Dengue

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Mortality from severe dengue is low, but the economic and resource burden on health services remains substantial in endemic settings. Unfortunately, progress towards development of effective therapeutics has been slow, despite notable advances in the understanding of disease pathogenesis and considerable investment in antiviral drug discovery. For decades antibody-dependent enhancement has been the prevalent model to explain dengue pathogenesis, but it was only recently demonstrated in vivo and in clinical studies. At present, the current mainstay of management for most symptomatic dengue patients remains careful observation and prompt but judicious use of intravenous hydration therapy for those with substantial vascular leakage. Various new promising technologies for diagnosis of dengue are currently in the pipeline. New sample-in, answer-out nucleic acid amplification technologies for point-of-care use are being developed to improve performance over current technologies, with the potential to test for multiple pathogens using a single specimen. The search for biomarkers that reliably predict development of severe dengue among symptomatic individuals is also a major focus of current research efforts. The first dengue vaccine was licensed in 2015 but its performance depends on serostatus. There is an urgent need to identify correlates of both vaccine protection and disease enhancement. A crucial assessment of vector control tools should guide a research agenda for determining the most effective interventions, and how to best combine state-of-the-art vector control with vaccination.

Introduction

Dengue is an acute arthropod-borne viral infection that places a heavy socioeconomic and disease burden on many tropical and subtropical regions, and is the most frequent arboviral disease globally.[1] The Global Burden of Disease study reported that dengue is increasing at a higher rate than any other communicable disease, with a 400% increase over just 13 years (2000–13).[2] Although dengue is still listed as a neglected tropical disease, investments in vaccine development and novel vector control measures have increased exponentially in the past decade.[3] We review recent updates and insights into dengue prevention and control measures, its pathogenesis relevant for vaccines and therapeutics, its clinical manifestations, and patient management.

Epidemiology and drivers for the geographic expansion of dengue

Transmitted by mosquitoes of the genus *Aedes*, dengue is found mainly in the tropics and subtropics, with over 3 billion people living in *Aedes*-infested areas.[4] The annual incidence of dengue infections was estimated to be around 400 million per year, of which about 25% were clinically apparent[1] and accounted for 1·1 million disability-adjusted life-years (DALYs) globally.[5] Asia accounts for 75% of the dengue disease burden, followed by Latin America and Africa.[1] In highly endemic areas, approximately 10% of all febrile episodes are due to dengue, with 4·6 episodes per 100 person-years occurring in Asia and 2·9 episodes per 100 person-years in Latin America.[6] The percentage of dengue-induced febrile episodes requiring hospital treatment was 19% in Asia and 11% in Latin America.[6]

The principal vector *Aedes aegypti* is a diurnal peridomiciliary mosquito, capable of stinging several people in a short timeframe and able to breed in various types of human-made containers that collect water. *Aedes albopictus*, although a less efficient vector, is continuing its geographic expansion into tropical and temperate climates. Global warming facilitates the wider geographic distribution of *Aedes* mosquitoes, thereby increasing dengue epidemic potential in temperate regions.[7] However, the main drivers for their proliferation and rising dengue incidence are population growth and high population density, rural-to-urban migration, degraded urban environments, absence of reliable piped water, and disorganised and inadequately funded mosquito control programmes.[8,9]

The rapid geographic spread of dengue viruses globally is the result of increasing human mobility via modern means of transportation.[10–14] Although imported dengue cases to the USA have resulted in small disease clusters for many years,[15] the first autochthonous sporadic cases in Europe (Croatia and France) were reported only in 2010,[16,17] and the first major outbreak was reported in 2012 in Madeira, Portugal.[18] Viraemic travellers to non-endemic areas constitute the main source for triggering autochthonous transmission.[19] International travellers are increasingly at risk of dengue,[20–24] with attack rates reported as high as 5·51 cases per 1000 travel-months.[25] Dengue is now the leading cause of fever in returning travellers, having overtaken malaria for travellers to Southeast Asia.[26]

Search strategy and selection criteria

We searched the Cochrane Library and MEDLINE for the years Jan 1, 2013, to July 30, 2018. We used the search terms "dengue", in combination with the terms "Aedes", "prevention", "control", "clinical management", "clinical trials", "anti-virals", or "vaccines". We largely selected publications in English from the past 5 years but did not exclude commonly referenced and highly regarded older publications. Furthermore, we searched relevant WHO sites.

The virus

Dengue viruses (DENV) belong to the genus Flavivirus, family Flaviviridae, with four serologically and genetically distinct serotypes.[27] DENV is an enveloped virus with a single positive-strand RNA genome, encoding three structural (capsid [C], pre-membrane [prM], and envelope [E]) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The C protein encapsidates the genome that is then surrounded by a lipid bilayer membrane, in which the E and M proteins are embedded. The E protein binds cellular receptors to enable virus entry into susceptible cells, and thus contains the epitopes crucial for neutralisation by antibodies that develop following infection. NS1 to NS5 form the replication complex that amplifies the viral genome. They also play important roles in interacting with host proteins necessary for successful virus replication.

