e4 Dengue virus

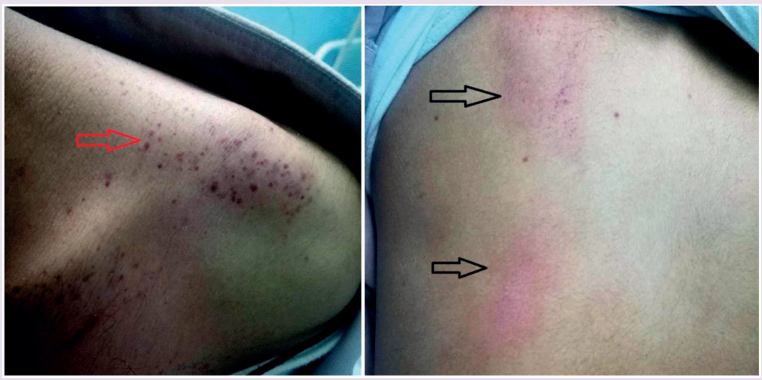

Figure e4.1 (A) Multiple purpura and petechiae. (B) Two large ecchymotic lesions on the left shoulder of the patient's back. *Slightly adapted from Raza M, Khan M, Ejaz K, et al. (2020) Cureus 12: e8581. DOI 10.7759/cureus.8581. Published under CC BY 4.0.*

A 38-year-old male with no significant past medical or surgical history presented to the emergency department with complaints of fever, headache, retro-orbital pain, and myalgia of 1-week's duration. The patient reported high-grade intermittent fever for the past 7 days that was associated with rigors and chills. His other complaints included reddish-colored urine with clots for 2 days and two episodes of gum bleeding. He denied having a sore throat, chest pain, shortness of breath, vomiting, abdominal pain, diarrhea, dysuria, urinary frequency, and contact with animals. On physical examination, the patient's vital signs were stable, oriented to person, place and time, and had mild conjunctival pallor. On skin examination, there were multiple purpura and petechiae on the left shoulder of the patient (**Figure e4.1A**), trunk, and both legs and two large ecchymotic lesions (bruising) on his back (**Figure e4.1B**). The rest of the systemic examination was unremarkable. He had a low platelet count ($20\,000 \times 10^9$/L) and WBC count (2.4×10^9/L).

Dengue hemorrhagic fever (DHF) was later confirmed on serologic evidence and the tourniquet test was positive. The patient was treated conservatively with acetaminophen (paracetamol) and one liter infusion of normal saline twice daily. The patient improved clinically on the 11th day of his admission with no hemorrhagic manifestations and normalization of blood count (platelet and WBC). The patient was discharged home with a follow-up appointment 1 month later.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

Dengue virus (DENV) is a single-stranded positive-sense RNA virus of the Flaviviridae family belonging to the *Flavivirus* genus. It consists of an outer icosahedrally symmetrical lipid envelope and a nucleocapsid core that encapsulates the genome. The genome is made up of around 10700 nucleotides and encodes a 3411 amino acid long precursor polyprotein containing three structural proteins, capsid, precursor membrane and envelope, and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (**Figure e4.2**). The NS proteins are responsible for viral replication and host immune evasion (see the host response). The exact roles of most of them are not well characterized but NS1 is responsible for most of the pathophysiologic features of severe dengue. There are four distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). These four antigenically related viruses share approximately 65% of their genomes and there is also some variation within a single serotype. Despite

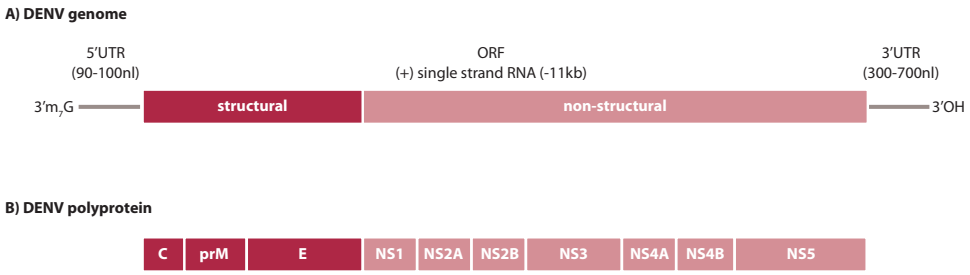

Figure e4.2 (A) The 5' end is capped with N7 methylated guanosine cap while the 3' end is hair looped. (B) A single polypeptide chain.

these variations, primary infection with each of the dengue serotypes results in the same disease and range of clinical symptoms.

ENTRY AND SPREAD WITHIN THE BODY

Mosquito-to-Human Transmission

DENV is transmitted to humans through the bites of infected female mosquitoes. The vector is *Aedes aegypti* and lives in intimate association with humans, rests in their homes and lays its eggs in various water containers. Other species, for example *albopictus*, can also act as vectors but their contribution is secondary to *Aedes aegypti*.

