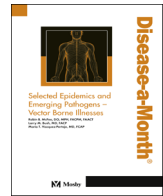




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Selected mosquito borne illnesses - Dengue



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Overview – Dengue Virus

Dengue virus (DENV) (Fig. 1), also sometimes referred to as Dengue virus disease (DVD) is a viral hemorrhagic fever virus (VHF).^{1–11} VHF are a diverse group of viral illnesses characterized by fever, sometimes extreme, and various degrees of bleeding risk, including hemorrhage, shock, and death.^{1–20} DENV is among the most common global mosquito borne illnesses, and is one of the fastest spreading infections worldwide.^{1,4,5,13,14,21–26} It is the second most commonly reported mosquito borne illness infecting humans, after malaria. Although the exact epidemiology of VHFs and DENV remain elusive, it is estimated 3 billion persons live at risk in areas where dengue virus (Fig. 1) can be transmitted.^{13,26,27} With upwards of half the global population possibly at risk, including parts of the United States, great attention to this emerging pathogen is vital, not only for policy makers, but clinicians. DENV infection is a major cause of disease in tropical and subtropical areas worldwide, including Cuba, and the Caribbean.²⁷ Outbreaks can involve thousands of people, as demonstrated in Indonesia, where by mid 2004 58,000 cases occurred, with a 1.1% case-fatality rate (CFR) [4]. CFR of dengue related illnesses vary widely, depending upon region, population density and demographics, ready access to health care, and host factors. Brazil, a diverse and large country has noted a CFR ranging from 1–12%. Not surprisingly, patients who are early diagnosed and quickly treated are likely to have a CFR less than 1%. Vietnam has reported a 97 percent increase in DENV cases in 2016 compared with 2015. Vietnam reported 63,504 cases of dengue in 44 out of 63 provinces, resulting in 20 deaths. Dengue is endemic in over 100 countries, including the Americas and American Tropics (Map 1).¹⁷ In the last twenty years there has been a dramatic increase in disease penetration in South America and the Caribbean.²¹ In 2010 local transmission of dengue occurred in France, underscoring the importance of preparedness for emerging pathogens long thought to be exotic and remote.^{1,12,13,17,28–40} Although pre-1970 only 9 countries experienced severe dengue, WHO considers Europe to be at risk for an outbreak. This is consistent with the dramatic increase in global incidence of dengue over the last few decades. While most recognize that Dengue

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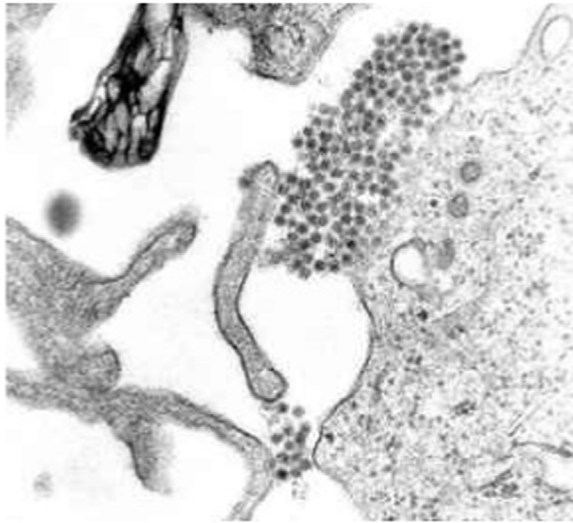
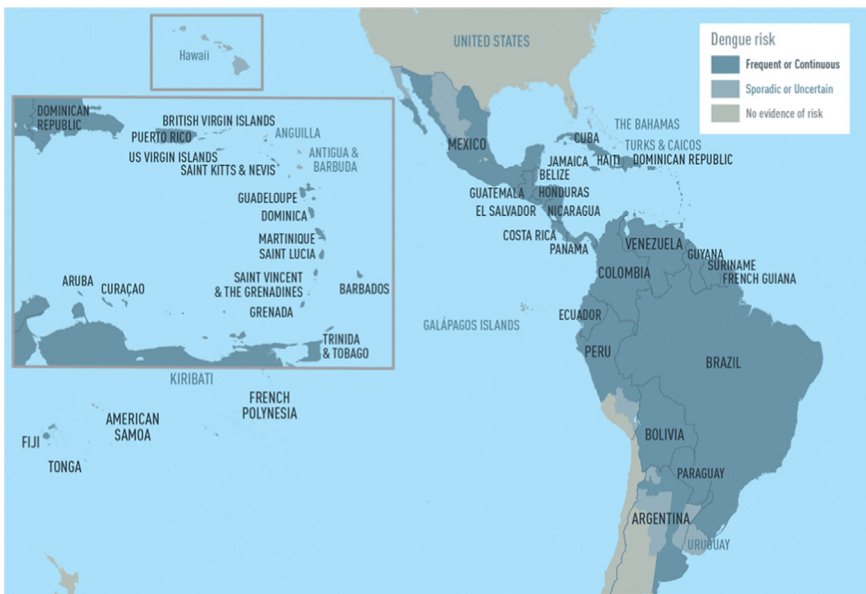


Fig. 1. Note cluster of DENV virions center top, and center bottom. (CDC.gov from University S. Carolina Biomedical Sciences).



Map 1. Dengue in the Americas (From CDC <https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/dengue#5154>).

is found in tropical and sub-tropical climates worldwide, what is often overlooked is the risk of DENV in urban and semi-urban areas.

VHFs are a group of infectious illnesses caused by several distinct families of RNA viruses with a worldwide distribution, that include Ebola, Lassa, dengue, Crimean Congo, and others.^{1–4,7,9,11,41–50} Most VHFs are capable of causing a syndrome that ranges in severity from mild illness to potentially fatal multisystem involvement.^{1,3–12,21,26,27,47,50–56} The clinical presentation – biodrome^{9,56} (cascade of signs and symptoms associated with a pathogen or family of pathogens), varies by virus, and host

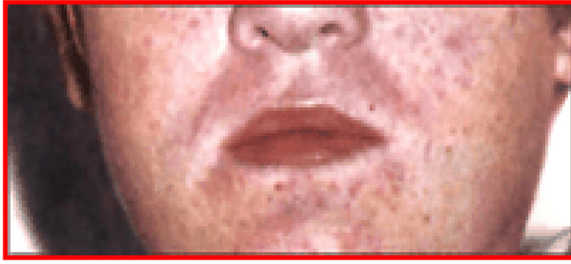


Photo 1. Marburg VHF exanthema.



Photo 2. Measles exanthem.



Photo 3. Ocular manifestations of VHF.

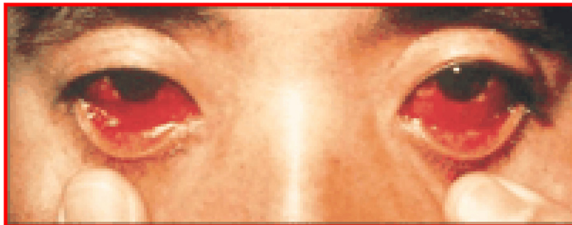


Photo 4. Ocular Manifestations in Bolivian Hemorrhagic Fever.

characteristics. This biodrome which will be discussed in more detail later involves fever, the circulatory system, rashes ([Photo 1](#))⁹ which can be mistaken for other exanthema, such as measles ([Photo 2](#)),⁹ fatigue, and the musculoskeletal system. In some cases ocular manifestations ([Photos](#)

3 and 4)⁹ are possible, as well as other organ involvement of varying degrees of severity, including the gastrointestinal tract, and kidneys.

Viral hemorrhagic fevers (VHFs) are caused by several distinct families of viruses, not surprisingly called the viral hemorrhagic fever viruses that include Ebola, Lassa, Dengue and others. VHFs are distributed worldwide.^{8–10,42} VHFs are a taxonomically diverse group of viruses capable of causing high morbidity and mortality. In addition to being a significant public health threat as endemic illnesses worldwide, VHF remain of considerable interest as possible biological weapons.^{9,10}

Regardless of the pathogen, VHF can lead to a severe multisystem syndrome that results primarily in fevers and bleeding risks.^{1–11,26,27,43–45,54,55} Some form of hematological event secondary to microvascular damage and changes in vascular permeability can occur, plus other symptoms. Inherent with VHFs the overall vascular system is affected, resulting at times in severe dysregulation of coagulation; depending upon the underlying viral illness, a variety in severity of bleeding can occur from petechiae to circulatory collapse.^{5,8–10,26,27,58,59} Some VHF viruses cause primarily relatively mild illnesses, others cause a broad range (Dengue) of manifestations from asymptomatic to death, and others result in life-threatening disease (Ebola).^{9,10,17,26,27,57} Most VHF are considered biosafety level 4 (BSL – 4) pathogens – the highest level of security and threat, usually associated with pathogens for which there is no treatment and/or preventive measure. Exceptions to BSL 4 are Dengue and Yellow Fever.

VHF viruses belong to four distinct families (Table 1): arenaviruses, filoviruses, bunyaviruses, and Flaviviruses which share common features^{8,9,11,34,44,60}:

- RNA viruses enveloped in a lipid coat.
- Survival is dependent upon a host (animal, insect) i.e. natural reservoir.
- Geographically restricted to regions populated by their respective host species.
 - Humans are not the natural reservoir. Humans become infected through contact with infected hosts. Of note, in some cases, humans can transmit the virus via a variety of mechanisms.
- Human outbreaks occur sporadically and cannot be predicted

In rare cases, other pathogens can cause a hemorrhagic fever, for example scrub typhus.

