The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

Clinical and Laboratory Diagnosis of Dengue Virus Infection

David A. Muller,[1,2] Alexandra C. I. Depelsenaire,[1] and Paul R. Young[2]

[1]Australian Institute for Bioengineering and Nanotechnology and [2]Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia

Infection with any of the 4 dengue virus serotypes results in a diverse range of symptoms, from mild undifferentiated fever to life-threatening hemorrhagic fever and shock. Given that dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Virus detection and serological conversion have been the main targets of diagnostic assessment for many years, however cross-reactivity of antibody responses among the flaviviruses has been a confounding issue in providing a differential diagnosis. Furthermore, there is no single, definitive diagnostic biomarker that is present across the entire period of patient presentation, particularly in those experiencing a secondary dengue infection. Nevertheless, the development and commercialization of point-of-care combination tests capable of detecting markers of infection present during different stages of infection (viral nonstructural protein 1 and immunoglobulin M) has greatly simplified laboratory-based dengue diagnosis. Despite these advances, significant challenges remain in the clinical management of dengue-infected patients, especially in the absence of reliable biomarkers that provide an effective prognostic indicator of severe disease progression. This review briefly summarizes some of the complexities and issues surrounding clinical dengue diagnosis and the laboratory diagnostic options currently available.

Keywords. dengue virus; dengue diagnosis; dengue disease; NS1 antigen capture; dengue serology.

Dengue virus (DENV) infection is responsible for the most significant mosquito-borne viral disease in the world today. Like other flaviviruses, its genome comprises a single strand of positive-sense RNA encoding 3 structural and 7 nonstructural (NS) proteins [1]. Unlike the other flaviviruses, there are 4 serotypes, referred to as DENV1–4, that are genetically similar but antigenically distinct [2], defined by the inability of individually elicited antibodies to cross-neutralize. Dengue is spread primarily by the female *Aedes aegypti* mosquito, a vector that can be found throughout the tropical and subtropical regions of the world [3, 4]. However, an increasing number of outbreaks have been attributed to transmission by the temperate climate mosquito *Aedes albopictus* [5], presenting the possibility of further geographical incursions. Mass global travel has resulted in many regions of the tropical world displaying hyperendemic dengue activity, with multiple serotypes circulating at any one time [6–9]. It is estimated there are up to 390 million DENV infections annually, with more than 500 000 hospitalizations and 25 000 deaths [10]. Infection with any of the 4 DENV serotypes can result in a range of clinical outcomes, with the majority of infections (70%–80%) being asymptomatic [10]. Clinical presentation can range from a mild fever to classical dengue fever with hemorrhage (DHF) and/or shock (dengue shock syndrome [DSS]) [11]. Classical dengue fever is an acute infection presenting clinically 4–10 days following the bite of an infected mosquito. The disease is characterized by elevated temperature (up to 40°C), severe headache, retro-orbital pain, malaise, severe joint and muscle pain, nausea, and vomiting, with a rash appearing after 3–4 days after fever onset [12]. Following a primary infection, the patient is immunologically protected from disease caused by that particular dengue serotype [13].

The severe forms of dengue disease are seen primarily in individuals experiencing a secondary infection with a different dengue serotype [13]. However, primary infection in young infants may also be associated with severe disease outcome [14]. Early in the acute febrile period of disease, dengue fever presents with the same clinical symptoms as primary dengue. Later, during defervescence, patients can rapidly deteriorate, progressing to hemorrhage with or without vascular leak. During this period, patients can experience bleeding, thrombocytopenia with <100 000 platelets/μL, ascites, pleural effusion, increased hematocrit concentrations, severe abdominal pain, restlessness, vomiting, and sudden reduction in temperature with profuse perspiration and adynamia [11]. Currently, there is no therapeutic option for dengue, with treatment being purely supportive. Nevertheless, the symptoms of DHF and DSS can be effectively managed in most cases by fluid replacement [15]. In

Correspondence: P. Young, PhD, Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia QLD 4072, Australia (p.young@uq.edu.au).

The Journal of Infectious Diseases® 2017;215(S2):S89–95

DOI:10.1093/infdis/jiw649

addition to early and accurate diagnosis, early markers of progression to severe disease are urgently needed.