Pathogenesis of severe dengue

Antibody-dependent enhancement

DENV serotypes 1–4 share a sizeable proportion of their structural antigens that, following infection with one DENV, induce antibodies that are type-specific, as well as cross-reactive with other DENVs. Upon infection with any DENV, the adaptive immune response that develops provides long-term immunity to the homologous virus, but protection against heterologous DENVs is short-lived. Human challenge studies conducted by Albert Sabin indicated that this cross-protection lasted approximately 3 months.[28] Conversely, epidemiological observations suggest that the cross-protection might be longer, lasting up to 2 years.[29,30] However, priming with one DENV serotype actually increases the risk of severe dengue upon secondary infection with a heterologous virus.[31] Likewise, DENV infection in infants at a time when maternal antibodies wane to sub-neutralising concentrations also appears to increase the risk of severe dengue,[32–34] although this risk has not been observed universally.[35] The underlying mechanism for increased disease severity is explained by antibody-dependent enhancement (ADE). First postulated by Halstead and colleagues,[36,37] this model states that cross-reactive antibodies or sub-neutralising concentrations of antibodies bind heterologous DENV to facilitate virus entry through Fc receptors expressed on target cells, such as monocytes, macrophages, and dendritic cells. Mechanistically, ADE is a more efficient pathway for viral entry than cognate receptor-mediated endocytosis.[38] Moreover, virus-host interaction during ADE also enables the virus to evade host antiviral and immune responses that would otherwise limit infection.[38,39] Collectively, ADE thus results in a greater burden of infection that induces imbalanced pro-inflammatory and anti-inflammatory responses,[40] which are thought to induce capillary endothelial pathology and vascular leakage, potentially leading to hypovolaemic shock—ie, dengue shock syndrome.[41]

ADE has been widely used to explain dengue pathogenesis, on the basis of the association between higher viraemia and NS protein antigenaemia in patients with secondary infection.[42,43] It has also been demonstrated in vivo in mice[44,45] and non-human primates.[46] Clinical studies provide a more nuanced view.[47–49] Investigators observed an enhancement in yellow fever vaccine immunogenicity, evidenced through prolonged viraemia associated with higher neutralising antibody titres, upon immunising naive volunteers against flaviviruses in two rounds with inactivated Japanese encephalitis virus, followed by a live yellow fever vaccine.[47] However, this enhancement was limited to patients with cross-reactive antibodies within a specific range of titres.[47] Likewise, results from a long-term cohort study in Nicaragua showed that not all children who were seropositive for dengue before an acute DENV infection were at equal risk of severe dengue; severe dengue occurred only in those who had baseline pre-infection antibodies within a narrow range of titres.[48] Another study using a different serological assay also arrived at the same conclusion: that the risk of ADE is restricted to those with cross-reactive antibodies expressed within specific limits.[49]

This limited range of antibody titres that are able to enhance infection is consistent with the biology of Fc receptors, which are predominantly activated when two or more Fc receptors are co-ligated by antibody-bound viral aggregates. On the one hand, at the lower range of antibody concentrations, the antibody-bound DENV will co-ligate the more abundantly expressed activating Fc receptors. On the other hand, high antibody concentrations will form larger viral aggregates to co-ligate the less abundantly expressed inhibitory Fcγ receptor, FcγRIIB.[50] FcγRIIB signals inhibit phagocytosis and so reduce viral entry into target cells.[51] Hence, the risk of ADE is not universal among all secondary DENV infections, but requires appropriate antibody-to-virus ratios. Only a fraction of infections occurring in the presence of reactive non-neutralising immunoglobulin G (IgG) advance to severe dengue, which means that pre-existing IgG titres cannot exclusively predict disease severity. A recent study identified patients with severe dengue who respond to infection by preferentially producing IgG1s with afucosylated Fc glycans, which incurred enhanced affinity for the activating FcγRIIIA receptor.[52] These antibodies reduced platelet counts in vivo, and the afucosylated glycans were a significant risk factor for thrombocytopenia.

Viral determinants

Whereas the molecular mechanism and the effects of ADE have been systematically examined, the role of viral non-structural factors encoded in the DENV genome is less understood. Long-term epidemiological observations suggest that viral factors might have important roles in pathogenesis. In Puerto Rico, the sequence of the DENV-2 envelope gene that was isolated during the 1994 outbreak was significantly different by several amino acid codons

compared with the DENV-2 that had circulated endemically since 1982.[53] Similarly, the DENV-2 epidemic in Santiago de Cuba in 1997 was attributed to a single amino acid substitution in the NS1 protein, compared with the DENV-2 that had been present on the island before the epidemic.[54] In Sri Lanka, DENV-3 was responsible for the dengue haemorrhagic fever outbreaks in 2000 and 1989, even though DENV-3 had been common in the country before 1989 and cases had been few;[55] the outbreaks were caused by the emergence of genetic differences that clustered DENV-3 into two distinct clades.[55] Similar clade replacement events associated with outbreaks have also been observed elsewhere.[56–58]

DENV evolves constantly because of its error-prone RNA-dependent RNA polymerase, although perhaps not as rapidly as other RNA viruses. Positive selections have been observed in the NS2B and NS5 genes, but other parts of the genome appear to be more constrained.[59] As a general trend, findings from molecular investigations suggest that DENVs that evolve more efficient methods to evade host antiviral responses gain epidemiological fitness by being able to grow to higher titres in both human and mosquito hosts.[60,61] In direct contrast, DENV strains that are less evasive are clinically attenuated.[62] Studies to define the viral determinants of clinical and epidemiological fitness using DENV isolates with corresponding and well documented clinical and epidemiological phenotypes remain much needed.