The clinical presentation – signs and symptoms associated with VHFs vary by virus and host characteristics, but initial presentation can include fever usually above 100.4°F, significant fatigue, muscle involvement (pain, weakness), and exhaustion.^{3–5,9,34,43,61}

Table 1

Viral Hemorrhagic Fever Virus Families & Examples Of Member Viruses.

-
- Arena Viruses:
 - Lassa fever
 - Argentine hemorrhagic fever
 - Bolivian hemorrhagic fever
 - Flaviviridae
 - Yellow fever
 - Dengue fever
 - Bunyaviridae
 - Crimean-Congo fever
 - Filoviruses
 - Marburg and Ebola hemorrhagic fevers
-



Photo 5. Crimean Congo VHF.

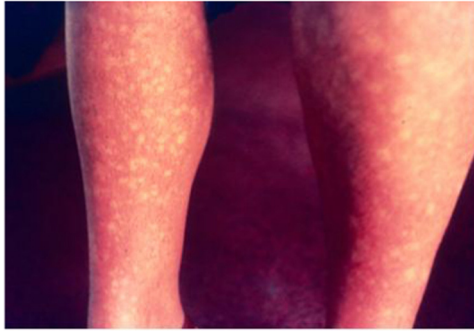


Photo 6. Rash on legs due to dengue fever; image from eMedicine.com, 2009, photo courtesy of Duane Gubler, PhD.

A variety of rashes are possible with VHF. For example, there is the maculopapular rash found in Marburg Disease (Photo 1).⁹ It is a nonpruritic maculopapular rash, that can resemble the rash of measles (Photo 2)⁹ and may occur in up to 50% of patients infected with the Ebola or Marburg viruses within the first week of illness.^{9,47,58,62} Extensive bleeding can cause a dramatic level of ecchymosis and extensive visual manifestations (Photo 5).⁹ The rash is more common in light-colored skin and desquamates on resolution (Photo 6).

Ocular manifestations can also be associated with hemorrhagic fever viruses (Photos 3, 4),⁹ and range from conjunctival injection to subconjunctival hemorrhage, in this case associated with Bolivian Hemorrhagic Fever virus (Photos 3 and 4).⁹

Evidence of bleeding is variable, again depending upon the pathogen, and host.^{27,59,63,64} DENV can infect a multitude of cells from dendritic, monocytes, lymphocytes, hepatocytes, endothelial cells as well as mast cells in vitro; studies are still underway to fully characterize DENV behavior in vivo, such as activation of memory T cells, which can catalyze the inflammatory cascade.^{27,63–65} Dengue severity can be affected by a variety of contributing factors, including whether this was a primary infection or reinfection with a different strain of DENV. Patients with severe cases of VHF often show signs of bleeding under the skin (Photo 5),⁹ in internal organs, or various orifices - mouth, eyes, ears or rectum. Severely ill patient cases may experience circulatory collapse, multisystem organ failure (renal failure is not rare) shock, nervous system malfunction, coma, delirium, seizures and ultimately death.^{3–6,9,60}

Dengue Fever Virus (DENV)

Dengue^{3–6,34,41–46} is a member of the genus *Flavivirus*^{8–11,34,41,50,60} and member of the family *Flaviridae*, along with West Nile virus, yellow fever virus, and tick – borne encephalitis virus, all of which are single stranded RNA viruses enclosed in a protein capsid which is enclosed in a host cell membrane-derived envelope.

Dengue like other Flaviviruses, has a positive single stranded RNA genome packed inside a core protein, surrounded by an icosahedral scaffold, encapsidated by a lipid envelope.^{27,41,47,50,66–72} Its 11 kb genome functions similar to mRNA. It encodes a polyprotein, that with translation gets cleaved into structural proteins (C, prM/M, E), and seven nonstructural proteins by viral or host proteases.⁷³ Because dengue viral genome can function as mRNA, once viral RNA is delivered into the cell cytoplasm utilizing bioactive vesicles, translation and genome synthesis can occur.

The sequence of the Flavivirus life cycle starts with viral proteins translated from genomic RNA within the first 1 to 5 hours post infection (HPI). Then viral RNA synthesis occurs approximately 5 HPI, where progeny virus assembly and release occur after 12 HPI.^{74,75}

Dengue virus (DENV) (Fig. 1) is transmitted to humans by *Aedes* mosquitoes, mainly *Aedes aegypti*. Among clinically important Flaviviruses (family Flaviridae), in addition to DENV, also transmitted by mosquito or tick vectors are the West Nile, yellow fever, and tick-borne encephalitis single stranded RNA viruses.

There are four serologically distinct Dengue viruses (DENV). Infection (and recovery) from one of the 4 serotypes confers lifelong immunity to the same serotype and only partial, temporary cross immunity to the other types; subsequent infection by another serotype may in fact increase the risk of severe dengue illness.^{1–11,43–45,68,69}

Based on neutralization assay data, the four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) can be distinguished. These serologically distinct dengue viruses (DENV 1–4) have important implications for disease severity and vaccine development.^{43–45,68–73} Although the four serotypes are closely related, antigenic differences between them may allow infection by the other three serotypes. Infection and recovery from one of the four serotypes confers lifelong immunity to the same serotype, but may only confer partial, even temporary cross immunity to the other types. Paradoxically, and what makes vaccine development somewhat difficult, subsequent infection by another DENV serotype may increase the risk of severe dengue illness, such that inadequate protection within a vaccine for any of the DENV 1–4 can have serious implications clinically.

The four DENV serotypes differ by 30–35%; interestingly there appears to be similarity between DENV and Zika. Apparently the DENV serocomplex differs from ZIKV by 41–46% (in amino acid sequence of the envelope protein).^{76–78} Serologically according to various reports, there appears to be difficulty in distinguishing DENV infection versus ZIKV infection serologically, suggesting a degree of antigenic similarity between the two viruses.^{76–80}

Not surprisingly, in DENV-endemic areas, all four serotypes of DENV frequently circulate together or in some cases will cyclically replace each other, the threat being that multiple infections are common.^{80,81} Problematic to the risk of subsequent infection with DENV infection is that the potential for life-threatening complications of dengue hemorrhagic fever are more common after secondary infection than after primary infection.⁷⁷ A variety of host factors are suggested as contributory to this, including the concept of antibody-dependent enhancement (ADE).⁷⁷ ADE involves antibodies generated during the primary infection with one of the DENV 1–4 will not provide a sufficient concentration or avidity to neutralize secondary infection with a DENV of a different serotype differing in amino acid sequence by 30–35%. If another subtype of dengue virus subsequently infects the individual, once again DNV activates the immune system. Owing to the similarity of DNV 1–4 surface antigens, the immune system initially treats this infection as if it was from the initial subtype. These antibodies will bind to the surface proteins but do not inactivate the virus so that when macrophages appear DENV proceeds to infect them. For example, once infected with DENV, an immune response occurs to that specific (DENV 1 – 4) dengue subtype. Antibodies to subtype-specific surface proteins are supposed to prevent DNV from binding to macrophage cells, which are a target cell for DNV infection, thus preventing viral entry. Some response may occur – opsonization of the secondary virus and targeting Fc-receptor-mediated endocytosis into myeloid cells (monocytes and macrophages). Macrophages are the principal site of DENV replication. The result of this is potentially higher viral loads.^{27,63,64} During the process, cytokine release can cause the endothelial tissue to become permeable which contributes to the hemorrhagic effects of Dengue.⁸² ADE can be readily demonstrated *in vitro* and has also been shown to drive higher viral loads of DENV in animal models.^{78–84} To be sure, the underlying health of the patient, inherent host immunity, and other factors, will determine disease severity.

Severity of dengue disease therefore is thought to be predicted according to the effect of this antibody-dependent enhancement (ADE) in different serotype cross-infections.^{2,5,6,76–83} ADE was first recognized nearly 50 years ago in DENV infection and is believed to be one of the factors that drive increased severity of secondary infections, which is a hallmark of DENV.⁸⁴ Although not necessarily unique to Dengue, it is a hallmark of DENV disease; the pathophysiology of Dengue associated ADE demonstrates the challenges associated with this viral illness, not the least of which is vaccine development. Vaccination that does not adequately protect against all four serotypes of DENV can predispose the recipient to severe illness, as has been demonstrated during early clinical trials of various candidate vaccines.

Like other pathogens (malaria, West Nile Virus) – dengue utilizes a mosquito vector – the *Aedes*. Prior to 1981 dengue was not widely diagnosed in the Americas. Since then it is found throughout South, Middle and North America, including the Caribbean. Dengue is endemic in over 100 countries. It can be transmitted via blood, including needle sticks, organs, transfusion or transplant, including bone marrow.

In the United States Dengue became a reportable infection in 2009, and according to the CDC, infection rates were 3 – 8%. Of note, dengue was a leading cause of febrile, systemic illness afflicting those returning from the Caribbean, South America, South Central and southeast Asia.

Transmission

Aedes aegypti, *Aedes albopictus*, as well as *Aedes polynesiensis* mosquitoes (Photo 7) are the most common vectors.^{7,8,47,49,61,75} *Aedes* mosquito resides nearly year round in Southeastern US states. They bite a viremic person, and after an incubation period, *Aedes* can transmit the virus for the insect's lifespan, which is approximately 1 month. *Aedes* can live indoors, and can be found in cool dark places such as bathrooms, closets, under beds, and behind curtains. A person can become infected from the bite of just one infected mosquito. While a mosquito can feed and bite anytime of the day, it is more likely during the predawn and dusk hours. Of concern the mosquito vector capable of transmitting dengue has spread to at least 26 states in the US, making the risk for dengue and dengue fever a potential public health threat. With climate change, public concern about chemical approaches (mosquito spray) to vector control, importation of items that harbor mosquitoes and other insects such as plants, population movement from regions where dengue is endemic, and more regions becoming capable of supporting *Aedes* and other mosquitoes, the spread of dengue and other mosquito borne illnesses in the United States is to be expected.