Saliva from the mosquito containing DENV is delivered into the dermis (and part epidermis) where it can infect/replicate in keratinocytes, and Langerhans cells (LCs) in the epidermis and dendritic cells (DCs), macrophages and mast cells residing in the basal and suprabasal layers of the epidermis (**Figure e4.3**). DENV is endocytosed using several receptors to bind to and infect host cells, including heparan sulfate, mannose receptor, and DC-SIGN. Some DENV can also enter the bloodstream directly while the mosquito takes a blood meal. Chemokines released by the infected cells recruit and infect more cells from the bloodstream to the site of the bite (see innate immunity) and infected DCs and macrophages (plus free virus) travel to the draining lymph nodes where further infection takes place (of predominantly myeloid derived cells) leading to viremia.

Symptoms develop during the disease – incubation period of 3–14 days with an average of 4–7 days. Only about 20% or less of infected individuals (depending on the DENV infective type) go on to develop symptoms of fever etc., (see clinical presentations) and can go on to develop severe disease with infection of other cell types including hepatocytes, muscle cells and endothelial cells.

Human-to-Mosquito Transmission

Asymptomatic individuals have viremia to different extents, with the highest overlapping with symptomatic individuals that is sufficient to infect mosquitos when they take a blood meal.

Mosquitos taking a blood meal from infected viremic individuals automatically become infected with DENV themselves. The risk of mosquito infection is associated with high viremia and high fever in the patient while high levels of DENV-specific antibodies are associated with a decreased risk of mosquito infection (see Section 2). People are generally viremic for about 4–5 days, but viremia can last as long as 12 days.

The virus first infects cells of the mosquito midgut, replicates and infects secondary tissues and then 8–10 days later (the extrinsic incubation period) infects the salivary glands. Variations in the extrinsic incubation period are influenced by ambient temperature, the magnitude of daily temperature fluctuations, virus genotype, and initial viral

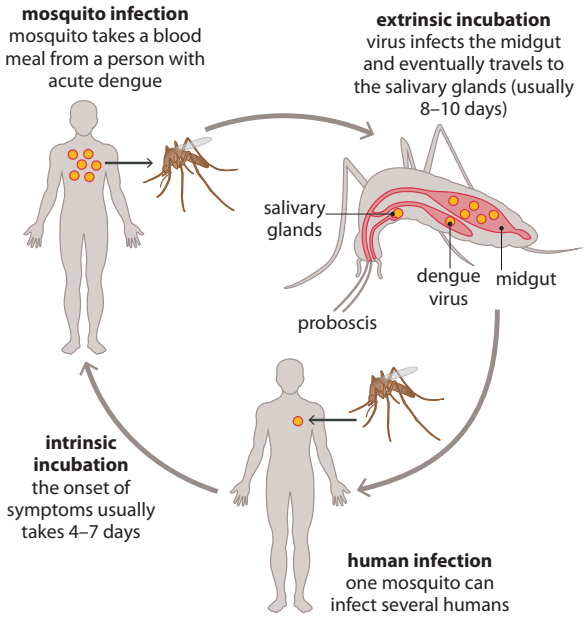

Figure e4.3 An *Aedes aegypti* mosquito can become infected by feeding on a person in the viremic phase of infection. Dengue viruses first infect mosquito midgut cells and other tissues before disseminating to the salivary glands – the extrinsic phase of the cycle. The infected mosquito can then transmit the dengue virus to several humans as it feeds or attempts to feed. Once infected, it takes an average of 4–7 days for the onset of symptoms and for a person to become capable of transmitting dengue virus to a new mosquito. Both symptomatic and asymptomatic individuals can transmit dengue virus to mosquitoes. *From Guzman MG, Gubler DJ, Izquierdo A et al. (2016) Nat Rev Dis Primers 2:16055, https://doi.org/10.1038/nrdp.2016.55. With permission from Springer Nature.*

concentration. The mosquito is now able to pass the DENV on to unsuspecting individuals. It remains infective for the life of the mosquito and can infect every person it subsequently feeds on or probes while trying to feed. The average female mosquito lives for approximately 1 week, but some females can live for up to 2 weeks.

The infected female mosquito can transmit the virus through the eggs to her offspring, but the epidemiologic importance of this vertical transmission is unclear.

PERSON-TO-PERSON SPREAD

It is rare for DENV to be spread through blood transfusion, organ transplant, or through a needle stick injury.

There is evidence of transmission of DENV from a pregnant mother to her baby with the risk being linked to the timing of the dengue infection during the pregnancy. If a pregnant mother is infected during pregnancy, babies may suffer from pre-term birth, fetal distress, and low birthweight.

EPIDEMIOLOGY

DENV is the most prevalent **arbovirus** worldwide, found in over 100 tropical and sub-tropical countries in Asia, the Pacific, the Americas, Africa, and the Caribbean.