CLINICAL DIAGNOSIS

Clinical diagnosis of dengue can be challenging, depending largely on what stage in the infection process a patient presents. Depending on the geographic region of the world, there can be a number of disease-causing pathogens or disease states that can mimic the disease spectrum arising from dengue infection (Supplementary Table 1). In the early stages of clinical disease, dengue can present as a mild undifferentiated "flu-like" fever with symptoms similar to those of other diseases such as influenza, measles, Zika, chikungunya, yellow fever, and malaria [16]. Correct diagnosis of the pathogen responsible for the later manifestation of shock is of particular importance, as treatment for dengue-induced shock vs that arising from sepsis traditionally requires different approaches [16]. However a potential, paradigm-shifting observation that DENV infection activates similar innate immune pathways as those induced in sepsis may suggest alternative, common targets for treatment [17]. Because the clinical symptoms of dengue are so diverse, accurate clinical diagnosis is challenging. As such, it is essential that laboratory or point-of-care diagnostics be used in conjunction with assessment of clinical presentation.

Clinical Presentation

In 2009 a working group coordinated by the World Health Organization (WHO) set out a series of guidelines for clinical management of dengue [16]. They also modified the existing dengue disease classifications from dengue fever, DHF, and DSS to dengue (with or without warning signs) and severe dengue (Figure 1). The aim of these guidelines was to establish uniform and simpler clinical criteria that provided a standardized global approach to disease classification.

Dengue virus infection can result in either asymptomatic or symptomatic infection [18]. Roughly 20% of all infections are symptomatic, with individuals experiencing disease symptoms that cover a broad clinical spectrum of nonsevere to severe clinical manifestations [19]. Illness caused by dengue has an abrupt onset with 3 broadly identifiable phases: febrile, critical, and recovery [16]. Appropriate viral diagnosis and evaluation of warning signs of progression to severe disease are critical for effective patient management.

The initial febrile phase is characterized by rapid onset, initially with sudden high-grade fever [18]. This phase lasts between 2 and 7 days, with the febrile phase of the disease being characterized by a facial flushing skin erythema, generalized body ache, myalgia, arthralgia, retro-orbital eye pain, photophobia, rubeliform exanthema, and headache [20]. Sore throat, anorexia, nausea, and vomiting are also common [20]. During this phase, a positive tourniquet test is able to differentiate dengue from other diseases presenting with similar symptoms [21]. The acute febrile phase may also be accompanied by hemorrhagic symptoms ranging from a positive tourniquet test and petechiae to spontaneous bleeding from the gastrointestinal tract, nose, gums, and other mucosal sites [16]. The severity of symptoms during this phase is not a predictor of progression to severe dengue; therefore, monitoring of early warning signs needs to be undertaken during the critical phase of disease [16].

The majority of DENV-infected patients make a full recovery after the febrile period and do not enter the critical phase of disease. However, patients that do enter the critical phase may develop warning signs that indicate increased capillary permeability leading to plasma leakage. Generally, patients worsen at the time of defervescence (from illness day 4) when their temperature drops to 37.5°C–38°C [22], and it is during this period that early symptoms of vascular leakage may be seen.

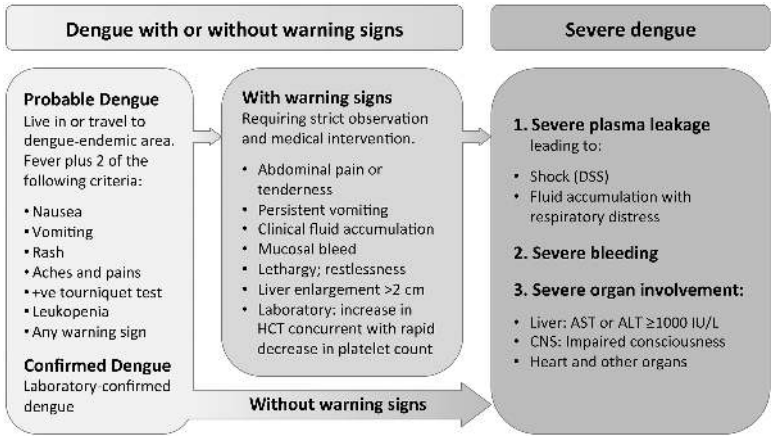

Figure 1. Classification of dengue disease progression. Criteria for dengue disease progression with and without warning signs are listed, as are the symptoms that define severe dengue. Adapted from World Health Organization guidelines [16]. Abbreviations: +ve, positive; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CNS, central nervous system; DSS, dengue shock syndrome; HCT, Hematocrit.