Not surprisingly the disease incidence seems to be increasing. Researchers suggest the surge in dengue fever may be due to several factors:

- Urban crowding; as population density increases, there are more sites for mosquitoes to thrive
- International commerce that contains infected mosquitoes, thus introducing the disease to areas previously free of the disease
- Environmental changes that allow mosquitoes to survive the winter months
- Travelers who carry the disease to areas where mosquitoes have not been previously infected



Photo 7. *Aedes* mosquito (www.cdc.gov).

Preventive measures include making certain window screens are intact, with holes repaired, ensuring no gaps between window and window-air conditioners, removing stagnant water outside, and using mosquito repellent, along with avoiding times when mosquitoes are prevalent – early morning, several hours after daybreak, late afternoon and early evening are times when the risk of being bitten are the highest, can reduce the risk of bites. Mosquitoes may feed/bite anytime during the day, but less likely than predawn and dusk. Interestingly *Aedes* mosquitoes live indoors, found in cool, dark places such as bathrooms and closets, under the bed, behind curtains.

PATHOPHYSIOLOGY OF HEMORRHAGE

As noted earlier, DENV infections result in a wide array of symptoms and forms of severity. A common feature is increased capillary permeability which can lead to hypovolemic shock. The exact mechanism of DENV pathology remains to be elucidated. Target cells for dengue include dendritic cells, macrophages/monocytes; these release chemokines and cytokines capable of activating endothelium. Endothelial cells are a fluid barrier of blood vessels. DENV vascular permeability may be mediated therefore by this interplay with endothelium. Viral non-structural protein 1 (NSP1) and antibodies may also be involved in endothelium dysfunction. Dengue hemorrhagic fever and dengue shock syndrome are associated with changes in vascular permeability.^{1–9,55,56} Microvascular and endothelial dysfunction are associated with DENV disease severity, and precede the appearance of clinical events.

Thrombocytopenia, platelet dysfunction, low fibrinogen, and other coagulation cascade factors (prolonged APTT) are associated with bleeding risk. Disseminated intravascular coagulation and vasculopathy also may contribute.

The most worrisome forms of Dengue clinical syndromes.^{4,6,9,10,43–46,60,61}

1. Dengue hemorrhagic fever (break bone fever) (DHF) is an acute febrile disease characterized by sudden onset, fever, intense headache, myalgia, retro-orbital pain, arthralgias, anorexia, GI affects can occur. A maculopapular rash may appear as the fever subsides. With this form, death is uncommon.
2. Dengue shock syndrome (DSS) is the most severe form, and is characterized by hypovolemia, a variety of bleeding manifestations that range from cutaneous to internal. Relative bradycardia and shock are possible, and the mortality rate is 10% or more. Aggressive, early supportive care including rehydration and attention to blood loss are critical.

Dengue fever and DHF/DSS have some commonalities – viremia lasting 5–8 days, fever of 2 – 7 days, headache, myalgia, bone/joint pain, and rash. Leucopenia is possible. Thrombocytopenia and cutaneous hemorrhage can be observed in varying degrees. Break bone fever – bone/joint pain that can be incapacitating is seen; commonly in adults. Abnormal hemostasis can be profound.

CLINICAL PRESENTATION

As global infections start emerging as domestic challenges to the United States, inquiring of patients their travel and occupation, which often involves business travel, becomes critical to emergency medicine and primary care physicians – the front line of health care. The challenge of competing demands in health care, including time per patient, may act as a barrier, but we are in an era where emerging infections against the backdrop of globalization with a growing magnitude in travel associated infections (TAI) justify the effort.

A viral hemorrhagic fever should be considered in any person who presents with a severe febrile illness and clinical evidence of vascular involvement - hypotension, petechiae, easy bleeding, facial/chest flushing, nondependent edema, and who has traveled to a region where VHF are known to occur. Although all VHFs present with febrile illness and hemorrhagic potential as does the Flavivirus, the incubation periods and clinical presentations of other VHF families do vary ([Table 2](#))

Table 2General Incubation Periods Other Families VHFs,^{8-11,34,42,44,60}

Virus family	Disease (Virus)	Natural distribution	Usual source of human infection	Incubation (Days)
Arenaviridae				
Arenavirus	Lassa fever	Africa	Rodent	5–16
	Argentine HF (Junin)	South America	Rodent	7–14
	Bolivian HF (Machupo)	South America	Rodent	9–15
	Brazilian HF (Sabia)	South America	Rodent	7–14
	Venezuelan HF (Guanarito)	South America	Rodent	7–14
Bunyaviridae				
Phlebovirus	Rift Valley fever	Africa	Mosquito	2–5
Nairovirus	Crimean-Congo HF	Europe, Asia, Africa	Tick	3–12
Hantavirus	Hemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome	Asia, Europe, worldwide	Rodent	9–35
Filoviridae				
Filovirus	Marburg and Ebola	Africa	Unknown	3–16
Fliviviridae	Dengue	Global		–14+

Dengue should therefore be considered as part of the differential diagnosis for patients who have travelled to the tropics and subtropics in the 2 weeks prior to the onset of symptoms. This takes into account the incubation period (Table 2) which, though typically is 4–7 days, can be as short as 3 days or upwards of 14 days. Of note, mild febrile infection with Dengue may not be identified as such. This is owing to the fact that many persons infected with Dengue for the initial time often have mild febrile illness or are asymptomatic. As discussed earlier, subsequent infections with Dengue are usually associated with severe disease.

Over the years there have been a variety of approaches to clinically diagnose, characterize and categorize various forms of DENV illnesses, in order to guide the clinician in terms of predicting severity and guiding levels of treatment. As the epidemiology of DENV has expanded, and changed over the years, with greater interest in studying the prevalent signs, symptoms and characteristics of various outbreaks globally, the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) have provided updated guidance, including assistance with laboratory

Table 3

Early onset, and Later Disease. Note the spectrum of illness ranges from asymptomatic to severe clinical symptoms, even death.

- Early onset (few days)
 - High fever
 - Headache/muscle aches/ stomach pains
 - Fatigue
 - Diarrhea/bloody diarrhea
 - Vomiting blood
 - Sore throat/red, itchy eyes
 - Hiccups
- Febrile illnesses – typically > 101°F
- Hematuria and other dyscrasias
- Relative bradycardia
- Constitutional symptoms

diagnostics, and expertise for health care professionals. The reader is invited to regularly visit www.who.int and www.cdc.gov for updates on DENV, and other emerging pathogens.

Regardless of diagnostic schema, dengue can cause anything within the range of asymptomatic to fulminant illness, life threatening disease, and death. An infection by any DENV 1–4 serotype usually results in asymptomatic illness in the majority of cases, but a variety of severity within the spectrum of clinical symptoms^{85,87} should be anticipated when the patient presents (Tables 1 and 3); this is especially true upon reinfection with a different serotype of DENV.

One of the most common schema distinguishing the various forms of DENV include DF, DHF, and DSS.

A mild flu-like syndrome, with minimal arthropathic pain has been referred to as dengue fever (DF). More severe forms of disease, characterized by coagulopathy, increased vascular fragility, and permeability is referred to as dengue hemorrhagic fever (DHF), which can progress to hypovolemic shock, referred to as dengue shock syndrome (DSS).

As discussed earlier, severity is dependent on a combination of factors, including host immunity. For example, it has been noted in Asia the risk of developing severe disease is greater in DENV-infected children (≤ 15 years) than in adults.^{86–89} However, in the Americas the adult population is primarily affected,^{90–92} and it appears there is an increasing trend progressing towards more serious (DHF/DSS) illness which has been observed in adult cases.^{93,94}

DHF is manifested as an incapacitating disease in older children, adolescents, and adults. It is characterized by the rapid onset of fever in combination with severe headache, retro-orbital pain, myalgia, arthralgia, gastrointestinal discomfort, and usually rash. Minor hemorrhagic manifestations may occur in the form of petechiae, epistaxis, and gingival bleeding. Leukopenia is a common finding, whereas thrombocytopenia may occasionally be observed in DF, especially in those with hemorrhagic signs.^{88,95}

Another schema that has been utilized by the World Health Organization (WHO) classified DHF in four grades (I to IV). DHF grades I and II represent relatively mild cases without shock, whereas grade III and IV cases are more severe and accompanied by shock. DHF is characterized by all the symptoms of DF, in combination with hemorrhagic manifestations (positive tourniquet test or spontaneous bleeding), thrombocytopenia, and evidence of increased vascular permeability (increased hemoconcentration or fluid effusion in chest or abdominal cavities).

While most DENV infections are asymptomatic, or minimal symptoms, those associated with symptomatic disease have been historically classified into

- Undifferentiated fever
- Dengue fever
- Dengue hemorrhagic fever
- Dengue shock syndrome

With greater research, the changing manifestations and emerging patterns of disease, epidemiology and viral behavior are being better illustrated, resulting in the updated criteria being presented.

Regardless of schema, DENV typically includes the following symptoms:

- 1 Fever
- 2 Severe frontal and/or retro-ocular headache
- 3 Muscle pain
- 4 Joint pain
- 5 Abdominal pain
- 6 Nausea
- 7 Vomiting

Given many of these symptoms are associated with other travel related infections and illnesses, a thorough history is essential when approaching a patient who may have done business in, been stationed at, or travelled within regions where dengue, and other vector borne illnesses are endemic.