Around 2.5 billion people, or 40% of the world's population, live in areas where there is a risk of dengue transmission. The incidence of dengue has grown dramatically in recent decades with cases reported to the WHO having increased eight-fold just over the last two decades from 505 430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032, affecting mostly the younger age group. The total number of cases appeared to have decreased during years 2020 and 2021, as well as for reported deaths. However, the data are not yet complete and the COVID-19 pandemic might have also hampered case reporting in several countries.

One estimate of current incidence indicates that there could be around 400 million DENV infections per year and, of these, around 100 million would have clinical disease (with any severity of disease). This would suggest that around 75% of cases are asymptomatic or under-reported.

Figure e4.4 shows the incidence of dengue cases between October and November 2021.

There are distinct epidemiologic patterns associated with the four serotypes of the virus. Different serotypes can exist within a region, but many countries are now endemic for all four serotypes. Dengue has an alarming impact on both human health and the global and national economies. It is a notifiable disease in many countries.

As well as the dengue infections in the endemic areas, a smaller number of the cases are travel related. For example, there were 117 (travel associated) new cases reported in the US for 2021 with 510 local cases in its territory (Puerto Rico).

In 2021, up to the end of November, the number of cases reported worldwide was 1 612 850, the majority from Brazil (916 096), India (123 106), Vietnam (68 268), Philippines (66 655), and Colombia (50 582). Since then 140 791 new cases have been reported, the majority from Brazil (52 446), Colombia (13 130), Pakistan (23 428), Peru (7334), and Vietnam (6964).

In Europe from 2015 to 2019, there were 11 478 travel-related dengue cases reported who had been infected in 110 different countries around the world.

The COVID-19 pandemic has placed enormous pressure on healthcare and management systems globally. The WHO has emphasized the importance of sustaining efforts to prevent, detect, and treat vector-borne diseases such as dengue and other arboviral diseases during this pandemic, as case numbers increase in several countries and place urban populations at

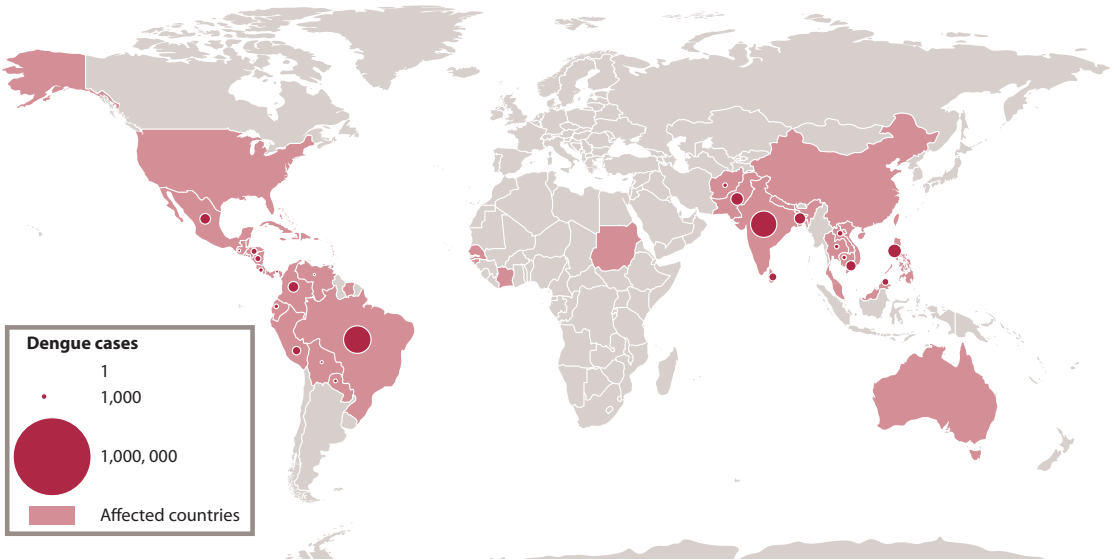

Figure e4.4 Geographic distribution of dengue cases reported worldwide in October to November 2021. *With permission from the European Centre for Disease Prevention and Control.*

the highest risk for these diseases. The combined impact of the COVID-19 and dengue epidemics could have devastating consequences on the populations at risk.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

THE IMMUNE RESPONSE AND PATHOGENESIS

Both innate and adaptive immune responses are important in the clearance of DENV in primary and subsequent responses. However, antibodies produced to different serotypes in subsequent responses can themselves lead to more severe infections (see Section 3).

