as early as the clinical symptoms appear and may remain measurable for nine days or longer, allowing its identification during the early febrile phase alongside viral RNA and before the development of detectable antibodies. A widely adopted diagnostic approach is to detect NS1 antigen in serum using ELISA.[44] For commercial purposes, rapid test strips and NS1 capture ELISAs have been developed as a result of the demonstration of high amounts of NS1 secretion by quantitative-capture ELISA. These commercial NS1-based assays are simple to perform and demonstrate strong sensitivity and specificity, and can aid in early dengue diagnosis with an excellent diagnostic performance.[45]

Serology

There are multiple approaches to serological diagnosis available, including hemagglutination inhibition (HI) assays, complement fixation tests, dot-blot assays, Western blotting, indirect immunofluorescent antibody tests, and plaque reduction neutralization tests, as well as IgM and IgG antibody-capture ELISAs. The most useful serological diagnostic methods for routine DENV detection are HI assays along with IgM and IgG antibody-capture ELISAs. The HI test has been implemented for dengue diagnosis for many years, with most laboratories developing in-house methodologies, although commercial kits are also available. The early acute disease period usually presents a negative window of detection because all assays based on antibody detection require the need for the relevant antibody response to be elicited. For this purpose, IgM and IgG antibody-capture ELISAs have become relatively routine, particularly following assay automation. IgM can be detected as early as day 3–5 in primary infection, peaking several weeks after recovery and remaining at detectable levels for several months.[46] IgG does not usually appear during the acute phase of primary disease. However, during secondary infection, the IgG response is rapid and anamnestic to shared epitopes on multiple viral proteins between the first and second infecting serotypes, with IgG appearing as early as 3 days after the onset of illness [S66]. Consequently, when performed in parallel, IgM and IgG detection can provide a diagnostic indication of primary or secondary infection, which is based on the ratio of IgM and IgG during the acute phase of disease.

Future approaches

Many new tests for the rapid diagnosis of dengue are currently under development. These include micro/paper fluidics, in vivo micropatches, isothermal PCR[47], and electrochemical and piezoelectric detection. All of these technologies are in the early stages of development, requiring continued refinement to make them practical solutions in real-world settings. In our view, the ideal goal for dengue diagnosis would be a test that differentiates primary from secondary dengue infection with IgM and IgG capture, along with quantitative serotype-specific NS1 detection.

Conclusion:

NS1 is a pathogenically important molecule as well as a valuable diagnostic biomarker in dengue. Targeting NS1 offers the development of therapeutics or immunotherapies that mitigate vascular pathology. Advances in laboratory tools, ranging from viral RNA detection to NS1 antigen assays and serology, have significantly improved diagnostic precision across different illness phases. Strengthening these diagnostic capabilities is critical for effective surveillance, outbreak control, and the reduction of dengue-related morbidity and mortality.

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