In addition to the above, skin rashes which can be seen; their form may be maculopapular or scarlatiniform rashes that can emerge in approximately 50% of persons infected, and usually are noted to occur three to four days into the infection.^{9,10,47}

Other criteria for Dengue Hemorrhagic Fever (DHF) include:

- Fever
- Hemorrhagic manifestations (not all will attend each patient)
 - Ecchymosis
 - Petechial hemorrhage
 - Epistaxis
 - Gum bleeding
 - Vaginal bleeding
 - Gastrointestinal (GI) bleeding
- Thrombocytopenia
 - < 100,000 platelets per uL blood
- Plasma leakage (increased vascular permeability)

Interestingly, though not a common symptom, nevertheless, hiccups have been noted with VHF. Dengue Shock Syndrome (DSS) includes the four DHF categories above, along with circulatory failure, hypotension, and shock.

Since 2009 the newer dengue case classification is based upon the following categories ():

- Dengue without warning signs
- Dengue with warning signs
- Severe dengue

The revised system also includes organ related symptoms that include:

- Liver failure
- Cardiac involvement
- CNS manifestations

CDC

According to the CDC overview involving persons with symptoms and exposures consistent with DENV infection (dengue), the illness occurs in three phases ().

Phase 1 - Acute DENV Phase:

- Principal symptom is 2–7 days of fever
 - Which may be accompanied by 1 or more
 - headache
 - retro-orbital eye pain
 - joint pain, muscle and/or bone pain
 - rash
 - mild bleeding manifestations (e.g., nose or gum bleed, petechiae, or easy bruising)
 - low white cell count.

Phase 2 - Critical Phase aka Severe Dengue (previously referred to as Dengue Hemorrhagic Fever /DHF or Dengue Shock Syndrome/DSS)

- Defervescence which marks usually a 24 to 48 hour period (in some patients this may be longer)
 - Which compensated or decompensated shock may occur due to increased capillary permeability with plasma leakage that produces
 - ascites

- pleural effusions
- “third spacing” of fluids

Without appropriate treatment, patients with severe dengue are at risk of death. As noted in other schema, warning signs of severe dengue include abdominal pain, vomiting, thrombocytopenia and mild to severe hemorrhagic manifestations, including tendency to bruise easily, petechiae, menorrhagia and mucous membrane bleeding of the nose or gums.

Phase 3 – The Convalescent Phase

- Lasts for 4-7 days.¹

World Health Organization 2009 Case Classification Dengue^{47,48}

The following are criteria for dengue and warning signs (in the setting of risk or endemic DENV)

Probable Dengue

- Fever
- **And 2 of the following**
- Nausea or vomiting
- Rash Aches and pain (headache, myalgia, arthralgia)
- Tourniquet test positive
- Leucopenia
- Any warning sign

Warning Signs

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleeding
- Lethargy, restlessness
- Liver enlargement more than 2 cm
- Increase in packed cell volume concurrent with rapid fall in platelet count

Criteria for severe dengue

Either

- Severe plasma leakage leading to
 - Shock (dengue shock syndrome)
 - Fluid accumulation with respiratory distress

Or

- Severe bleeding (assessed by clinician)

Or

- Severe organ involvement
 - Liver enzyme concentrations, aspartate aminotransferase or alanine aminotransferase $> = 1000$ U/L
 - CNS involvement, impair consciousness
 - Heart or other organ involvement

Vital signs can be very telling in DENV and other VHF. Tachycardia may be a compensatory mechanism for hypovolemia and/or a response to fever. However in some VHF a pulse temperature dissociation, sometimes referred to as relative bradycardia or Faget’s Sign can occur whereby the

Table 4

Dengue hemorrhagic fever (DHF) also known as “break bone fever.” Note the spectrum of illness ranges from asymptomatic to severe clinical symptoms, even death.

-
- Fever lasting 2 – 7 days
 - Significant headache, including retro-orbital pain
 - Severe muscle
 - Severe joint pain (break bone fever)
 - Rash
 - Hemorrhagic manifestations (mild to outright circulatory collapse and organ failure)
 - Thrombocytopenia ($< =100,000$ cells/mm³)
 - Evidence of plasma leakage including hemoconcentration (increase in hematocrit $> =20\%$ above the age average or a decrease in hematocrit $> =20\%$ after fluid replacement)
 - Pleural effusion
 - Ascites
 - Hypoproteinemia
 - Leukopenia
-

heart rate response to fever is diminished. This may also be related to chronotropic inhibition from medications or underlying comorbidities. Nevertheless, heart rate, respiratory rate, temperature, blood pressure, and pulse oximetry are important basics to guide early management (Table 4).

It is important to recognize that once the vascular permeability phase is over (usually 96 hours or less) the resorption phase where the patient symptoms improve, appetite returns, widened blood pressure occur and vitals overall stabilize, there remains the risk of fluid overload; the clinician will adjust replenishment accordingly.

Neurodenv

Neurological involvement from DENV infection was initially considered rare. Dengue in the CNS was detected in the 1990s – most frequently DENV2 and DENV3 serotypes are associated with the neurological events.^{21,47,50} DENV 4 serotype has been noted in neurons, microglia, and endothelial cells, albeit the mechanisms associated with neuro-virulence remain to be fully characterized. Encephalitis was the most commonly reported clinical neuro event associated with direct viral involvement, and usually develops in the acute phase of DENV infection. Also associated with neuro disease in the acute febrile period and related to DENV invasion include meningitis, myositis, and myelitis. DENV invasion of spinal cord produces a local immune response.^{47–50}

Just as the epidemiology has expanded, newer clinical manifestations are being recognized. More recently increasing numbers of neurological signs and symptoms are being reported. It is important to avoid underestimating the neurological involvement. These include encephalitis,^{47,50,96,97} encephalopathy, meningitis, Guillian-Barre’ Syndrome (GBS),^{50,98} myelitis,^{50,98} acute disseminated encephalomyelitis (ADEM), polyneuropathy, mononeuropathy, and cerebromeningeal hemorrhage. Hypokalemic paralysis may occur.

Acute encephalopathy is a commonly noted neurological manifestation associated with DENV. As with encephalopathy from other etiologies, this involves an altered level of consciousness. Causes include prolonged shock, anoxia, cerebral edema, metabolic derangements such as hyponatremia, hemorrhages, acute liver failure, renal failure. Basic CSF analysis is often unremarkable.⁵⁰

Although the exact epidemiology of DENV neuro manifestations remains elusive, studies suggest the incidence ranges between 0.5% and 6.2%.^{47,50,97}

Among neuroDENV cases, encephalitis is also a commonly occurring neurological clinical manifestation associated with DENV infection, with a frequency between 4.2% to 51% depending upon the sources,^{50,97} patient population, and serotype during the outbreaks.

CSF analysis may suggest inflammatory reaction. Lymphomononuclear pleocytosis and normal glucose levels have been reported – normal CSF has been seen in more than 50% of DENV encephalitis. As such. Normal CSF cellularity may not rule out dengue encephalitis. The presence of lymphocytic pleocytosis may support the diagnosis of Dengue neuro, but again CSF results may not be fully predictive.⁴⁷

Although initial diagnosis is presumptive, based upon clinical findings, neuroimaging can provide important clues as to neurological complications. Electroencephalogram (EEG) abnormalities have been seen in Dengue neuro.⁴⁷

During the first days of infection, DENV virus is in the blood; detection of NS1 antigen or RNA by RT PCR are options. IgM – ELISA are widely available tests. Recognize antibodies against other Flaviviruses such as Japanese encephalitis, WNV, or yellow fever are possible, and may lead to false positives.⁴⁷

Clinical criteria of DENV encephalitis⁴⁷:

1. Fever
2. Acute cerebral involvement
 - a. Altered consciousness
 - b. Altered personality
3. Anti-dengue immunoglobulin M antibodies or DENV genomic material in CSF
4. Exclusion of other etiologies of viral encephalitis/encephalopathy

Symptoms of DENV neurological involvement include:

1. Seizures
2. Altered consciousness
3. Headaches

In a recent study of acute disseminated encephalomyelitis (ADEM), the prevalence among dengue patients was 0.4%, with the most frequently noted manifestation included altered sensorium/consciousness, seizures, and urination problems. Problems with vision, slurring of speech, gait abnormalities, including ataxia, were also reported. One of the predictors of outcome was high temperature, and earlier onset of neurological illness.

Of note, when neurological DENV presents itself, the usual symptoms associated with Dengue – myalgias, diarrhea, joint pain, abdominal pain, rash and bleeding occur concomitantly in ~50% of encephalitis cases. An index of suspicion in terms of DENV when neurological signs and symptoms present, especially in regions where dengue is endemic, in order to reduce misdiagnosis or delayed diagnosis.

Radiographic studies for neuro-dengue such as computed tomography (CT) and magnetic resonance imaging (MRI) are highly variable. Hemorrhages, cerebral edema, and abnormalities of the globus pallidus, hippocampus, thalamus and internal capsule may be found – such lesions may appear as hyper intensities on MRI.^{47,50}

Dengue associated myelitis has been reported in 9.5–15% of cases, and is noted to appear between 7 and 30 days post onset of infection. Paraparesis and sphincter dysfunction have been noted.^{47–50}

Guillain Barre' Syndrome (GBS) accounted for about one third of the neuro-associated illness from DENV. Symptoms are consistent with GBS from other infections – ascending paraparesis being the primary manifestation.⁴⁷ The prognosis after treatment is usually positive.

Given the general lack of antiviral interventions for DENV, the treatment of DENV associated neuro illness depends upon the diagnosis; the diagnosis and prognosis of ENV GBS is similar to that reported from other infectious processes.