INNATE IMMUNITY

Following a bite by an infected female mosquito, keratinocytes, Langerhans cells (LC), dendritic cells (DC), and macrophages become infected with DENV. Cytokine and chemokine production by the infected cells is mediated through pattern-recognition receptors (PRRs) on/in these cells that recognize microbial associated molecular patterns (**MAMPS**). Pyrogenic cytokines TNFα and IL-1-β are produced during this first wave of infection. Intracellular PRRs that include retinoic acid-inducible gene I (RIG-I)-like receptors, Toll-like receptors TLR3, and TLR7 that recognize ss and dsRNA respectively, trigger an antiviral response, which is initially characterized by the induction of the type 1 interferons (IFNα and IFNβ) designed to reduce the viral infection. Complement is also activated through the mannose binding lectin (MBL) pathway through recognition of DENV by the MBL complex leading to lysis, recruitment of phagocytes, and inflammation.

Following this first wave of local skin infection, cytokines and chemokines produced by the infected cells lead to edema and to traffic from the bloodstream (mediated by acute inflammation and mast-cell degranulation) of more monocyte-derived macrophages, monocyte-derived DCs and circulating plasmacytoid dendritic cells (**pDCs**). These recruited cells also become infected with DENV. Activated pDCs produce large amounts of type 1 interferons even without being infected. Natural killer (NK) cells and natural killer T (NKT) cells are also recruited to the site of infection by the chemokines and produce type II interferon (IFN-γ) that inhibits infection. In addition, they kill DENV-infected cells either alone or through antibody dependent cytotoxicity (if antibodies to a previous infection are present at this time – see responses to subsequent DENV infection). Infected macrophages, LCs, and DCs pass through the lymph to draining lymph nodes to infect more cells where the adaptive immune response is initiated.

DENV has several strategies for avoiding innate immunity. The NS proteins of the virus (see **Figure e4.2**) NS2a, NS4a, and NS4b block the transcription of IFN-β while NS1 inhibits the activation of complement through MBL.

ADAPTIVE IMMUNITY

The adaptive immune response is initiated through T-cell recognition of processed DENV in association with MHC (HLA) molecules on LCs and DCs within lymph nodes. CD4+ T cells and CD8+ T cells are both increased in DENV infection. The virus polarizes T-cell responses to Th-1 CD4+ cells which activate the cell-mediated arm of the adaptive immune response inducing cytotoxic CD8+ T cells directed against virus infected cells. CD8+ T cells peak at the time of febrile illness but there is some debate as to how efficient they are at killing virus-infected cells. For the humoral arm of the adaptive immune response, CD4+ **T follicular helper cells** are increased in number and interact with and activate virus serotype-specific B cells in germinal centers of lymph nodes giving rise to specific memory B cells and plasmablasts that develop into plasma cells. Virus-specific IgM, IgG, and IgA antibodies are produced, some of which bind viral envelope specific antigens and neutralize virions, thereby preventing entry into target cells. Complement enhances viral clearance via the classical pathway. Recovery from infection through the innate and adaptive immune response is believed to provide lifelong immunity against that serotype although there is now some evidence that this protection might not be complete.

Subsequent Response to a Heterologous DENV Serotype and Mechanisms Leading to Severe Disease

On second infection with DENV, T- and B-cell responses are enhanced through activation of serotype cross-reactive memory cells but also with some naive serotype specific T and B cells. DENV-specific memory B cells differentiate rapidly and secrete large amounts of virus-specific IgG. These antibodies detected during the acute phase of a secondary DENV infection also show cross reactivity for multiple serotypes as do T-cell responses. DENV-specific CD8+ T-cell frequencies in the blood are similar during primary and secondary DENV infection. In this setting, cross-reactive T cells, specific for the primary infecting serotype, become predominant during a secondary heterologous infection. This expansion of preexisting cross-reactive and low-affinity memory T cells is thought to hamper effective viral control and contribute to severe disease through enhanced production of inflammatory cytokines (see below). The main difference between primary and secondary infection is the presence of preexisting antibodies, which are thought to increase infection and disease severity. At this stage, the virus might be cleared successfully, and the patient recovers without complications. However, these antibodies have been shown to mediate a phenomenon termed antibody-dependent enhancement (ADE). The mechanism involves the opsonization of DENV virions through binding to non-neutralizing antibodies and the immune complex binding to IgG Fc receptors on DENV target cells such as DCs and monocytes, leading to high levels of viral replication. Since antibody titers (and their affinity or avidity) vary, the neutralizing versus enhancing properties of

these antibodies also fluctuate. In one study, it was shown that the risk of severe dengue is increased only when preexisting antibody titers are within a fairly narrow "intermediate" range, while individuals with either higher or lower titers showed no increased risk of developing severe dengue in the form of DHF possibly leading to dengue shock syndrome (DSS). Particular antibody isotypes may also influence disease severity. For example, a high IgG1:IgG2 ratio, together with other factors, was shown to be a risk factor for thrombocytopenia in individuals with DENV infection. As well as increasing virus uptake and replication, viral innate immune pathways would be activated in the infected cells that could lead to production or release of large amounts of potentially harmful inflammatory products. Cytokines such as TNFα have been shown in animal models of DENV infection to play a role in increase in vascular permeability and in hemorrhage via production of reactive oxygen species. Although the mechanisms of DHF and DSS are unclear it seems likely that a "cytokine storm" is involved.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

Most dengue infections are asymptomatic or show mild symptoms, but it can manifest as a severe flu-like illness that affects infants, young children, and adults, seldom causing death. After an incubation period of 4–10 days following the bite from an infected mosquito, symptoms appear and usually last for 2–7 days. The WHO classifies dengue into two major categories: dengue (with/without warning signs) and severe dengue. The sub-classification of dengue with or without warning signs is put in place to aid health practitioners to triage patients for hospital admission, ensuring close observation, and to minimize the risk of developing the more severe dengue.