A viral factor that has recently been shown to possibly play a crucial role in dengue pathogenesis is the NS1 protein.[63] NS1 forms part of the replication complex of the DENV genome and is located on the endoplasmic reticulum as a dimer.[63] There, it interacts with a myriad of host proteins.[64] A hexameric form of NS1 is secreted from infected cells and appears to exert multiple functions,[65] including protecting the virus from complement and lectin-mediated neutralisation.[66,67] More recently, independent studies also suggest that NS1 might have toxic properties that disrupt the endothelial glycocalyx through either inflammatory-dependent or inflammatory-independent pathways.[68–71] Disruption of the endothelial glycocalyx increases vascular permeability and is likely to contribute to dengue-associated vascular leakage. Finally, secreted NS1 could also play a role in augmenting flaviviral infection in the mosquito vector; NS1 in viraemic blood could function to inhibit the reactive oxygen species response in the mosquito midgut that would otherwise limit infection.[72] However, clinical observations indicate that NS1 antigenaemia appears to be longer in primary than in secondary DENV infection, in contrast to the greater risk of severe disease with secondary than with primary dengue.[73,74] Thus, although the exact role of NS1 in clinical pathogenesis remains to be defined, NS1 has become a target of interest for antiviral therapy[65] and vaccination.[69] The figure outlines several of these key interactions in the context of the DENV lifecycle in human cells.

Host factors

Besides ADE and viral factors, host factors also contribute to clinical outcomes of DENV infection. Several studies have identified genetic polymorphisms that are associated with more severe disease. These polymorphisms include the activating Fc gamma receptor FcγRIIA,[75,76] inflammatory and anti-inflammatory cytokines,[77,78] human leucocyte antigens (HLA),[79,80] as well as genes in the lipid and steroid metabolism pathways.[81] Indeed, polymorphisms in the genes oxysterol binding protein-like 10 (OSBPL10) and retinoid x receptor alpha (RXRA), which encode proteins that function in pathways that link lipid metabolism with immune response, might explain the reduced susceptibility of people from African descent to severe dengue.[81] In a genome-wide association study comparing more than 3500 cases of dengue shock syndrome in Vietnamese people with almost 5000 healthy controls, susceptibility loci were identified in the MHC class I chain-related protein B (MICB) and phospholipase C epsilon 1 (PLCE1) genes.[82] Follow-up studies in Thailand arrived at a similar conclusion, in which single-nucleotide polymorphisms in MICB and PLCE1 genes were associated with increased and decreased risks for DSS, respectively.[83]

Transmission

Dengue is transmitted by the bite of an infected female mosquito. Non-vector transmission can also occur, for example, through blood transfusion, organ transplantation, needle stick injuries, and mucosal splashes.[84–86] Unlike Zika virus,[87] sexual transmission of dengue has not yet been reported. However, a recent single-case report documented prolonged shedding of dengue virus in the semen.[88] In contrast, another study in five patients with dengue confirmed by PCR, dengue virus was not detected in semen.[89] Persistent shedding of DENV-RNA was demonstrated in vaginal secretion up to 18 days from symptom onset.[90] Vertical transmission is common among mothers who are viraemic at delivery;[91] transplacental transmission is not thought to happen from infections that occur earlier during gestation, but formal data are lacking. Dengue virus was retrieved from 75% of 12 infected breastfeeding mothers, so transmission via breastfeeding is plausible, although no cases have been reported.[91]

Clinical manifestations

Disease classification

Although most people with DENV infection remain asymptomatic or develop only very minor symptoms, about 25% experience a self-limited febrile illness, accompanied by mild-to-moderate haematological and biochemical abnormalities. Clinically relevant complications develop in a small proportion of these patients, including a systemic vascular leak syndrome, coagulation abnormalities that can be associated with bleeding, and organ involvement, typically hepatic or neurological.[92]