Although DENV remains primarily a hemorrhagic fever infection often with associated rash, central (CNS) and peripheral nervous system (PNS) illness can be associated with dengue. Given the infection is associated with cerebral anoxia, shock, edema which is caused by liver failure, thrombocytopenia leading to hemorrhages and electrolyte dysfunction which can determine dengue encephalopathy/ encephalitis. Dengue associated coagulopathy and vasculopathy could also lead to ischemic stroke.

DENV neurological illness has been categorized as DENV direct invasion, para infectious disease and post infectious disease. The post-infection period and immune-allergic response may set the stage for acute disseminated encephalomyelitis, neuromyelitis optica, optic neuritis, myelitis or encephalopathy.

Such neuropathology may be related to direct nervous system viral involvement, an autoimmune reaction, as well as metabolic and hemorrhagic derangements. Intrathecal synthesis of dengue antibodies have been detected in cerebrospinal fluid. The actual mechanism of dengue infection related neurological disturbances may be related to the specific type of neurologic disease. Also viral and host factors play a part in the pathogenesis.

Treatment strategies vary, depending upon the etiology and manifestation of DENV associated neuro – from treating underlying metabolic derangements, circulation/bleeding, hydration, nutrition, and immune mediated influences. In the latter some suggest intravenous methylprednisolone for several days. However in the absence of controlled trials, this strategy must be guided by risk, benefit.^{47,99} For GBS associated with DENV, as with other etiologies of post infectious GBS, high dose immunoglobulin has been suggested.⁴⁷

Other Clinical Considerations

Clearly DENV is a complex infectious disease capable of producing a large variety of clinical presentations in addition to hemorrhagic fever.

Recognize the range of “look alike” etiologies - early stage rashes can pose a clinical challenge. Consider the study conducted in Brazil involving children with exanthema (with or without fever) who presented to the emergency department of a hospital in a Dengue endemic region. Among study participants, a protocol exam, thorough history as well as collection of blood samples subjected to a wide variety of serology and other studies.

The results were not surprising¹⁰⁰; Dengue Virus (DV) was detected in nearly 78% of the children. Herpes virus type 6 (8.4%), and parvovirus (2.8%) were the second and third most commonly identified viruses in the study respectively. No positive serology was noted for measles, rubella or toxoplasmosis. Among children infected with DV, the most common clinical manifestations included fever, itching, prostration, and myalgia. The tourniquet test was positive in nearly 60% of the confirmed DV cases. Other signs and symptoms include nausea, vomiting, and flushed skin – the latter occurring during the first 24–48 h.

Moreover, the astute clinician will recognize that co-infections with infectious agents transmitted from similar vectors within endemic regions is a clinical possibility, as was discussed in the Zika and Chikungunya sections of this edition.

Taking into consideration underlying comorbidities, vaccine status and medications (herbal, over the counter, and prescribed) patients presenting with symptoms consistent with DENV and/or VHF is critical to take into consideration their impact on the overall infection process and response to treatment.

With the expanding range of dengue infections, and a significant proportion of the world at risk, including the Americas, there is the potential for more severe DENV manifestations with patients who are taking anticoagulation therapy, especially in those with underlying comorbid conditions.^{26,100}

Special care and attention to hemostasis, volume replenishment, potential for shock, and multisystem organ stability are important especially with patients who are taking warfarin or other medications that can impair coagulation. A recent case documenting bilateral rectus sheath hematomas have been observed in DENV hemorrhagic infection associated with warfarin; though rare, it is worth the additional attention to such patients.

Diagnostic Testing

Clinical Considerations^{1–9,12,16,21,47,49,52,78,88–90,92,96,100–104}

An index of suspicion and careful attention to travel history, occupation, and status of comorbidities, along with timeline of illness, vector bites, and biodrome associated with the patient are critical to making a presumptive diagnosis, and importantly providing the appropriate level of acuity in treatment, especially given Dengue potential to cause severe pain, hemorrhage, even death. Moreover the evolution of illness has multiple grades of severity, the most significant associated with shock, and organ failure. While it is true that most cases are low acuity and resolve without sequelae, the prudent clinician will be alert for rapid changes in clinical status.

Initial testing, depending upon presentation and underlying host factors can include complete blood counts (CBC), arterial blood gas (ABG), coagulation panel, liver and kidney function tests, along with metabolic and electrolyte panels can help guide the clinician.

Certain biomarkers may be useful in predicting severity of illness.^{54,55} Total plasma cholesterol, high density lipoprotein and low density lipoprotein were significantly decreased in children presenting with severe disease compared with the levels of those having mild dengue infection. Liver injury from severe dengue is associated with elevated AST, ALT, gamma glutamyl transpeptidase (GGTP), ALP, and serum albumin. High levels of AST and ALT were associated with liver injury DENV. A decrease in lymphocytes and platelets have also been noted.^{9,27,47,50,101}

Lactate and lactate dehydrogenase (LDH) are associated with shock – whether from cardiopulmonary failure, sepsis, shock, and hepatic injury, including that from dengue shock syndrome (DSS).⁵⁴ According to one study, the majority of dengue patients had impaired liver function, with elevated AST and ALT, in contrast with non DENV patients with minimal elevation. Serum lactate was not found to be elevated in the early stages of DENV. But increased in DSS patients nearing the end of the febrile phase. The mean LDH levels in dengue patients was greater than 500 IU, and less than 500 IU non dengue. Of note the increasing levels of LDH towards the end of the febrile phase were seen in DHF, and DSS, not dengue fever and non dengue patients.

Most patients with DENV and dengue shock syndrome, as well as some non shock patients exhibit DIC (thrombocytopenia, prolonged partial thromboplastin time, decreased fibrinogen levels, and increased levels of fibrinogen degradation products.

The differential diagnosis is extensive in terms of symptoms consistent with dengue, especially in the early stages. Among infectious etiologies, these include scrub typhus, murine typhus or other typhus infection, Japanese encephalitis, other Flavivirus and typhoid.

DENV Specific Testing¹⁰¹

In regions with ample public health resources, DENV can be readily diagnosed using laboratory testing; this is often based upon ELISA serology for the detection of specific IgM and IgG antibodies, or the NS1 antigen during the acute phase, a fourfold rise in antibody titre in paired era samples.

There are rapid DENV tests available, but their sensitivity and specificity may be variable.

Testing

The potential survival of persons who might be infected with DENV, and the treatment of dengue initially rests upon early suspicion of illness. The challenge with some presentations of DENV, especially DF can be difficult, as early signs and symptoms can be confused with those of other diseases, including malaria, leptospirosis and typhoid fever.

According to the CDC Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods. Diagnosis of acute (on-going) or recent dengue infection can be established by testing serum samples during the first 5 days of symptoms and/or early convalescent phase (more than 5 days of symptoms). Acute infection with dengue virus is confirmed when the virus is isolated from serum or autopsy tissue specimens, or the specific dengue virus genome is identified by reverse transcription-polymerase chain reaction (RT-PCR) from serum or plasma, cerebrospinal fluid, or autopsy tissue specimens during an acute febrile illness. Methods such as one-step, real time RT-PCR or nested RT-PCR are now widely used to detect dengue viral genes in acute-phase serum samples. This detection coincides with the viremia and the febrile phase of illness onset. Acute infections can also be laboratory confirmed by identification of dengue viral antigen or RNA in autopsy tissue specimens by immunofluorescence or immunohistochemical analysis, or by seroconversion from negative to positive IgM antibody to dengue or demonstration of a fourfold or greater increase in IgG antibody titers in paired (acute and convalescent) serum specimens.

IgM antibodies for dengue may remain elevated for 2 to 3 months after the illness. The elevated IgM observed in a sample could be the result of an infection that occurred 2 to 3 months ago. In addition, there is cross reactivity with other flaviviruses including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV) and yellow fever virus (YFV). The provider should review the patient's past medical history, recent travel history, and vaccination record

(especially yellow fever vaccination) to determine the likelihood that the current acute febrile illness is due to an infection with dengue virus.

Paired samples may be required to confirm illness; both acute and convalescent phase specimens may be needed to make a diagnosis of dengue infection. This is especially true for those who submit a day 5 acute specimen because the virus and IgM antibodies may at that time be at undetectable levels.

Definitive diagnosis relies on specific virological diagnosis – detection of viremia or IgM.⁹ Precautions should be observed in collecting, handling, transporting and processing samples from suspected VHFF patients. A patient presumptively considered to have DF, DHF or DSS can be confirmed using a serum specimen^{2,4,5,7–10,21,38}:

1. Detection of Dengue virus genomic sequence
2. Detection of Dengue virus antigens (nonstructural protein 1; NS1 antigen)
3. Serologic testing for IgM anti-Dengue virus

Of note, detection of Dengue virus genomes or NS1 antigen is utilized in the acute febrile stage of illness (<=5 days after the onset of symptoms). Testing for IgM anti-Dengue virus is most primarily > 5 days after the onset of fever.

Dengue can be isolated in cell culture derived from serum, cerebrospinal fluid (CSF) or tissue specimens. However Dengue virus genomes can be readily identified by reverse transcriptase polymerase chain reaction (RT – PCR) from CSF, plasma, serum or autopsy tissue specimens. There are immunoassays available to detect NS1 antigen which is found in blood during the viremia stage. Dengue antigens can be detected in tissue by immunofluorescence, immunohistochemical analysis. In patients with acute illness, serological testing for Dengue is IgM anti-Dengue virus; this should become positive usually after 5 days from the onset of symptom/s.