DENGUE

Patients with dengue present with high fever (40°C/104°F) (**DF**) accompanied by two of the following symptoms during the febrile phase (2–7 days): severe headache; pain behind the eyes; muscle and joint pains; nausea; vomiting; swollen lymph nodes, or a maculopapular rash. They may have flushing, a characteristic feature is commonly observed on the face, neck, and chest. Coryza (rhinitis) may also be a prominent symptom especially in infants. These patients will start to recover within a week or so of starting symptoms but can remain weak for some time.

SEVERE DENGUE

Severe dengue, also called DHF, is more likely to occur during a secondary or tertiary infection where the DENV subtype is different from the primary DENV subtype. A patient enters what is called the critical phase of infection about 3–7 days after illness onset. During the 24–48 hours of critical phase, a small portion of patients may suddenly show deterioration of their symptoms. This occurs at the time when the fever in the patient is dropping (below 38°C/100°F), and when warning signs associated with severe dengue can manifest themselves. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment.

Warning signs that doctors need to look for include: severe abdominal pain; persistent vomiting; rapid breathing: bleeding gums or nose; fatigue; restlessness; liver enlargement; blood in vomit or stool.

If patients manifest these symptoms during the critical phase, close observation for the next 24–48 hours is essential so that proper medical care can be provided, to avoid complications and risk of death. Close monitoring should also continue during the convalescent phase.

Typical cases of severe dengue present with four major clinical manifestations: high fever and hemorrhagic phenomena but often hepatomegaly and circulatory failure. There is moderate to marked thrombocytopenia with hemoconcentration. Children often present with a sudden rise in temperature, facial flush and anorexia, headache, and muscle, bone, and joint pain. The most common hemorrhagic phenomena include bruising (as seen in our patient in **Figure e4.1**). In the majority of cases, discrete fine petechiae present over the extremities, axillae, face, and soft palate usually seen in the early febrile phase. Gingival (gum) bleeding can occur, and the liver is usually palpable. Many patients recover spontaneously or after a course of fluid and electrolyte therapy.

In patients who have circulatory failure, typical signs include the skin becoming cool and blotchy and a rapid weak pulse rate. Acute abdominal pain frequently occurs prior to the onset of shock. The patient is also hypotensive and may go into a complete shock state with blood pressure and pulse rate imperceptible. This is called dengue shock syndrome (DSS). However, most patients do remain conscious almost to the terminal stage. The duration of shock is short and the patient either dies within 12–24 hours or recovers rapidly with appropriate treatment (see Section 5).

The mortality of patients in severe cases can be up to 20%.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

CLINICAL DIAGNOSIS

Patients with dengue will have a history of living in, or recent travel to, a region where the disease is endemic. The incubation period is 3–14 days (average, 4–7 days); symptoms that begin more than 2 weeks after a person departs from an endemic area are probably not due to dengue.

Clinical diagnosis of dengue depends largely on what stage in the infection process a patient presents and which country the patient presents in or has been to recently. 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, and Zika, and up to 50% are misdiagnosed as other febrile illnesses. Correct diagnosis of the pathogen that could be responsible for the later manifestation of shock is of particular importance, as treatment for dengue-induced shock versus that arising from sepsis traditionally requires different approaches.

Since 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.

LABORATORY DIAGNOSIS

Several methods can be used for diagnosis of DENV infection. These include virologic and serologic tests. Depending on the time of patient presentation, different diagnostic methods may be more or less appropriate. Patient samples collected during the first week of illness should be tested by both serologic and virologic methods (RT-PCR). Serology is the method of choice for diagnosis at the end of the acute phase of illness. Various commercial rapid diagnostic tests (RDTs) kits are available, but currently most are reported to be of relatively low specificity and sensitivity.

VIROLOGIC METHODS

The virus can be isolated from the blood during the first few days of infection. Various reverse transcription–polymerase chain reaction (RT–PCR) methods are available. In general, RT–PCR assays are sensitive, but specialized equipment is required, and they are not always available in all medical facilities. RT–PCR products from clinical samples may also be used for genotyping of the virus, allowing comparisons with virus samples from various geographic sources.