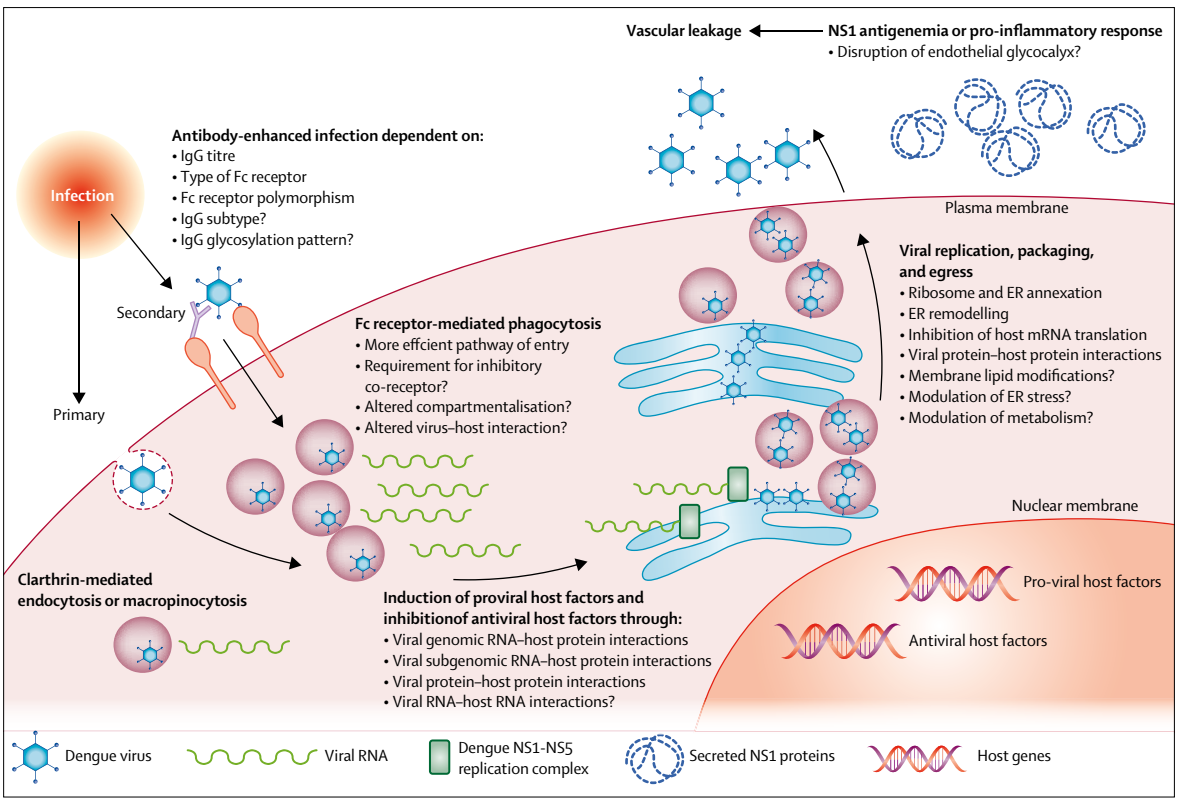

Figure: **Pathogenesis of severe dengue**
Schematic overview of the lifecycle of dengue virus in a mammalian cell. Upon entry, the virus uncoats to release its RNA genome and replicates in the cytoplasm. Successful replication depends on mulitple interactions between viral RNA and proteins with host factors. Newly synthesised viruses assemble in the ER and are transported through the trans-Golgi network to be released from infected cells via exocytosis. Indicates hypothesised mechanisms or involvement. ER=endoplasmic reticulum.

However, although these severe complications are infrequent and are usually readily identifiable, the spectrum of clinical manifestations is broad and can be quite subtle. The original classification system advocated by WHO in 1997 separated clinical dengue disease into two distinct entities, dengue fever and dengue haemorrhagic fever,[93] but the utility of this system has been questioned.[94] Following a multicentre study,[95] the 2009 WHO dengue case classification now identifies symptomatic individuals as having dengue if they have no major complications, or as having severe dengue if they experience complications in any of three categories, (1) plasma leakage severe enough to cause dengue shock syndrome or respiratory distress, (2) severe bleeding, or (3) severe organ impairment.[92] The system is dynamic, intended to facilitate more effective triage and clinical management and to improve the quality of epidemiological data collected globally, but remains controversial.[96–98]

Risk groups

In hyperendemic areas, symptomatic dengue is primarily a disease of older children and young adults, probably reflecting the timing of initial exposure in childhood. However, the risk for symptomatic or severe disease is also high among infants who are born to dengue-immune mothers and are first exposed to DENV at a time when maternal anti-dengue antibodies (acquired transplacentally) are waning to sub-neutralising titres.[32–34] Pregnant women are another group at high risk for severe disease, especially during the third trimester,[99,100] and perinatal transmission to infants is recognised.[101] Two large epidemiological studies from Brazil indicate that symptomatic dengue during pregnancy is associated with an increased risk of preterm birth and fetal death, although not with congenital malformations or low birthweight.[102,103] Additionally, in regions with relatively low endemicity, clinical disease is reported more often among adults than in children, including in people aged above 60 years.[104] The frequency and pattern of complications observed reflect the greater likelihood of underlying comorbidities in these adults.[105]

Clinical phases

After an incubation period of 4–7 days (maximum 14 days), symptoms typically begin abruptly and follow three phases, the febrile, critical, and recovery phases (panel). Classically, the febrile phase begins with sudden onset of high fever and chills, often with severe malaise, vomiting, and constitutional symptoms. Flushing of the

Panel: Clinical manifestations of the three dengue phases

Febrile phase

- High fever and chills. Typically persistent or unremitting, although a saddleback pattern can be observed. Children experience high fever and vomiting but are usually less symptomatic than adolescents and adults, except that febrile convulsions can occur.
- Fever lasts for 3–7 days from illness onset.
- Systemic symptoms such as headache, malaise, retro-orbital pain, arthralgia, myalgia, bone pain, nausea, vomiting, and altered taste sensation.
- Presence of upper respiratory symptoms helps to differentiate influenza from dengue.
- Examination findings can include rash, flush, conjunctival or pharyngeal injection, mild bleeding manifestations, generalised lymphadenopathy, and a palpable liver.
- A tourniquet test can be positive but is a non-specific finding.

Critical phase

Vascular leak syndrome

- Onset of clinically detectable plasma leakage occurs usually between days 4–6 of illness, often around defervescence. However, in one large series, about 10% of DSS patients were still febrile at presentation with shock.
- Plasma leakage can lead to intravascular volume depletion, hypoproteinaemia, and serosal effusions.
- If leakage is severe, DSS can ensue, diagnosed with PP ≤20 mm Hg or hypotension for age, with a rapid weak pulse and poor perfusion. Children have a lower threshold for leakage and are thus at greater risk for DSS than adults.
- Respiratory distress due to fluid overload is seen in severe cases and after overly aggressive fluid resuscitation.
- Warning signs for cardiovascular decompensation include persistent vomiting, severe abdominal pain, tender hepatomegaly, serosal effusions, mucosal bleeding, and lethargy or restlessness.
- There is a strong association with secondary infections.
- Leakage usually resolves within 48–72 h.

Bleeding

- Minor bleeding is common—eg, skin petechiae, easy bruising, epistaxis, gingival, GI or PV bleeding—but not universal.
- In children, major bleeding (usually GI) is seen only in association with profound or prolonged shock and can be the terminal event.
- Mucosal bleeding is more common and more severe in adults and can result in haemorrhagic shock (as distinct from DSS).
- Severe menorrhagia can occur in women, and life-threatening uterine haemorrhage has been reported during pregnancy.
- Intracranial haemorrhage is very rare but often fatal.
- Adults are more likely than children to have underlying disorders—eg, chronic liver disease, peptic ulcer, and gastritis—that influence the risk for bleeding. Dengue-related organ involvement (especially liver) is also more common in adults, potentially affecting haemostasis.
- Healthy adults also have intrinsically lower platelet counts than children, increasing the risk for bleeding with dengue.