Laboratory confirmation also includes seroconversion from negative to positive IgM anti-Dengue virus from specimens obtained from acute phase (< 5 days after fever begins) to convalescent phase (> 5 days post symptom/s onset). Also a $\geq 4x$ rise in reciprocal IgG anti-Dengue virus titer or hemagglutination inhibition titer to Dengue virus antigens in serum obtained from acute and convalescent phase, or IgM anti-Dengue virus detected in CSF.

IgM anti-Dengue virus in a solitary serum sample is suggestive of probably recent Dengue infection whereas IgG anti-Dengue virus can indicate in a single sample either a recent or past Dengue infection. It is important to recognize Dengue virus antibodies can cross react with antibodies from other Flaviviruses including West Nile, yellow fever and Japanese encephalitis viruses, especially when using only antibody testing (IgM or IgG anti-Dengue) from a single sample. Also prior vaccination or infection with another Flavivirus may also result in false positive IgG or IgM anti-Dengue.

According to the most recent updates from the CDC guidance on laboratory detection of Dengue (<https://www.cdc.gov/Dengue/clinicalLab/laboratory.html>)

Testing Algorithms for Dengue (from CDC Dengue):

A. PCR

DENV can be detected in the blood (serum) from patients for approximately the first 5 days of symptoms. Currently, several PCR tests are employed to detect the viral genome in serum. In addition, virus can be isolated and sequenced for additional characterization. Real time RT–PCR assays have been developed and automated; but none of these tests are yet commercially available. Because antibodies are detected later, RT–PCR has become a primary tool to detect virus early in the course of illness. Current tests are between 80–90% sensitive, and more that 95% specific. A positive PCR result is a definite proof of current infection and it usually confirms the infecting serotype as well. However, a negative result is interpreted as “indeterminate”. Patients receiving negative results before 5 days of illness are usually asked to submit a second serum sample for serological confirmation after the 5th day of illness (bellow).

B. MAC ELISA

IgM antibody capture ELISA (MAC-ELISA) format is most commonly employed in diagnostic laboratories and commercial available diagnostic kits. The assay is based on capturing human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of

dengue virus specific antigen (DENV1–4). The antigens used for this assay are derived from the envelope protein of the virus. One of the limitation of this testing is the cross reactivity between other circulating flaviviruses. This limitation must be considered when working in regions where multiple flaviviruses co-circulate. IgM detection is not useful for dengue serotype determination due to cross-reactivity of the antibody.

C. IgG ELISA

The IgG ELISA

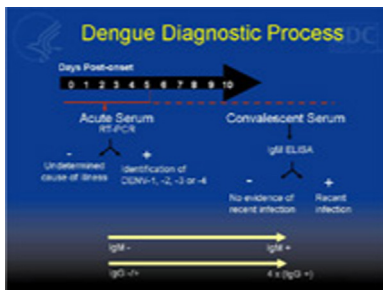
A. used for the detection of a past dengue infection utilizes the same viral antigens as the MAC ELISA. This assay correlates with the hemagglutination assay (HI) previously used. In general IgG ELISA lacks specificity within the flavivirus serocomplex groups. Primary versus secondary dengue infection can be determined using a simple algorithm. Samples with a negative IgG in the acute phase and a positive IgG in the convalescent phase of the infection are primary dengue infections. Samples with a positive IgG in the acute phase and a 4 fold rise in IgG titer in the convalescent phase (with at least a 7 day interval between the two samples) is a secondary dengue infection.

B. NS1 ELISA

The non-structural protein 1 (NS1) of the dengue viral genome has been shown to be useful as a tool for the diagnosis of acute dengue infections. Dengue NS1 antigen has been detected in the serum of DENV infected patients as early as 1 day post onset of symptoms (DPO), and up to 18 DPO. The NS1 ELISA based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay.

C. PRNT

Plaque Reduction and Neutralization Test (PRNT) and the microneutralization PRNT can be used when a serological specific diagnostic is required, as this assay is the most specific serological tool for the determination of dengue antibodies The PRNT test is used to determine the infecting serotype in convalescent sera. This assay measures the titer of the neutralizing antibodies in the serum of the infected individual and determines the level of protective antibodies this individual has towards the infecting virus. The assay is a biological assay based on the principle of interaction of virus and antibody resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture. Some of the variability of this assay is differences in interpretation of the results because of the cell lines and virus seeds used as well as the dilution of the sera.



(<https://www.cdc.gov/dengue/clinlab/diagnosticprocess.html>)

The CDC has a Dengue Branch for further clinical assistance. The General CDC phone number is 800-CDC-INFO (800-232-4636)

Tourniquet Test

The Tourniquet Test or Rumpel-Leede Capillary-Fragility Test, which is sometimes call just the capillary fragility test, is an easily administered bedside test to determine capillary fragility, which may give a clue for the patient risk of hemorrhage. In revealing the fragility of capillary walls, it may be used to suggest thrombocytopenia. This technique has been widely utilized, especially in the developing world, with WHO including this test in earlier diagnostic criteria. In certain studies it has had variable degrees of sensitivity and specificity.

Procedure

A blood pressure (BP) cuff is applied on the contralateral side to the arm with venipuncture sites. The (BP) cuff is inflated to the mean of systolic and diastolic pressures for a timed 5 minutes. At 5 min the cuff is removed, and the total number of petechiae visible in a 2.5 cm² region. The tourniquet test was considered positive when 20 or more petechiae were observed in a 2.5 cm² square.

Alternatively, the CDC guidance for the Tourniquet test is as follows:

How to do a Tourniquet Test

1. Take the patient's blood pressure and record it, for example, 100/70.
2. Inflate the cuff to a point midway between SBP and DBP and maintain for 5 minutes. $(100 + 70) \div 2 = 85$ mm Hg
3. Reduce and wait 2 minutes.
4. Count petechiae below antecubital fossa. (Image 1):

• A positive test is 10 or more petechiae per 1 square inch

The CDC notes that the tourniquet test was part of the WHO case definition for dengue. The CDC guidance for the use of the test as a marker of capillary fragility and it can be used as a triage tool to differentiate patients with acute gastroenteritis, for example, from those with dengue. Even if a tourniquet test was previously done, it should be repeated if:

- It was previously negative
- There is no bleeding manifestation

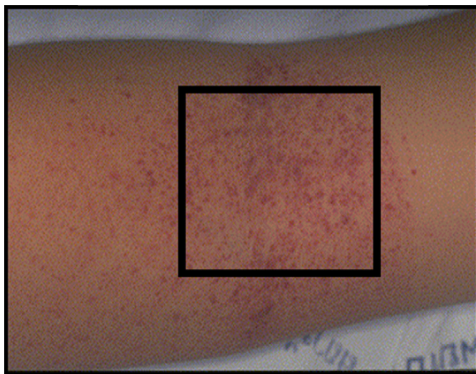


Image 1. Results of tourniquet test¹⁰². <https://www.cdc.gov/dengue/training/cme/ccm/page73112.html>. Note the 1 square inch box and number of petechiae present, which is a positive test.

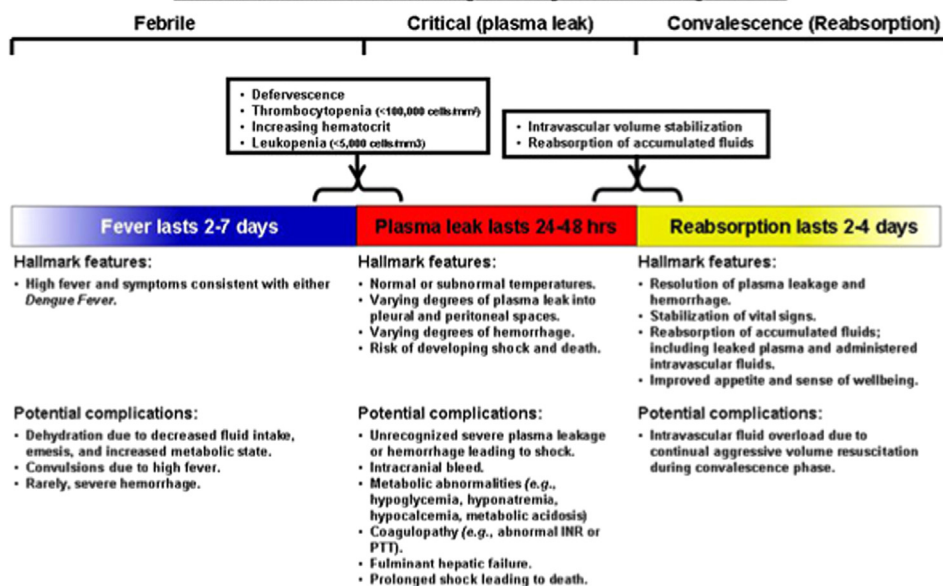
The results of various studies evaluating this test have been variable depending upon location, severity, and observer. Fragile skin, aging, use of platelet inhibitors may influence the test results. A negative tourniquet test does not rule out DENV. A positive test may underscore the risk of bleeding.

Treatment

Currently there are no approved antiviral medications. Many antimicrobials and other medical therapeutics are currently under investigation, including nutraceuticals, but as of this manuscript none are FDA approved. Pain management and fluid replenishment, along with aggressive and intensive medical care – usually at an advanced health care facility, are required for all but the most minor cases. Treatment by professionals experienced in DENV infections can significantly reduce the likelihood of death.

Each phase of illness is associated with clinical manifestations, and specific treatment considerations. The CDC provides a useful diagnostic and treatment algorithm for the clinician (Algorithm 1): <https://www.cdc.gov/dengue/clinicallab/clinical.html>

Phases of infections resulting in Dengue Hemorrhagic Fever



DENV is characterized by vascular permeability, thrombocytopenia, and coagulation disorder, associated with capillary fragility.^{55,57} Easy bruising and positive tourniquet testing are relatively easy identifiers of bleeding issues. Therefore fluid management and intensive supportive care are mainstays of treatment.