Leukopenia followed by a rapid drop in platelet count generally leads to plasma leakage [20]. Coincident with dropping platelet counts is a corresponding rise in hematocrit level. Leakage can last 24–48 hours and, during this time, hematocrit levels need to be closely monitored as an indicator of the need for intravenous fluid adjustment [20, 23]. During this time, and unless profound leak is clinically apparent, other methods such as ultrasound detection can be employed to detect free fluid in the chest or abdominal cavities [24]. Warning signs (Figure 1) are almost always present in patients before the onset of shock [24]. Shock occurs when a patient loses a critical volume of plasma through vascular leakage. The hypoperfusion that occurs during profound/prolonged shock results in metabolic acidosis that can lead to progressive organ impairment and eventual intravascular coagulation [25].

Once patients pass through the critical 24–48 hour period, disease recovery can be remarkably rapid. Reabsorption of extravascular fluids accompanies an increase in the general well-being of the patient, and appetite returns along with cessation of other symptoms [25]. Patients can develop what has been referred to as a "recovery rash" with characteristic patches of normal skin likened to "isles of white in a sea of red" that develop on the trunk, then spread to the head and extremities [26]. Patient blood counts stabilize and return to normal during this recovery phase.

The severe forms of disease are defined as a patient that has dengue with one of the following: severe plasma leakage that leads to shock and/or fluid accumulation with respiratory distress; severe bleeding, and severe organ impairment (Figure 1) [16]. As noted above, dengue-induced shock occurs at defervescence and at a time when viral levels are falling (Figure 2), indicating likely immune-mediated pathology [27]. The hypovolemic shock that occurs is a result of prolonged increased vascular permeability causing plasma leakage [28]. Patients with DSS initially suffer from asymptomatic capillary leakage progressing to compensated shock to hypotensive shock, eventually leading to cardiac arrest [29, 30]. Dengue shock patients need to be closely monitored, as the time between warning signs and the development of compensated shock and hypotensive shock may only be a matter of hours [28]. Only minutes may separate hypotensive shock and cardiorespiratory collapse and cardiac arrest [28]. For a more in-depth review of the clinical presentation of severe dengue and patient management, consult the WHO Handbook for clinical management of dengue [16].

Predictive Algorithms

Given the wide presentation and dynamic nature of DENV-related illness, a number of studies have been performed to assess the potential of using an algorithm approach to the prediction of patient progression from dengue fever to severe dengue disease [19, 24, 25]. These studies have looked at a combination of clinical and laboratory markers, both viral and host derived, that have previously been shown to vary significantly between dengue fever and severe dengue patients [31–34]. Virological markers such as the virus or viral genome and the secreted NS1 protein along with host factors including virus-specific immune responses, liver enzymes (aspartate aminotransferase and alanine aminotransferase) [35], and hematological factors such as platelet and hematocrit counts have all been considered [32, 33, 36]. While some of these studies have shown promise, a comprehensive multicenter clinical study is yet to be evaluated and reported [34, 37–39]. Such a study has been under way since 2011 [40] and its findings, due in early 2017, are keenly awaited.