Liver impairment

- Hepatomegaly and liver dysfunction are very common but rarely clinically important. AST titres typically exceed ALT titres.
- Chronic liver disease (eg, HBV) could aggravate the hepatic dysfunction.
- Isolated acute liver failure (without DSS) is rare and has a poor prognosis.

CNS impairment

- Seizures, encephalitis, encephalopathy, neuropathies, Guillain-Barré syndrome, and transverse myelitis have all been reported.
- DENV can invade the CNS, but the underlying pathogenic mechanisms are variable.

Cardiac impairment

- Sinus bradycardia and minor or asymptomatic arrhythmias are common.
- Myocardial impairment contributes to fluid overload in DSS patients.
- True myocarditis is rare, with some evidence for viral invasion.

Eye impairment

- Ocular manifestations include retinal haemorrhages, retinal oedema, macular ischaemia, and optic neuritis.
- Patients typically complain of painless visual impairment, often around the time of the platelet nadir.
- Gradual improvement occurs over several weeks, although sometimes visual impairment is permanent.
- Steroids might be beneficial in severe cases.

Impairment of other organs

- Microscopic haematuria has been noted in 20–30% of inpatients with dengue but AKI is rare generally.
- Renal failure is sometimes seen in profound DSS, or in association with rhabdomyolysis.

Recovery phase

- With good supportive care full recovery is usual within 1–2 weeks.
- Post-viral fatigue and depression are reported, but few studies have evaluated these outcomes prospectively.
- A florid convalescent rash can develop, resolving slowly over several weeks.
- Fever persisting for >10 days can indicate bacterial superinfection or development of rare complications, such as secondary haemophagocytic lymphohistiocytosis.

ALT=alanine transaminase. AKI=acute kidney injury. AST=aspartate transaminase. DENV=dengue virus. DSS=dengue shock syndrome. GI=gastrointestinal. HBV=hepatitis B virus. PP=pulse pressure. PV=per vaginal.

face and trunk might become apparent from day 2–3, and some patients have a transient macular rash.

Most patients improve when the fever settles. Crucially, however, various complications can develop around the time of defervesence, requiring prompt identification to facilitate effective case management. This marks the onset of the critical phase. Most notable is a poorly defined vasculopathy, characterised by increased vascular permeability, plasma leakage, and intravascular volume depletion, which can progress to life-threatening dengue shock syndrome.[106,107] Conventionally, haemoconcentration of 20% or more is accepted as evidence of dengue-associated plasma leakage,[92] but for individual case management this criterion is hard to apply, since the baseline haematocrit is rarely known. Similarly, serosal effusions (pleural, peritoneal, and sometimes pericardial) reflect the severity of the vasculopathy but are difficult to detect clinically until shock is established.[106] Ultrasound studies indicate that pleural effusions, ascites, and gall bladder wall oedema are commonly present during the critical phase, and have also demonstrated signs of minor leakage as early as day 2–3 of fever.[108] With increasing volume depletion, an unusual and highly characteristic phenomenon can be observed when the diastolic pressure rises while the systolic pressure is maintained, resulting in narrowing of the pulse pressure (PP). When the PP narrows to 20 mm Hg or less, conventionally the patient is defined as having dengue shock syndrome. Surprisingly, the individual can appear alert and deceptively well, but the significance of the narrow PP must not be ignored, since without prompt fluid resuscitation the patient can deteriorate rapidly. Recurrent episodes of shock (reshock) can occur in the 48–72 h before the vasculopathy resolves, with repeated episodes associated with a substantial increase in mortality.[106] In infants, older people, pregnant women, and those with underlying hypertension or vascular disorders, early clinical indicators of shock are sometimes not readily apparent.

Haemorrhagic manifestations are also often seen during the critical phase but are usually minor. Clinical evidence of organ impairment is observed less frequently, except as a secondary phenomenon or in individuals with underlying diseases.[109] Asymptomatic hepatomegaly and mild-to-moderate transaminitis are common, but acute liver failure is rare.[110] Neurological involvement is also uncommon, although cases are well documented.[111] Diverse cardiac manifestations have been reported, including myocardial dysfunction and a broad range of rhythm disturbances.[112] Involvement of other organs is less well documented, but with increasing awareness among physicians, a broader spectrum of complications is now being recognised, such as ocular complications.[113]

Even among those who develop complications, with good supportive care full recovery is usual within 1–2 weeks. The increased vascular permeability and abnormal haemostasis are transient and resolve within 48–72 h of becoming clinically apparent, but the individual often remains tired and lethargic for several days. In some cases a florid convalescent rash can persist for several weeks. The recovery phase can be quite prolonged in adults, who can experience profound tiredness, weakness, myalgia, and depression for weeks to months after the acute illness has resolved.[114,115]

Laboratory investigations

Some degree of thrombocytopenia and leucopenia are almost universal during the febrile phase, often associated with an increase in atypical lymphocytes. Significantly lower total white blood cell, neutrophil, and platelet counts have been observed in laboratory investigations of dengue cases than among comparable patients with other febrile illnesses.[116] Severe neutropenia can occur but is not associated with severe disease, secondary bacterial infections, or fatal outcome; prophylactic antibiotics are not recommended without additional evidence for bacterial infection.[117] Coagulation derangements are also common, with increased activated partial thromboplastin times and reduced fibrinogen concentrations reported most frequently.[118] These various abnormalities demonstrate a characteristic temporal evolution, reaching their peak or nadir during the critical phase, before returning to normal levels towards the end of the second week.