RT-PCR can provide relatively fast results (unlike virus isolation), and using only a single sample. This test is not susceptible to the cross-reactivity with other flaviviruses seen with serologic testing.

The virus may also be detected by testing for the virus-produced protein NS1. There are commercially-produced RDTs available for this that are reported to have high specificity and sensitivity and it only takes ~20 mins to determine the result.

SEROLOGIC METHODS

Using the enzyme-linked immunosorbent assays (**ELISA**) to measure IgM and IgG anti-dengue antibodies may confirm a recent or past infection. IgM antibodies are detectable ~1 week after infection and are highest between 2 and 4 weeks after the onset of illness. They remain detectable for about 3 months. The presence of IgM indicates a recent DENV infection while the presence IgG antibody levels, that take longer to develop than IgM, indicates a past infection. IgG antibodies remain in the body for some time.

Diagnosis of Severe Dengue

A tourniquet test is helpful in diagnosing severe dengue. Blood pressure should be measured using an appropriate cuff size. Cuff pressure is increased to halfway between systolic and diastolic pressure for 5 minutes, then released. After 1 minute, or after normal skin circulation is observed, the result can be read. The test is considered positive if there are >20 petechiae/inch^2. Unlike dengue fever (DF) patients, severe dengue patients have vascular permeability (plasma leakage) during the critical phase. This leakage is selective into the pleural and peritoneal cavities that results in pleural effusion and ascites. Chest X-ray or ultrasound can be carried out to confirm the plasma leakage. There are no current prognostic diagnostic laboratory tests for severe dengue but having these would allow physicians to predict the complications and thus prevent them. There is optimism based on measurement of a number of potential prognostic biomarkers and these include various host immune, endothelial activation, biochemical, and genetic markers. Other potential biomarkers are TNFα, proteases, and adhesion molecules. Angiopoietin-2 and soluble VEGFR-2 have been found to be strongly associated with clinically apparent vascular leakage compared to the other factors associated with endothelial activation or dysfunction. In addition, the soluble form of CD163, a scavenger receptor expressed on monocyte–macrophages, is increased in severe dengue patients compared to those with mild DF and is suggested as a predictive marker for severe dengue. These studies collectively suggest there is significant potential for the development of a diagnostic test for severe dengue.

DIFFERENTIAL DIAGNOSIS

The following include some of the many possibilities.

Dengue Fever

- Infectious mononucleosis;
- Chikengunya viral infections;
- Coxsackie and other enteroviral infections;
- Rickettsial infections;
- Rubella;
- Parvovirus B19 infections;
- Leptospirosis;
- Influenza.

Severe Dengue (Dengue Hemorrhagic Fever)

- Leptospirosis;
- Chikengunya viral infections;
- Kawasaki disease;
- Yellow fever;
- Hantaviral infections
- Other viral hemorrhagic fevers.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

Treatment of Dengue Fever

There are no specific antivirals for DF and therefore care is limited to the management of symptoms.

Pain killers and fever reducers are taken to control the symptoms of muscle aches and pains, and fever. Acetaminophen (paracetamol) is used but non-steroidal anti-inflammatory drugs (**NSAIDs**) such as ibuprofen and aspirin are contraindicated. These anti-inflammatory drugs act by thinning the blood, and in a disease with the risk of hemorrhage, blood thinners may exacerbate the prognosis. Antiemetics may be given if the patients have nausea/vomiting. Other supportive and symptomatic medicines may be given according to the clinical signs and symptoms, for example, anticonvulsants, an H2-blocker, or proton pump inhibitor.

Early detection of disease progression associated with severe dengue, and access to proper medical care now lowers fatality rates of severe dengue to below 1%.

Management of Severe Dengue

More severe dengue can become apparent during the critical phase following the febrile phase of infection and may lead to severe complications and death if there is not appropriate and timely management. Unlike DF, patients with severe dengue have vascular permeability (plasma leakage) during the critical phase. Volume replacement is indicated following early detection of plasma leakage. The principle of volume replacement is to give the minimal amount to maintain effective circulation (intravascular volume). It is important that volume replacement is carried out to avoid hypovolemic shock and even death (DSS). Indications for shock or impending shock include narrowing of pulse pressure to 20 mmHg, hypotension, rapid and weak pulse, leukopenia and/or thrombocytopenia, poor appetite, clinical deterioration, and significant bleeding during the late febrile stage. Blood transfusion is important for bleeding symptoms and platelet transfusion for thrombocytopenia.

The future: A number of studies using human monoclonal antibodies to different components of the virus, including NS1, have been carried out in recent years. Some do not induce **ADE**, at least, *in vitro*. Using these to reduce the viral load in patients with dengue infection appears feasible from animal studies.

PREVENTION

Vector Control

Dengue prevention and control depends on effective vector control and sustained community involvement can improve vector control efforts substantially.