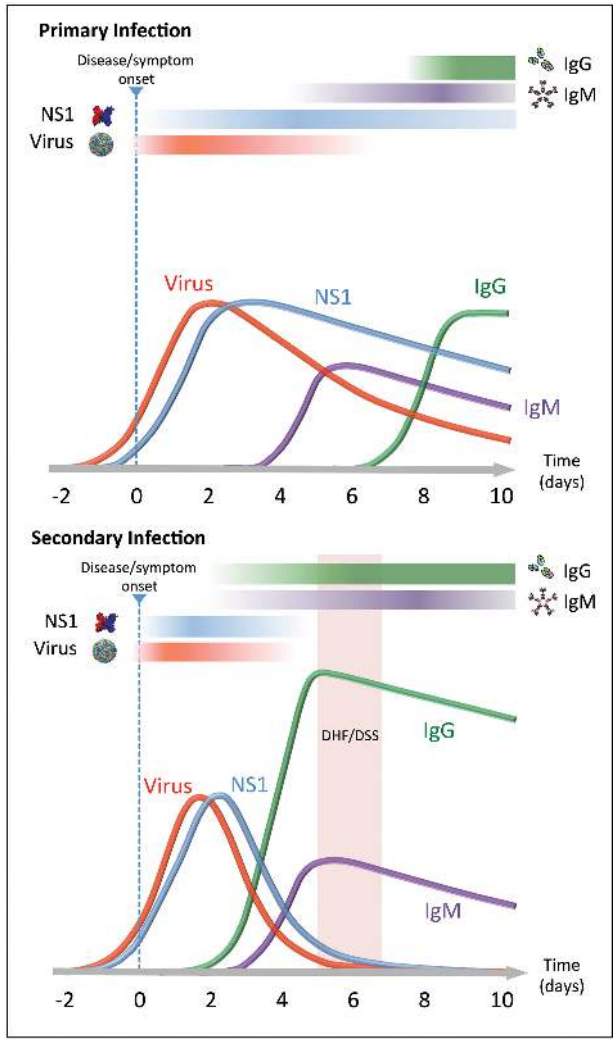

Figure 2. Timeline of dengue biomarker appearance in patients experiencing primary and secondary infection. In primary infection (top panel), both nonstructural protein 1 (NS1) and virus can be detected from the onset of disease, with immunoglobulin M (IgM) appearing around day 3 of illness and immunoglobulin G (IgG) appearing toward the end of the acute period. Secondary infections (bottom panel) are characterized by the presence of IgG early in the acute phase of disease and a shorter duration of NS1 and virus detection. Note onset of severe dengue (dengue hemorrhagic fever [DHF]/dengue shock syndrome [DSS]), primarily in secondary infections and at a time when virus and NS1 levels are falling.

However, the development of a universal predictive algorithm for severe disease progression presents numerous challenges given the significant variables introduced by local virus evolution, the subtleties of virus–host interactions, geographic spread of the disease, and regional host genetics. Nevertheless, the current WHO guidelines aid clinicians in providing a clear set of clinical warning signs to help predict severe disease onset. Although not all the warning signs appear early in disease, when appropriately implemented in the clinic, along with accurate laboratory diagnosis, the WHO guidelines provide a strong framework for effective monitoring of severe disease.

LABORATORY DIAGNOSIS

Biomarkers that have been targeted for diagnosis include the virus itself (virus isolation in culture or mosquitoes or the direct detection of viral genomic RNA), viral products (capture and detection of the secreted NS1 protein), or the host immune response to virus infection (through measurement of virus-specific immunoglobulin M [IgM] and immunoglobulin G [IgG]). The timing of the appearance and duration of these biomarkers in both primary and secondary dengue infection is graphically presented in Figure 2. In the following, we briefly review both traditional and more recent approaches to measuring the presence of these markers of infection.

Virus Isolation

Virus isolation has been the traditional diagnostic method for detecting DENV infection. However, it has gradually been replaced by reverse-transcription polymerase chain reaction (RT-PCR) and, more recently, by NS1 antigen-capture enzyme-linked immunosorbent assays (ELISAs) for more rapid diagnosis [41]. For virus isolation, clinical samples taken from patients are cultured in a variety of cell lines of either mosquito (AP-61, Tra-284, AP64, C6/36, and CLA-1 cells) or mammalian (LLCMK2, Vero, and BHK-21 cells) origin or in live mosquitoes [41, 42]. Blood samples taken from infected patients experiencing febrile illness up to 5 days after the onset of disease yield the most successful results. However, virus isolation from secondary infected patients is made more difficult by the rapid anamnestic production of cross-reactive antibodies early during the acute phase of disease that form immune-complexes with circulating virus [43]. Although detection of DENV by virus isolation is definitive, it is not particularly practical, as isolation can take days to weeks to perform [44].

RT-PCR

Molecular methods such as RT-PCR and nucleic acid hybridization have been used to great effect in successfully diagnosing DENV infection. PCR-based methods provide same- or next-day diagnosis of DENV during the acute phase of disease. Lanciotti et al [44] originally reported a 2-step heminested RT-PCR assay that was highly sensitive. This method was then modified to a single-step multiplex real-time RT-PCR assay, which was adopted worldwide. A major advantage of PCR-based techniques is that viral RNA can be detected from the onset of illness and is sensitive, specific, fast, less complicated, and cheaper than virus isolation methods [45]. Although PCR-based methods are fast and accurate, they require 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 lack center-to-center standardization [46]. 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 [47].