In the vascular permeability stage most of the complications from DENV arise during this period. These include hemorrhage and metabolic abnormalities (hypocalcemia, hypoglycemia, hyperglycemia, lactic acidosis, hyponatremia) occur. Such metabolic derangements are usually related to prolonged shock. Treatment during this period is aimed at preventing prolonged shock, and the organ failure/death that can result, as well as supporting multiple systems until the plasma leak phase subsides.

Not surprisingly, attention must be paid to the type of intravenous fluid used to support the patient, which may include blood products should the clinical situation deteriorate requiring transfusion. Type of fluid administered, flow rate, and overall volume must be carefully managed.

This intensive monitoring of intravascular volume, various organs' function, and timely clinical response are critical for this phase of DENV illness.

The choice in resuscitation - blood, blood products (fresh frozen plasma, concentrated platelets) or various forms of crystalloid fluid (lactated Ringers, saline) depends upon clinical situation. Internal bleeding may be a challenge to identify in the presence of hemoconcentration.

Transfusion of volume-replacing blood products should be considered, especially with substantial hemorrhage, significant decline in hematocrit and clinical status.

It is important to be alert for both overt and occult hemorrhage, being vigilant for another source of volume/intravascular depletion.

Rapid resuscitation of DSS, the correction of metabolic and electrolytic disturbances can prevent DIC and reverse DSS.

An acute change from fever to hypothermia, severe abdominal pain, vomiting, bleeding, shortness of breath and/or difficulty breathing, or altered mental status are potential medical emergencies. These patients belong in the hospital, as do patients with rapidly progressive fever. Addressing metabolic derangements such as acidosis can reduce the risk of DIC. Recognizing multisystem organ involvement is possible, especially kidney damage, allows timely implementation of interventions by anticipating potentially rapid clinical deterioration.

Pain control is important. However owing to the nature of DENV infections, avoid certain pain medications such as aspirin containing products and nonsteroidal anti-inflammatory medications (NSAIDs), as they can increase the risk of bleeding. Acetaminophen is a useful treatment for fevers.

Narcotics may be necessary for severe joint or muscle pain.

Given DENV can cause dysfunction of other systems, both due to hypovolemia and direct viral influence, such as the neurological system, careful attention to deteriorating function, mental status and other organ efficiency, with rapid treatment. Collaboration with specialty expertise will likely be necessary. Corticosteroids or immune globulins may be required under certain neuroDENV circumstances, but risk/benefit must be taken into consideration.

The clinician should also be alert to co-infection with malaria, or Zika, as well as other potentially treatable vector borne illnesses given the ecology of dengue infection, and the ability of Dengue carrying mosquitoes to transmit other pathogens of human concern.

Hospitalization should be considered for patients with:

- Tachycardia
- Increased capillary refill time greater than 2 seconds
- Mottling, cooling and/or pallor to the skin
- Diminished peripheral pulses compared with baseline
- Change in mental status
- Oliguria
- Sudden elevation in hematocrit despite fluid resuscitation
- Narrowing pulse pressure (< 20 mm Hg)
- Hypotension
- Other evidence of shock

VACCINES

As discussed earlier, the potential for ADE has made the development of vaccines against DENV particularly difficult.

Dengvaxia[®] (CYD-TDV), is among the few candidate vaccines that has advanced into utilization.^{103,104} It was developed by Sanofi Pasteur. Dengvaxia is a chimeric yellow fever–DENV tetravalent dengue vaccine, and is licensed in several countries. The vaccine contains sequences encoding the precursor membrane protein and envelope proteins that make up the glycoprotein shell of the DENV are combined with sequences encoding the non-structural proteins of the attenuated vaccine against the 17D strain of yellow fever virus.⁷⁷ Dengvaxia has demonstrated some protection from DENV infection. Moreover Dengvaxia seems to give protection to people who have been previously infected with DENV, but its efficacy is lower when given to DENV-naive

vaccinees.^{77,103} Estimates suggest this vaccine has the potential to reduce the burden of disease by 10–30% over a 30-year period if it is utilized in DENV-endemic countries.^{103,104}

Dengvaxia seems to give protection to people who have been previously infected with DENV, but its efficacy is lower when given to DENV-naïve vaccinees.^{77,103,104}

Subgroup analysis of Dengvaxia trials raised safety concerns.⁷⁷ In patients under 9 years of age, the hospitalization rates from a DENV infection were greater for vaccinated children than for non-vaccinated children in the control group. ADE phenomenon may be involved. Consider in children who at study entry were DENV naïve had been primed but not protected by the vaccine. Based upon this analysis, the vaccine is not licensed for use in children under 9 years of age. Moreover, Dengvaxia is recommended only in populations where a seroprevalence of 70% or greater of prior DENV exposure exists in the group to be vaccinated.^{103,104}

Dengvaxia – also known as CYD-TDV was the first dengue vaccine to be licensed, although there have been multiple attempts to develop effective vaccines to date. CYD-TDV was first licensed in Mexico in December 2015 for use in individuals 9–45 years of age living in endemic areas. CYD-TDV is a live recombinant tetravalent dengue vaccine. It must be given as a 3-dose series on a 0/6/12 month schedule.

According to WHO, there are approximately five additional dengue vaccine candidates under development, including tetravalent live-attenuated vaccines in phase III clinical trials. Other vaccine candidates (based on subunit, DNA and purified inactivated virus platforms) are at earlier stages of clinical development.

The effectiveness of CYD-TDV has been evaluated in two Phase 3 clinical trials (CYD14 in five countries in Asia and CYD15 in five countries in Latin America). Together, these trials included over 35,000 participants aged 2 to 16 years: ages at first vaccination were 2 to 14 years in CYD14, 9 to 16 years in CYD15. In each of these trials, participants were randomized to vaccine and placebo in a 2:1 ratio. The study protocols included an active phase of follow-up for one year after the last dose of vaccine in the series (25 months from dose 1) and include a hospital-based follow-up period of four additional years, which is ongoing.

Results have been published for each trial separately, as well as pooled. Trial results include children aged < 9 years old, which is an age group that is not included in the current indication. This is due to results that were observed during the Phase 3 trials in the youngest age group in the CYD14 Phase 3 Trial.

Vaccine efficacy against confirmed dengue pooled across both trials was 59.2% in the year following the primary series (per protocol analysis). During this initial time period, pooled vaccine efficacy against severe dengue was 79.1%. Efficacy varied by serotype: vaccine efficacy was higher against serotypes 3 and 4 (71.6% and 76.9%, respectively) than for serotypes 1 and 2 (54.7% and 43.0%). Vaccine efficacy also varied by age at vaccination and serostatus at baseline (i.e., previous exposure to dengue prior to vaccination).

When limited to older age groups (ages included in the current licensure), pooled vaccine efficacy amongst all participants aged 9 years or over was 65.6%, and in participants aged < 9 years it was 44%.

Within the randomized subset of participants for whom pre-vaccination blood samples were collected, pooled vaccine efficacy against VCD in those seropositive for a prior exposure to dengue virus was 78.2%, while in those seronegative at baseline it was 38.1% (not statistically significant). In a post-hoc analysis in those ≥9 years of age, vaccine efficacy in those seronegative at baseline was 52.5% (95% CI 5.9%, 76.1%).

While efficacy was reported against hospitalized and severe dengue in Years 1 and 2 post-dose 1, an excess of cases of hospitalized and severe dengue cases in those receiving CYD-TDV was seen in Year 3 in some subgroups, although it is based on relatively small numbers of cases. The excess was mostly observed in those vaccinated aged 2–5 years in CYD14 in Asia, for which the relative risk of hospitalized dengue in vaccinees was 7.45 (95% CI 1.15, 313.80) in Year 3, based on 15 cases in the CYD-TDV group and 1 case in the control group. This younger age group has not been included in the age indication of the vaccine. No safety signals were reported in the older age groups.

Other vaccine strategies

Virus vectored vaccines

There are vaccines using replication deficient adenovirus vaccine vector and single cycle Venezuelan equine encephalitis virus vaccine vector. West Nile Virus particles vaccine vector and measles virus vaccine vector are also under study to integrate various Dengue component proteins. To date these remain under study.

Adenovirus vaccine vectors have large insert capacity, efficient deliver, and high expression of antigens in a broad array of cells, and a documented human record of safety in humans.

Purified inactivated virus vaccines, live attenuated viruses, Recombinant subunit vaccines and DNA viruses, VLP vaccines as well live attenuated virus (LAV), virus-like particles (VLPs) and plasmid vectors are being researched. Although beyond the scope of this paper, each approach has enjoys advantages and faces barriers to success. For example, non-living vaccines can offer the advantage of better suitability for immunocompromised individuals – a not insignificant subpopulation considering HIV, cancer patients undergoing chemo and rheumatology as well as transplant patients on immunosuppressive therapy.⁶⁶

WHO recommends that countries should consider introduction of the dengue vaccine CYD-TDV only in geographic settings (national or subnational) where epidemiological data indicate a high burden of disease. Complete recommendations may be found in the WHO position paper on dengue vaccines.¹⁰³

Other preventive strategies

WHO recommends the following preventive strategies¹⁰⁵:

Vector control has been the key strategy to control or prevent the transmission of dengue virus. Strategies include:

- preventing mosquitoes from accessing egg-laying habitats by environmental management and modification;
- disposing of solid waste properly and removing artificial man-made habitats;
- covering, emptying and cleaning of domestic water storage containers on a weekly basis;
- applying appropriate insecticides to water storage outdoor containers;
- using of personal household protection such as window screens, long-sleeved clothes, insecticide treated materials, coils and vaporizers;
- improving community participation and mobilization for sustained vector control;
- applying insecticides as space spraying during outbreaks as one of the emergency vector-control measures
- active monitoring and surveillance of vectors should be carried out to determine effectiveness of control interventions.