Transaminase concentrations are almost invariably increased across the spectrum of clinical dengue disease, with aspartate aminotransferase concentrations typically higher than those of alanine aminotransferase, reflecting the combination of musculoskeletal and liver involvement.[119] Hypoproteinaemia, particularly hypoalbuminaemia, is a marker of the severity of leakage, but during the critical phase low plasma albumin concentrations can be masked by concomitant haemoconcentration. Acute kidney injury is uncommon and renal function is usually normal, except in those with profound shock and acute tubular necrosis, in older people, or in those with underlying diseases.[120]

Risk prediction

During the transition from febrile to critical phases, several clinical warning signs have been highlighted by WHO as potential signals of impending deterioration (panel). At present, however, the evidence base for these signs is scarce and some definitions are subjective.[121] Establishing a reliable early prediction algorithm for severe dengue is a high priority to facilitate triage and optimal use of limited resources in endemic areas. However, most efforts to date have assessed associations with severe disease, rather than attempting to identify true predictors. Nevertheless, in one large study an algorithm using data obtained on day 3 of illness (including history of vomiting, platelet count, aspartate transaminase concentrations, and NS1 rapid test status), had acceptable performance in identifying the 117 patients who subsequently developed severe dengue, out of 7544 children with suspected dengue.[122]

Development of dynamic prediction models that incorporate repeated observations is an alternative approach; in one large study, inclusion of serial daily platelet counts into a model to predict DSS showed promise.[123] Finally, various viral, immunological, and vascular biomarkers have been proposed and, if validated, could potentially be combined with clinical data to improve risk prediction algorithms.[124–126] For example, chymase titres could potentially become a prognostic biomarker of severe dengue.[127]

Diagnosis

A wide variety of conditions, primarily viral infections, but also bacterial and parasitic diseases must be considered in the differential diagnosis, depending on local disease epidemiology, travel history, and the clinical picture. The choice of laboratory test depends on the day after onset of illness. Before day 5, dengue can be diagnosed by virus isolation in cell culture, detection of viral RNA by nucleic acid amplification tests such as RT-PCR, or detection of viral antigens such as NS1 by ELISA or rapid tests.[92] After day 4–5, dengue viruses disappear from the blood, although NS1 antigens can persist longer, in particular in primary dengue infections.

Coincident with the appearance of dengue-specific antibodies, serological assays should be used. Dengue IgM antibodies start to rise from day 4 onwards, peak at about days 10–14, and then decline and disappear after about 3 months. In primary infections, anti-dengue IgG can be detected at low concentrations by the end of the first week (often only from day 10 onwards); the concentration increases slowly thereafter and is thought to persist for life. In patients with a past dengue (or other flavivirus) infection, dengue IgG titres rise rapidly within the first week of illness. Serological assays typically do not allow determination of the infecting virus serotype, are susceptible to cross-reactivity with other flaviviruses, and often require paired (acute and convalescent) samples. Although rapid diagnostic tests are available for NS1 antigen, IgM antibody detection, or both simultaneously, the sensitivities and specificities of the available tests have been uniformly lower than those of the equivalent laboratory-based ELISA assays.[128] Nevertheless, the combination of NS1 antigen and IgM testing at point of care offers a longer diagnostic window and has revolutionised the diagnosis of dengue. Cross-reactivity with Zika virus is reported for all serological assays and also for NS1 antigen.

Management

Identification of specific therapeutics for dengue has been a major focus of research in recent decades. Given that high viraemia is considered to be one risk factor for the development of severe disease, an antiviral drug started early in the course of illness has the potential to both shorten the duration of infection and mitigate severity. If effective, such a drug might also reduce onward DENV transmission by limiting the period of infectiousness to mosquitoes.[129] However, although the potential utility of several repurposed drugs with antiviral activity (chloroquine, balapiravir, celgosivir, and lovastatin) has been explored in randomised blinded clinical trials (table), no evidence has yet been demonstrated of a benefit in reducing plasma viraemia or preventing complications.[130–135,139] A trial of ivermectin is currently in progress (ClinicalTrials.gov number NCT02045069). In all the trials published to date, treatment started within 48 h or 72 h of fever onset, suggesting that intervention might need to start even earlier in the course of illness to influence viraemia.

In parallel with the current rapidly advancing knowledge of DENV structure and biology, major efforts are also being directed towards the discovery of drugs against a range of host and viral targets.[140] Small molecules that target viral entry are of particular interest. Currently, the most advanced small molecule drug is the NS4B inhibitor under development by Janssen Pharmaceuticals,[141] which is expected to enter clinical trials within the next couple of years. Another approach involves development of monoclonal antibodies that limit viral replication,[142] primarily through blocking viral attachment or invasion. The most potent neutralising antibodies identified so far map to domain III of the E protein, or to complex quaternary structural epitopes on the E protein dimer. Some are serotype-specific, but others can be broadly neutralising; none have as yet progressed to clinical trials and it might be that such antibodies are better suited for drug probe and pathogenesis research or for prophylactic use, rather than as therapeutics.

An alternative potential intervention strategy involves suppression of the host immune response, for example by using corticosteroids. However, corticosteroid therapy showed no convincing benefit on mortality from dengue shock syndrome in several small trials during the 1980s. In a more recent randomised blinded trial focused on safety, use of oral prednisolone during the early acute phase was not associated with delayed viral clearance or other adverse events, but neither was there a reduction in development of shock or other complications.[136] Here, too, it is possible that the intervention was administered too late to attenuate the infection-driven processes that result in severe dengue, or that a higher steroid dose might have been more effective. However, hyperglycaemia was observed in some recipients of high-dose prednisolone,[136] and this event probably precludes further development of this strategy. A Cochrane review concluded that there is insufficient evidence to evaluate the effects of corticosteroids, either for established dengue shock syndrome or in the treatment of early dengue.[143]