NS1 Antigen Capture

The viral protein NS1 is an ideal diagnostic target because it is secreted from infected cells, is found at high levels circulating in the blood of infected individuals, and can be detected from the onset of symptoms through to 9 days or longer after disease onset, at least in primary infections. NS1 can be detected at the same time as viral RNA and before an antibody response is mounted in primary infections. It can be viewed as a surrogate marker for viremia, with the level of NS1 shown to correlate with viral titer [48, 49]. Detection of NS1 in patient blood using an antigen-capture ELISA approach was first described in 2000 [48]. Using quantitative-capture ELISA, it has been found that NS1 is secreted at high levels, within the range of low nanograms per milliliter to micrograms per milliliter, with up to 50 μg/mL found circulating in some infected individuals. Subsequent studies investigating the kinetics of NS1 in secondary infections found that NS1 levels ≥600 ng/mL within the first 72 hours of disease was a strong predictor of progression to more severe disease [27]. These early reports led to the commercial development of NS1 capture ELISAs and rapid strip tests [48, 49]. The commercial development of NS1 as a diagnostic tool has revolutionized dengue diagnosis as it has provided simple and low-technology assays that have high sensitivity and specificity. These detection assays have now become the new standard for dengue diagnostics [14, 27, 50, S51–S57], allowing early diagnosis and more effective patient management. Despite the suggested predictive value of NS1 as a marker of disease progression, the required quantitation still remains the province of academic research, with all commercial tests only providing qualitative positive/negative readout. A limitation of NS1 detection for patients experiencing a secondary infection is the rapid anamnestic rise in NS1 cross-reacting antibodies during the acute phase of disease.

These antibodies sequester NS1 in immune complexes that cannot be readily detected in capture assays. Consequently, the kinetics of NS1 detection over the course of disease during secondary infections is shorter than that for primary infections (Figure 2).

Serology

There are numerous 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 [S58–S64]. HI assays along with IgM and IgG antibody-capture ELISAs have proven to be the most useful serological diagnostic methods for routine DENV detection. The HI test has been applied to dengue diagnosis for many years, with most laboratories developing in-house methodologies, although commercial kits are also available [S65]. As with all assays based on antibody detection, the early acute disease period usually presents a negative window of detection, given the need for the relevant antibody response to be elicited. Nevertheless, high-throughput IgM and IgG antibody-capture ELISAs have become relatively routine, particularly following assay automation. IgM can appear as early as day 3–5 in primary infection, peaking several weeks after recovery and remaining at detectable levels for several months [41, S66, S67]. IgG does not generally appear during the acute phase of primary disease. However, during secondary infection, there is a rapid anamnestic IgG response to shared epitopes on multiple viral proteins between the first and second infecting serotypes, with IgG appearing as early as 3 days after onset of illness [S66]. Consequently, when performed in parallel, IgM and IgG detection can provide a diagnostic indication of primary or secondary infection based on the ratio of IgM and IgG during the acute phase of disease [S66, S68].

Detection of DENV infection by serology is complicated in areas of the world where >1 flavivirus is circulating (eg, yellow fever, Japanese encephalitis, and, more recently, Zika virus), because of shared cross-reactive epitopes on the flavivirus E protein, and hence cross-reactivity of the antibody response. This is particularly problematic in the current Zika virus epidemic in Brazil, which is occurring in a background of complicating DENV infection and serology. Antibodies directed against these flaviviruses can cross-react in DENV serology assays, leading to false-positive results. To reduce these false-positive results, IgM and IgG serology should be paired with NS1 antigen capture. Commercial dengue NS1 antigen-capture ELISA and immunochromatographic (Rapid) strip tests have been designed to be highly specific, with no demonstrable cross-reactivity with other flavivirus NS1 species [S57, S69, S70]. However, with the possible cross-reactivity detected in a recent Zika virus–infected patient, the specificity of these assays will need to be revisited [S71].

Combined Approaches

Given the varying kinetics of each biomarker, no single assay can be used to definitively diagnose DENV in patients who may present at a different stage in their infection. Detection of NS1 antigen is perhaps the most robust of all the DENV diagnostic methods with a relatively long detection window, particularly in patients with primary infection. However, as noted above, NS1 detection can be obscured by immune complex formation in secondary infections, thereby shortening its window of detection (Figure 2). In this case, combining NS1 detection with IgM and/or IgG detection has been shown to dramatically improve positive dengue diagnosis [S72, S73]. There are several diagnostic kits currently available that take advantage of this

Table 1. Laboratory Diagnostic Methods for the Detection of Dengue Infection

	Clinical Sample	Diagnostic Approach	Methodology	Time to Results
Virus and virus product detection	Acute serum (1–5 d of fever) and necropsy tissue	Virus isolation	Mosquito or mosquito cell culture inoculation	1 wk or more
		Nucleic acid detection	RT-PCR and real-time RT-PCR	1–2 d
		Antigen detection	NS1 Ag rapid test	Minutes
			NS1 Ag capture ELISA	1 d
			Immunohistochemistry	2–5 d
Serological response	Paired sera • S1: acute serum from 1–5 d • S2: convalescent serum 15–21 d	IgM or IgG seroconversion (S1 to S2)	ELISA HI	1–2 d
			Plaque reduction neutralization test	>7 d
	Serum after day 5 of fever	IgM detection	MAC-ELISA	1–2 d
			IgM rapid tests (lateral flow)	Minutes
		IgG detection	IgG ELISA HI	1–2 d
			IgG rapid tests (lateral flow)	Minutes

Adapted from the World Health Organization [16].