Conclusion

Dengue is an important, emerging vector borne illness that is likely to cause increasing numbers of cases in the United States, and other Western countries owing to the expanding range of vectors – mosquitoes, and dramatic increase in human migration – whether from travel, work, military, refugee status or immigration.^{15,108–112}

This is underscored by the reality that regions typically unaffected by DENV are now hosting cases of infection. Imported cases – from Portugal to other European nations occurred in 2012. Of note, there is an upward trend in Dengue incidence among hospitalized patients in the US. One of the factors is the substantial number of travelers who enter the US from tropical and subtropical regions.

Dengue is ubiquitous in the tropics.^{12–16} Asia represents 70% of the global burden, within which India alone represents approximately 50% of the cases. The Americas represent 14% of the global burden, with approximately 50% of those cases occurring in Brazil and Mexico.^{108–112} The study published in Nature suggests there are more cases in Africa, than prior estimates suggested, representing 16% of worldwide Dengue. Rain, high temperatures and being located close to urban

centers increase the risk of infection, not a coincidence that ecology plays a key role in mosquito population. Not surprisingly the researchers note that climate and population migration are key factors in predicting the penetration of Dengue globally. As if 100 million cases don't suggest enough of a global threat, nearly 400 million underscores the immediate need to develop vaccines, and antivirals, as well as improved attempts to reduce the mosquito population globally.

As of June 2013 the Lao People's Democratic Republic recorded over 10,000 cases, which is seven times more than the number for cases during the same timeframe in 2012. Among those who died, to date most were children. Not surprisingly, the rainy season is associated with increased cases, given the attraction for mosquitoes. Vector control is critical to reduce the risk of transmission – internationally and in the United States.

Within the last few years Europe experienced its first sustained outbreak of Dengue since the 1920s, with approximately 2000 people infected in Madeira, Portugal. In the United States there have been several outbreaks; Hawaii in 2001, South Florida in 2009 and 2010. During 2009 and 2010, dengue fever emerged for the first time in decades in the contiguous United States, when an outbreak in the Florida Keys led to 93 cases.

World Health Organization^{103–105} estimates approximately 200 million cases occur worldwide, of which 200,000 to 500,000 are severe enough to be classified as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) which has a mortality rate 1 – 5 %, mostly among those under 15 yrs of age, depending upon medical care and host characteristics. Moreover, an estimated 500,000 hospitalizations and more than 20,000 deaths are annually associated with dengue. These numbers are likely significant underestimates. A study published in *Nature* estimates 390 million cases, 96 million of which have clinical symptomatology. Of concern – the number of asymptomatic patients is unknown. The significance of which – these individuals can allow mosquitoes to transmit the pathogen, which may result in a clinically significant infection, as an uninfected mosquito becomes infected by biting someone infected with dengue (or other mosquito vector disease pathogen). Clearly there is a potentially large reservoir of virus with people acting as multipliers of the virus, which is mostly found in tropical and subtropical climates, and in both urban as well as semi-urban areas. Not surprisingly the worldwide distribution is similar to that of malaria.

While there are a range of estimates in terms of the populations at risk, and magnitude of illness, the true burden remains unknown.⁶¹

Worrisome is the fact that the mosquito vector has spread to at least 26 states making the risk for dengue and dengue hemorrhagic fever a potential public health threat for the US. With climate change, and more regions becoming habitable for *Aedes* and other mosquitoes, expect Dengue and other mosquito borne illnesses to spread.^{18–20,28,37–40,108–112} The natural reservoirs are humans. Not surprising, the distribution is similar to that of malaria worldwide. Severe dengue is a leading cause of illness and death in children across certain Asian and South American countries. Access to proper healthcare can decrease the fatality rate to below 1%; it is much higher untreated. Outbreaks are not uncommon, and pose a challenge given the mainstay of treatment is symptomatic and supportive care – labor and resource intensive interventions, given there is no antiviral specific for this virus.

Given that the *Aedes* mosquito resides nearly year round in several Southeastern states, plus travelers returning from Dengue endemic regions, and a susceptible population, the conditions exist for more outbreaks in the United States and North America. Of concern from a disease containment perspective – Dengue was not a reportable infection until 2009 in the US. According to a study in Geo-Sentinel among surveillance network clinics, Dengue was the top cause of febrile, systemic illness afflicting those returning from the Caribbean, South America, South Central and Southeast Asia. While Malaria is the most common cause of hospitalization as a travel related illness among persons who return to the US from the tropics, in some studies, Dengue was the second most common among febrile travelers who were in Dengue endemic areas, based upon serological testing.

Not surprisingly mosquitoes that spread dengue fever tap into the domestic networks of humans, Rapid transmission of human infections can also occur from house-to-house, as people visit nearby friends and relatives, making it critically important to encourage consistent mosquito control across neighborhoods as well as regions.³⁵

Of course Dengue is not the only major public health infection spread by mosquitoes.^{5,106} Consider West Nile Virus in the United States, and Malaria worldwide.

Interestingly it is not just people but the globalized trade in materials; bamboo plants have been shown to carry the mosquito.

As with any vector control approach, removing standing water, as well as some well known sites that harbor mosquitoes, such as used tires, water storage tanks, even flowerpot trays and birdbaths.^{113,114} For the latter, garden supply stores sell a variety of agitators – solar or battery powered, that can keep birdbaths and backyard ponds from becoming standing water sources and mosquito breeding sites.

In addition to the most common cause of transmission, there are limited data suggesting needle stick, mucous membrane contact with blood and maternal – fetal transmission are also possible modes. Peripartum infections may increase the chance of symptomatic disease afflicting the newborn; although the data are limited in terms of the actual number of cases transmitted from mother to newborn. Of concern, the secondary infection effect may place infants at greater risk for Dengue if they are infected again by a different DENV in the baby's 6–12 month. Other than the above, human to human transmission has not been documented.

As discussed in the September 2017 *Disease-A-Month* edition on emerging pathogens, travel related illnesses are often initially misdiagnosed.¹⁰⁷ Travel, whether occupational or vacation related, is often overlooked in the history portion of clinical encounters, yet are frequently associated with illness.^{36,37,112} Not surprisingly malaria is the most common cause of hospitalization as a travel associated illness among persons returning to the US from the tropics.¹⁰⁷ Dengue was the second most common, based upon serology. This underscores the importance of a travel history for patients, especially those presenting with febrile illnesses.^{39,115}

A viral hemorrhagic fever should be considered in any person who presents with a severe febrile illness and clinical evidence of vascular involvement - hypotension, petechiae, easy bleeding, facial/chest flushing, nondependent edema, and who has traveled to a region where VHF are known to occur.

Travelers should be cautioned if planning to work/visit in mosquito environments,¹⁰⁸ make certain there are intact screens for windows and doors, air conditioning instead of open windows, and repellents such as DEET. Although often out of the purview of tourists, the use of insecticides to decrease the mosquito population can be effective preventive measure. The key is to avoid being in environments heavily populated by mosquitoes.

Dengue, like so many emerging pathogens, is no longer relegated to distance locations. In addition to travelers, and immigrants who can present with DENV, the potential for transmission within the United States exists.^{39,115}

The astute clinician will therefore be aware of changing patterns of emerging threats to patients at home, as well as referable to travel into regions with potentially dangerous endemic illnesses.¹¹⁵ Patients should be encouraged to share concerns and ask questions with regard to potential threats to health. Referral to a travel medicine clinic, the CDC traveler guide,^{4,36} as well as making certain routine vaccines are up to date, along with appropriate health related recommendations for the target regions (vaccines, safety, food hygiene), including the potential need for travel insurance, copies of prescriptions, and “medic alert” bracelets. Patients should be counseled to seek medical attention if developing a rash, fever or other significant symptoms within three weeks of travel, and to alert treating sources as to the specific locations visited.

Missing the diagnosis or the warning signs that alert the clinician of severe dengue approaching critical progression could lead to fatality. Warning signs include abdominal symptoms (pain, tenderness), gastrointestinal (vomiting), bleeding, hepatomegaly with enlargement greater than 2 cm, dramatic change in hematocrit (even elevation), and fluid accumulation. Admission to intensive care should be considered if concern about deteriorating clinical condition.⁷⁵ Other warning signs of plasma leakage, alterations in intra/extravascular spaces include tachycardia and narrowed pulse pressure.

Although there currently are no FDA approved antivirals specific to DENV, there is an FDA approved vaccine available, with others under research. The mainstay of care remains early diagnosis with aggressive cardiovascular support, symptomatic care, pain management, and maintaining other systems' support. Additionally there are situations where neurologic sequelae

of DENV (dengue myelitis, acute disseminated encephalomyelitis), some suggest intravenous methylprednisolone, albeit there remains a lack of randomized controlled trials to endorse this universally; risk/benefit must be balanced.^{75,116–118} Blood transfusions may be necessary.^{75,119}

DENV is a complex disease; infection can result in asymptomatic illness or fatality, as well as a wide range of severe symptoms including pain and hemorrhage. Dengue, as well as Zika, Chikungunya, and other emerging pathogens remind us how important it is for a more robust collaboration between public health and the wider medical community. For updates on Dengue, the clinician is recommended to regularly visit the CDC -Dengue website.¹⁰¹

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