Prevention/Reduction of Mosquito Breeding

This can be achieved by preventing mosquitoes from accessing egg-laying habitats through environmental management and modification; disposing of solid waste properly and removing artificial man-made habitats that can hold water; covering domestic water storage containers, emptying and cleaning them on a weekly basis and using appropriate insecticides to treat water storage outdoor containers.

Personal Protection from Mosquito Bites

This is achieved using personal household protection measures, such as window screens, repellents, coils, vaporizers and targeted indoor residual spraying for dengue outbreaks. In addition, hanging mosquito nets over the sleeping area and keeping infected patients under a bed net at all times to prevent *Aedes* mosquitoes spreading the virus to healthy people. It is important that these measures are carried out during the day both inside and outside of the home (e.g. at work/school) because the primary mosquito vector bites throughout the day. Individuals are also advised to wear clothing that minimizes skin exposure to mosquitoes.

Community Engagement

It is important to educate the community on the risks of mosquito-borne diseases and engage with the community to improve participation and mobilization for sustained vector control.

Active Mosquito and Virus Surveillance

Active monitoring and surveillance of vector abundance and identification of species composition should be carried out to determine effectiveness of control interventions. Prospectively monitoring prevalence of virus in the mosquito population, with active screening of mosquito collections should also be undertaken.

Vector Surveillance can be Combined with Clinical and Environment Surveillance

There is ongoing research among many international research groups in search of novel tools and innovative strategies that will contribute in global efforts to interrupt transmission of Dengue. Integration of vector management approaches is encouraged by the WHO with the aim of achieving sustainable, effective locally adapted vector control interventions.

VACCINES

Developing an effective vaccine is challenging since it needs to be able to neutralize all four serotypes of DENV. Up until recently, there was only one licenced dengue vaccine. Dengvaxia (CYD-TDV) is a live attenuated chimeric, tetravalent vaccine with a Yellow Fever 17D strain virus backbone prepared using DNA technology. It is approved for use in many countries for seropositive individuals between 9 and 45 years of age who live in dengue endemic areas. Despite its efficacy in preventing severe disease in seropositive individuals, it

has placed some individuals at risk for developing severe dengue disease. In the US, it is approved only for children aged 9–16 years who have laboratory-confirmed previous DENV infection and live in dengue endemic areas. Among children, Dengvaxia has an efficacy of about 80% against the outcomes of symptomatic virologically confirmed dengue, with hospitalization for dengue, and severe dengue.

In recent published results of a Phase 3 Tetravalent Immunization against Dengue Efficacy Study (TIDES) trial, two doses of DENVax/TAK003 prevented laboratory-confirmed symptomatic dengue fever of any severity caused by any of the four dengue virus serotypes in children and adolescents. TAK003, based on a live-attenuated dengue serotype 2 virus, has now been approved by EU regulators and European Medicines Agency (EMA) advisors have backed the use of this vaccine for those aged 4 and older to prevent any of the four serotypes of dengue.

At the time of writing, several promising other vaccines are in different phases of **clinical trials** including:

1. The TV003 vaccine candidate is a live attenuated vaccine containing a mixture of the four live attenuated dengue serotypes producing strong responses to all serotypes. TV005 is the same but with a higher dose of the DENV 2 component.
2. TDEN-LAV is a live attenuated tetravalent vaccine. During the trial, unprimed vaccine recipients did not develop responses to all four serotypes until the second dose: the level of neutralizing antibody was not measured and so is unknown.
3. TDEN-PIV (DPIV) is a purified, formalin-inactivated tetravalent DENV vaccine: moderately immunogenic against all four serotypes, however, there was a waning and a plateau of neutralizing antibodies.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- Dengue virus (DENV) is a single-stranded positive-sense RNA virus encoding three structural proteins (capsid, precursor membrane, and envelope) and seven non-structural proteins responsible for viral replication and host immune evasion. There are four serotypes that share around 65% of their genomes.
- The virus is transmitted to humans through the bites of infected *Aedes aegypti* female mosquitoes. It infects keratinocytes, Langerhans cells (LC), dendritic cells (DC) and macrophages. The vector picks up the virus from a human during the viremic phase of infection and replicates before being passed on to another human. The mosquito continues to infect other humans during its life (1 to 2 weeks).
- DENV is the most prevalent arbovirus worldwide, found in over 100 tropical and sub-tropical countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. Around 2.5 billion people, or 40% of the world's population, live in areas where there is a risk of dengue transmission. The incidence of dengue increased eight-fold just over the last two decades from 505430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. At least 75% of the cases are asymptomatic and underreported especially during the highly pressurized health systems seen throughout the COVID-19 pandemic.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- Both innate and adaptive immune responses are important in the clearance of DENV in primary and subsequent infections.
- Pro-inflammatory cytokines produced by infected cells and other cells such as mast cells in the skin through pattern-recognition receptors are particularly important in the initial stages resulting in recruitment of other cells, monocytes/macrophages, to the inflammatory site. Complement is also activated. Type I interferons (IFN-α and β) are important cytokines at this stage for reducing infection with DENV. TNFα is also produced. Infected cells migrate via the lymphatics to drain lymph nodes and then infect organs of the body.
- In the lymph nodes, the adaptive immune response is initiated to produce specific antibodies (IgM, IgG and IgA) and cytotoxic CD8+ T cells. During secondary infection, antibodies can enhance infection rates through binding via IgG Fc receptors especially in macrophages and DC (antibody-dependent enhancement, ADE).
- DENV has several strategies for avoiding the immune system including blocking the transcription of IFN-β by some of the non-structural proteins while NS1 inhibits the activation of complement through the mannose lectin binding pathway.
- It is unclear as to what causes severe dengue that is seen in a minor population of patients infected with a different DENV serotype from the primary infection, but it is likely to be through over production of cytokines.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Patients with symptomatic dengue fever present with high temperatures (40°C/104°F): possible other symptoms include severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen lymph nodes, and a maculopapular rash.
- Patients can also present with more severe signs and symptoms (severe dengue) during the "critical phase" and it is important to monitor as soon as they show warning signs that include severe abdominal pain; persistent vomiting; rapid breathing: bleeding gums or nose; fatigue; restlessness; liver enlargement; blood in vomit or stool.