Abbreviations: Ag, antigen; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition assay; IgG, immunoglobulin G; IgM, immunoglobulin M; MAC, immunoglobulin M antibody capture; NS1, nonstructural protein 1; RT-PCR, reverse-transcription polymerase chain reaction.

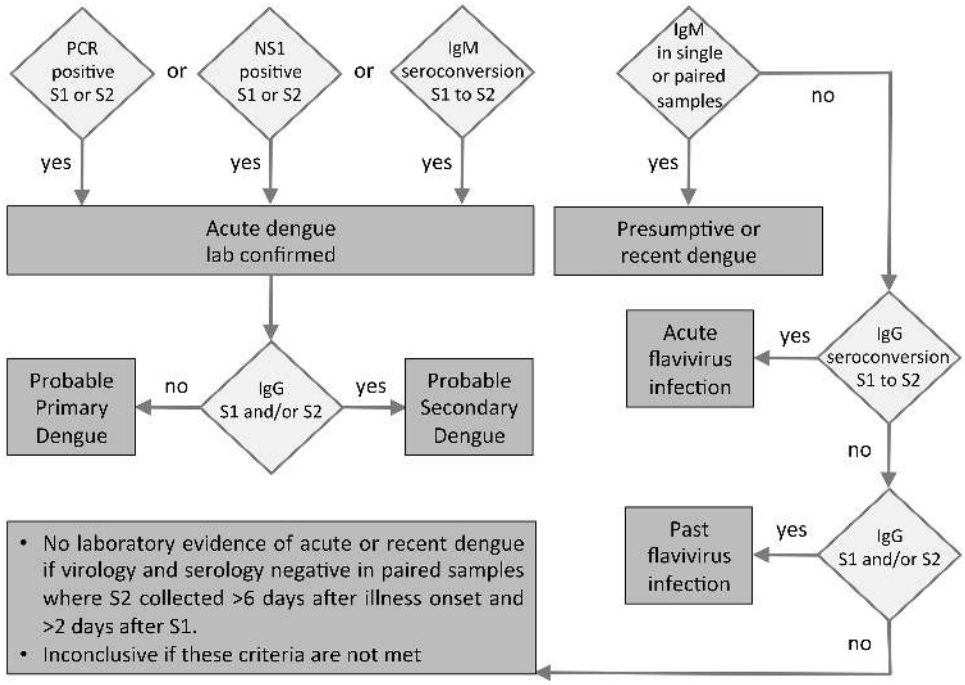

Figure 3. Diagnostic algorithm for laboratory confirmation of dengue virus infection. Polymerase chain reaction (PCR) or nonstructural protein 1 (NS1) positivity in either acute (S1) or convalescent (S2) clinical samples confirms current dengue infection. Seroconversion of S1 to S2 as measured by immunoglobulin M (IgM) enzyme-linked immunosorbent assay 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. Adapted from Jaenisch et al [40].

approach in rapid point-of-care devices, including those developed by SD Bioline Dengue Duo (NS1 Ag + Ab Kit). Using this combination approach, detection sensitivities nearing 100% have been reported from the onset of illness through recovery [S69].

FUTURE TESTS

There are many new approaches for rapid dengue diagnosis currently under development. These include micro/paper fluidics, in vivo micropatches [S74], isothermal PCR [47], and electrochemical [S75, S76] and piezoelectric [S77] detection. All of these technologies are in the early stage 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, with quantitative serotype-specific NS1 detection.

CONCLUSIONS

Early and accurate laboratory diagnosis of DENV infection is critical to effective patient management. While we already have the tools that allow us to reliably determine if a patient is suffering from dengue infection (Table 1 summarizes the diagnostic methods available, and a representative diagnostic algorithm is shown in Figure 3), we are still lacking effective predictive biomarkers of progression to severe disease. Early studies have suggested that elevated levels of NS1 and viremia may have predictive value [27]; however, in the absence of readily available quantitative NS1 assays, few validation studies have been performed. Beyond DENV biomarkers, host responses provide potential predictive markers of severe disease progression. Several candidate proteins [S78] have been identified; however, their predictive value is yet to be validated. To determine a set of predictive biomarkers, a large multicenter study is currently under way across multiple countries, with the aim of identifying a set of predictive parameters for severe disease. Results from this study are expected to be published in the early part of 2017 [40].