In the absence of effective antiviral or immunosuppressive therapy, good supportive care is the cornerstone of effective case management. For dengue shock syndrome, prompt recognition and immediate fluid resuscitation is crucial, aiming to provide just

sufficient fluid replacement to maintain adequate intravascular volume for 48–72 h until the vasculopathy reverses. Meticulous attention is necessary to limit iatrogenic complications, particularly development of fluid overload. A balanced crystalloid solution was shown to be as effective as colloid therapy for initial resuscitation of children with moderately severe dengue shock syndrome,[137] but no research has ever been carried out to investigate potential therapeutic options for the difficult problem of reshock. Current WHO guidelines, based primarily on expert opinion, recommend use of colloid boluses in these circumstances,[144] despite the fact that in high-income settings use of synthetic colloid solutions for volume expansion is no longer considered appropriate.[145] Another controversial area has been the frequent use of prophylactic platelet transfusions for moderate-to-severe thrombocytopenia, without clinical bleeding. In a trial in Asian adults[138] with platelet

	Drug remarks	Trial description	Inclusion criteria	Schedule	Primary outcome	Secondary outcomes	Comments
Chloroquine[130] (antiviral)	Cheap, safe 4-amino-quinoline derivative with broad antiviral effect	Randomised, placebo-controlled trial in 307 Vietnamese adults with suspected dengue	Suspected dengue with fever for <72 h	Standard 3-day course of chloroquine or identical placebo	No reduction in duration of viraemia or NS1 antigenaemia	Chloroquine was associated with a few more adverse events than placebo, but they were generally mild	84% patients were PCR-confirmed for dengue
Balapiravir[131] (antiviral)	Polymerase inhibitor initially developed for treatment of hepatitis C	Dose-escalating, randomised, placebo-controlled trial in 64 Vietnamese men	Confirmed dengue (NS1 positivity) and fever for <48 h	5-day regimen of 1500 mg or 3000 mg of balapiravir or identical placebo	Clinical and laboratory adverse event profiles were similar for all treatment regimens	No evidence of any effect on a range of virological, clinical, or immunological endpoints	Later in-vitro studies show that activation of peripheral blood mononuclear cells by infection with dengue virus depotentiates balapiravir[132]
Celgosivir[133,134] (antiviral)	An iminosugar that induces misfolding of viral proteins through inhibition of host α-glucosidase enzymes	Phase 1b, randomised, double-blind, placebo-controlled trial in 50 Singaporean adults	Confirmed dengue (via PCR or NS1 positivity) with fever ≥38°C for <48 h	1 dose of 400 mg then 8 doses of 200 mg twice daily of celgosivir or identical placebo (9 total doses)	Generally safe and well tolerated, but no reduction in viral load or fever demonstrated	Extended evaluation of trial data suggests a different dosing regimen may improve efficacy	The antiviral potency of related iminosugars may be greater than celgosivir
Lovastatin[135]	Statins have anti-inflammatory, endothelial-stabilising, and antiviral properties	Randomised, double-blind, placebo-controlled trial in 300 Vietnamese adults	Confirmed dengue (NS1 positivity) and fever for <48 h	5 days of 80 mg lovastatin or identical placebo once daily	Generally well tolerated and adverse events profiles similar between groups	No evidence of a beneficial effect on any clinical manifestations or dengue viraemia	..
Cortico-steroids[136] (immuno-suppressant)	Corticosteroids inhibit a broad range of immune responses but may impair viral control mechanisms	Randomised, placebo-controlled trial in 225 Vietnamese patients aged 5–20 years with suspected dengue	Suspected dengue with fever ≥38°C for ≤72 h	3 days of low-dose (0·5 mg/kg) or high-dose (2 mg/kg) oral prednisolone or identical placebo	No evidence of significantly increased or prolonged viraemia with prednisolone use	No association with any of the predefined clinical, haematological, or virological endpoints; adverse event profiles were similar, except for a trend towards hyperglycaemia in high-dose steroid recipients	PCR confirmation in 223/225 subjects
Shock resuscitation[137] (supportive care)	..	Randomised, double-blind trial of 3 fluids for initial resuscitation of 512 Vietnamese children with dengue shock syndrome	Stratified by severity into two groups on the basis of initial pulse pressure	For those with moderate severity, 25 mL/kg RL, 6% dextran 70, or 6% hydroxyethyl starch, given over 2 h, then a standard reducing intravenous fluid schedule; severe cases were not eligible for RL but received either one of the colloids	RL gave a less rapid reduction in the haematocrit count; minor differences in efficacy between the two colloids	Significantly more recipients of dextran than of starch had adverse reactions; bleeding, coagulation derangements, and severity of fluid overload were similar for all fluid treatment groups	One starch recipient died but all other patients recovered fully; requirement for rescue colloid (reshock) was similar for the different fluids in the two severity groups
Prophylactic platelet transfusions[138] (supportive care)	..	Randomised, open-label superiority trial in 372 adults with dengue and thrombocytopenia in Singapore and Malaysia	Platelet count ≤20 000 per μL with no serious bleeding	Transfusion group received supportive care plus 4 units of pooled platelets every day when the platelet count was ≤20 000 per μL *vs* supportive care alone in the control group	Prophylactic platelet transfusion was not superior to supportive care in preventing bleeding	Adverse events occurred significantly more often in the platelet group	..

NS1=non-structural protein 1. RL=Ringer's lactate.