Continued...

...continued

- Typical cases of severe dengue (also called DHF) present with four major clinical manifestations: high fever and hemorrhagic phenomena but often hepatomegaly and circulatory failure. There is moderate to marked thrombocytopenia with hemoconcentration. The most common hemorrhagic phenomena include bruising and, in the majority of cases, discrete fine petechiae present over the extremities, axillae, face, and soft palate usually seen in the early febrile phase. Many patients recover spontaneously or after a course of fluid and electrolyte therapy.
- Typical signs of circulatory failure include the skin becoming cool and blotchy and a rapid weak pulse rate. Acute abdominal pain frequently occurs prior to the onset of shock. The patient is also hypotensive and may go into a complete shock state with blood pressure and pulse rate imperceptible. This is called dengue shock syndrome (DSS). However, most patients do remain conscious almost to the terminal stage. The duration of shock is short and the patient either dies within 12–24 hours or recovers rapidly with appropriate treatment.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Clinical diagnosis of dengue can be challenging, depending largely on which country the patients are in or have visited and what stage in the infection process a patient presents with.
- 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, and Zika. Up to 50% of cases are misdiagnosed as other febrile illnesses.
- The virus can be isolated from the blood during the first few days of infection. Various reverse transcription–polymerase chain reaction (RT–PCR) methods are available. In general, RT–PCR assays are sensitive, but specialized equipment is required and they are not always available in all medical facilities.
- The virus may also be detected by testing for the virus-produced protein NS1. There are commercially available rapid diagnostic tests available for this.
- Using the enzyme-linked immunosorbent assays (ELISA) to measure IgM and IgG anti-dengue antibodies may confirm a recent or past infection.
- For diagnosis of severe dengue, the tourniquet test is helpful. An X-ray or ultrasound can be carried out to confirm plasma leakage.
- There are no current prognostic diagnostic laboratory tests for severe dengue but there are a number of potential biomarkers that include various host immune, endothelial activation, biochemical, and genetic markers.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- There are no specific antivirals for dengue fever and therefore care is limited to the management of symptoms.
- Pain killers and fever reducers are taken to control the symptoms of muscle aches and pains, and fever. Acetaminophen (paracetamol) is used but NSAIDs such as ibuprofen and aspirin are contraindicated.
- Patients with severe dengue who have leukopenia may be given blood transfusions; those with thrombocytopenia and bleeding are treated with platelet transfusions.
- It is important that those with plasma leakage are given volume replacement to avoid hypovolemic shock. Dengue prevention and control depends on effective vector control and sustained community involvement can improve vector control efforts substantially. This is achieved by: a) prevention/reduction of mosquito breeding; b) personal protection from mosquito bites c) community engagement d) active mosquito and virus surveillance and e) vector surveillance combined with clinical and environment surveillance.
- Dengvaxia (CYD-TDV) was, until recently, the only vaccine licensed and approved for use in many countries for seropositive individuals between 9 and 45 years of age who live in dengue endemic areas. In the US, it is approved only for children aged 9–16 years who have laboratory-confirmed previous dengue virus infection and live in dengue endemic areas.
- Among children, Dengvaxia has an efficacy of about 80% against the outcomes of symptomatic virologically confirmed dengue, hospitalization for dengue, and severe dengue.
- A new vaccine DENVax/TAK003, based on a live-attenuated dengue serotype 2 virus, has now been approved by EU regulators for use in children aged 4 and older to prevent any of the four serotypes of dengue.
- There are several other promising vaccines that are at various stages of **clinical trials**.