Combinatorial approaches employing virus and/or viral product detection along with serology currently yield the most reliable diagnosis of dengue infection and should be coupled with close clinical monitoring of warning signs to identify patients at risk of progression to severe dengue disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank Professor Cameron Simmons for his critical reading of this manuscript.

Financial support. This work was in part supported by the National Health and Medical Research Council of Australia and the Queensland State Government (Accelerate Fellowship to DAM).

Potential conflicts of interest. P. R. Y. has been a consultant to the biotechnology industry and received funding for the development of NS1-based diagnostics. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Perera R, Kuhn RJ. Structural proteomics of dengue virus. Curr Opin Microbiol **2008**; 11:369–77.
2. Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. Nat Rev Microbiol **2010**; 8:S7–16.
3. Smith CE. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. J Trop Med Hyg **1956**; 59:243–51.
4. Halstead SB. Dengue. Lancet **2007**; 370:1644–52.
5. Kraemer MU, Sinka ME, Duda KA, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife **2015**; 4:e08347.
6. Rathakrishnan A, Klekamp B, Wang SM, et al. Clinical and immunological markers of dengue progression in a study cohort from a hyperendemic area in Malaysia. PLoS One **2014**; 9:e92021.
7. Ocampo CB, Wesson DM. Population dynamics of *Aedes aegypti* from a dengue hyperendemic urban setting in Colombia. Am J Trop Med Hyg **2004**; 71:506–13.
8. Halstead SB, Streit TG, Lafontant JG, et al. Haiti: absence of dengue hemorrhagic fever despite hyperendemic dengue virus transmission. Am J Trop Med Hyg **2001**; 65:180–3.
9. Braga C, de Albuquerque MFPM, Cordeiro MT, et al. Prospective birth cohort in a hyperendemic dengue area in northeast Brazil: methods and preliminary results. Cad Saude Publica **2016**; 32.
10. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature **2013**; 496:504–7.
11. Mairuhu AT, Wagenaar J, Brandjes DP, van Gorp EC. Dengue: an arthropod-borne disease of global importance. Eur J Clin Microbiol Infect Dis **2004**; 23:425–33.
12. Kautner I, Robinson MJ, Kuhnle U. Dengue virus infection: epidemiology, pathogenesis, clinical presentation, diagnosis, and prevention. J Pediatr **1997**; 131:516–24.
13. Halstead SB. Etiologies of the experimental dengues of Siler and Simmons. Am J Trop Med Hyg **1974**; 23:974–82.
14. Simmons CP, Chau TN, Thuy TT, et al. Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. J Infect Dis **2007**; 196:416–24.
15. Rosenberger KD, Lum L, Alexander N, Junghanss T, Wills B, Jaenisch T; DENCO Clinical Study Group. Vascular leakage in dengue–clinical spectrum and influence of parenteral fluid therapy. Trop Med Int Health **2016**; 21:445–53.
16. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. New edition. Geneva, Switzerland: WHO, **2009**.
17. Modhiran N, Watterson D, Muller DA, et al. Dengue virus NS1 protein activates cells via Toll-like receptor 4 and disrupts endothelial cell monolayer integrity. Sci Transl Med **2015**; 7.
18. Rigau-Pérez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet **1998**; 352:971–7.
19. Yacoub S, Lam PK, Vu le HM, et al. Association of microvascular function and endothelial biomarkers with clinical outcome in dengue: an observational study. J Infect Dis **2016**; 214:697–706.
20. Kalayanarooj S, Vaughn DW, Nimmannitya S, et al. Early clinical and laboratory indicators of acute dengue illness. J Infect Dis **1997**; 176:313–21.
21. Mayxay M, Phetsouvanh R, Moore CE, et al. Predictive diagnostic value of the tourniquet test for the diagnosis of dengue infection in adults. Trop Med Int Health **2011**; 16:127–33.
22. Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W, et al. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. Pediatr Infect Dis J **2007**; 26:283–90; discussion 91–2.
23. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg **1969**; 18:954–71.
24. Yacoub S, Wills B. Predicting outcome from dengue. BMC Med **2014**; 12:147.
25. Yacoub S, Wills B. Dengue: an update for clinicians working in non-endemic areas. Clin Med (Lond) **2015**; 15:82–5.
26. Nimmannitya S. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health **1987**; 18:392–7.
27. Libraty DH, Young PR, Pickering D, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. J Infect Dis **2002**; 186:1165–8.
28. Lum LCS, Goh AY, Chan PW, El-Amin AL, Lam SK. Risk factors for hemorrhage in severe dengue infections. J Pediatr **2002**; 140:629–31.
29. Yacoub S, Wertheim H, Simmons CP, Screaton G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. Nat Rev Cardiol **2014**; 11:335–45.
30. Griffee MJ, Merkel MJ, Wei KS. The role of echocardiography in hemodynamic assessment of septic shock. Crit Care Clin **2010**; 26:365–82, table of contents.
31. Potts JA, Gibbons RV, Rothman AL, et al. Prediction of dengue disease severity among pediatric Thai patients using early clinical laboratory indicators. PLoS Negl Trop Dis **2010**; 4:e769.
32. Potts JA, Rothman AL. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Trop Med Int Health **2008**; 13:1328–40.
33. Brasier AR, Ju H, Garcia J, et al; Venezuelan Dengue Fever Working Group. A three-component biomarker panel for prediction of dengue hemorrhagic fever. Am J Trop Med Hyg **2012**; 86:341–8.
34. Tanner L, Schreiber M, Low JG, et al. Decision tree algorithms predict the diagnosis and outcome of dengue fever in the early phase of illness. PLoS Negl Trop Dis **2008**; 2:e196.
35. Mahmuduzzaman M, Chowdhury AS, Ghosh DK, Kabir IM, Rahman MA, Ali MS. Serum transaminase level changes in dengue fever and its correlation with disease severity. Mymensingh Med J **2011**; 20:349–55.
36. The TD, Thao LTT, Minh DN, et al. Clinical features of dengue in a large vietnamese cohort: intrinsically lower platelet counts and greater risk for bleeding in adults than children. Plos Neglect Trop D **2012**; 6:e1679.
37. Tamibmaniam J, Hussin N, Cheah WK, Ng KS, Muninathan P. Proposal of a clinical decision tree algorithm using factors associated with severe dengue infection. PLoS One **2016**; 11:e0161696.
38. Lee IK, Liu JW, Chen YH, et al. Development of a simple clinical risk score for early prediction of severe dengue in adult patients. PLoS One **2016**; 11:e0154772.
39. Hunsperger EA, Sharp TM, Lalita P, et al. Use of a rapid test for diagnosis of dengue during suspected dengue outbreaks in resource-limited regions. J Clin Microbiol **2016**; 54:2090–5.
40. Jaenisch T, Tam DT, Kieu NT, et al. Clinical evaluation of dengue and identification of risk factors for severe disease: protocol for a multicentre study in 8 countries. BMC Infect Dis **2016**; 16:120.
41. Shu PY, Chen LK, Chang SF, et al. Dengue virus serotyping based on envelope and membrane and nonstructural protein NS1 serotype-specific capture immunoglobulin M enzyme-linked immunosorbent assays. J Clin Microbiol **2004**; 42:2489–94.
42. Guzmán MG, Kourí G. Advances in dengue diagnosis. Clin Diagn Lab Immunol **1996**; 3:621–7.
43. Teles FR, Prazeres DM, Lima-Filho JL. Trends in dengue diagnosis. Rev Med Virol **2005**; 15:287–302.
44. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol **1992**; 30:545–51.
45. Deubel V, Laille M, Hugnot JP, et al. Identification of dengue sequences by genomic amplification: rapid diagnosis of dengue virus serotypes in peripheral blood. J Virol Methods **1990**; 30:41–54.
46. Najioullah F, Viron F, Césaire R. Evaluation of four commercial real-time RT-PCR kits for the detection of dengue viruses in clinical samples. Virol J **2014**; 11:164.
47. Teoh BT, Sam SS, Tan KK, et al. Detection of dengue viruses using reverse transcription-loop-mediated isothermal amplification. BMC Infect Dis **2013**; 13:387.
48. Young PR, Hilditch PA, Bletchly C, Halloran W. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. J Clin Microbiol **2000**; 38:1053–7.
49. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol **2002**; 40:376–81.
50. Bessoff K, Delorey M, Sun W, Hunsperger E. Comparison of two commercially available dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single clinical sample for diagnosis of acute DENV infection. Clin Vaccine Immunol **2008**; 15:1513–8.