Table: **Summary of randomised trials performed since 1999 investigating intervention strategies against dengue**

counts below 20 000 cells per µL, prophylactic platelet transfusion was not superior to supportive care in preventing bleeding, but was associated with more adverse events. Several other prophylactic and therapeutic interventions have been evaluated in small trials (plasma infusion, recombinant activated factor VII, anti-D globulin, immunoglobulin, and interleukin 11), but there is no evidence any of these interventions is associated with a beneficial effect in preventing or treating clinically significant dengue-associated bleeding.[146]

Vector control

Integrated vector management is the strategic approach promoted to countries by WHO to reduce dengue mortality and morbidity by 2020.[92] This management involves using a combination of approaches incorporating key elements of social mobilisation, integration of chemical and non-chemical control methods to target areas of high human-vector contact, evidence-based decision making to guide research and policy, as well as capacity building. Vector control methods can be broadly divided into biological, chemical, and environmental. Biological methods include using Bacillus thuringiensis israelensis, larvivorous fish, and copedods for the control of mosquito larval stages; chemical methods include using insecticides for residual sprayings and long-lasting insecticide treated materials; and temephos or pyriproxyfen to control larval stages. Environmental methods aim to reduce mosquito breeding sites.

Although community mobilisation and participation to reduce *Aedes* larval habitats have shown variable success,[147] a recent multicentre randomised trial provided the first evidence that community mobilisation can enhance dengue vector control and reduce dengue incidence.[148] Daytime personal protection against *Aedes* mosquito bites is challenging. Compliance rates with antivectorial protective measures by travellers were shown to be low,[149] and would not be scalable on a population basis. Meta-analyses showed that house screening significantly reduced dengue risk, as did combining community-based environmental management with water container covers, whereas indoor residual spraying did not affect infection risk.[150] Technologies that can be applied during the daytime to protect against mosquito bites should be a research priority, such as insecticide-treated clothing. A community-based trial in Thailand testing school uniforms impregnated with permethrin did not, however, have an effect.[151]

Two novel approaches to controlling *A aegypti* are in advanced development: the release of Wolbachia-infected mosquitoes and Release of Insects carrying Dominant Lethal genes (RIDL). Wolbachia infection is a self-sustaining invasive strategy that uses inherited endosymbiotic bacteria to render natural mosquito populations resistant to arboviruses.[152] Strains of the bacterium Wolbachia deliberately introduced into *A aegypti* can spread across mosquito populations in release trials, and insects infected with these strains show markedly reduced vector competence. Thus, Wolbachia represents an exciting potential new form of dengue biocontrol.[153] The RIDL approach uses the insertion of a lethal gene into the *A aegypti* genome.[154] When the carrier male mosquito mates with wild-type females the lethality trait is passed on to the offspring; field trials are ongoing. Based on the growing consensus that no single intervention will be sufficient to effectively reduce disease, there is increasing interest in combining mosquito interventions with vaccination.[155]

Vaccines

In late 2015, after decades of research, the world's first dengue vaccine by Sanofi Pasteur, CYD-TDV or Dengvaxia, was licensed.[156] CYD-TDV is a recombinant, live attenuated, tetravalent vaccine, based on the yellow fever 17D backbone. The structural genes (prM-E) of the YF17D virus vector are replaced by the structural genes of each of the four DENV serotypes. CYD-TDV is now registered in 20 countries, typically with an indication for individuals aged 9–45 years. Data generated by a large phase 3 trial in Asia and Latin America[157,158] showed unpredicted complexity of vaccine performance, with efficacy dependent on serotype, baseline serostatus, and age.[159] In 2018, Sanofi Pasteur released new long-term safety data, obtained from analyses of blood samples taken 13 months after the first dose, to retrospectively infer serostatus at baseline.[160] The analyses revealed an excess risk of severe dengue in seronegative vaccine recipients, compared with seronegative non-vaccinated individuals, while confirming long-term protection in seropositive individuals.[161] The most plausible hypothesis for the increased risk in seronegative individuals is that the live attenuated CYD-TDV initiates a first immune response to dengue that predisposes them to a higher risk of severe disease when they experience their first natural dengue infection.[162] The revised WHO Strategic Advisory Group of Experts recommendations from April 2018, stated that for countries considering CYD-TDV vaccination, pre-vaccination screening would be the preferred strategy, in which only dengue-seropositive persons are vaccinated.[163] Efforts are now underway to develop and validate rapid diagnostic tests to screen for dengue serostatus, but cross-reactivity with other flaviviruses will remain a challenge.[164] Research is also needed to evaluate vaccine schedules with fewer doses, assess the need for booster doses, and identify populations that will benefit most from this vaccine.[161,165]

Both homotypic and heterotypic dengue protective immunity might require fully competent CD4 and CD8 cells, derived mainly from presentation of non-structural protein epitopes to the immune system.[166–168] Dengue non-structural proteins are absent from the Sanofi dengue-yellow fever chimeric vaccine but (at least partially) present in second generation dengue vaccines. Two chimeric live attenuated dengue vaccines are now in

phase 3 trials: one developed by the National Institute of Allergy and Infectious Diseases (TV003/TV005; NCT02406729, ClinicalTrials.gov), and one developed by Takeda (NCT02747927, ClinicalTrials.gov). A single dose of either TV 003 or TV005 induced seroconversion to four DENV serotypes in 74%–92% (TV003) and 90% (TV005) of flavivirus seronegative adults, and elicited near-sterilising immunity to a second dose of vaccine administered 6–12 months later.[169] Takeda's live attenuated tetravalent dengue vaccine candidate comprises an attenuated DENV-2 strain plus chimeric viruses containing the prM and E genes of DENV-1, 3, and 4, cloned into the attenuated DENV-2 backbone.[170] Whether these second-generation dengue vaccines will encounter the same safety issue in vaccinated seronegative patients is unknown. Trial results are eagerly awaited, with the first readouts expected by 2019.

Future challenges and opportunities

Although mortality is generally low in countries with good clinical infrastructure and standard operating procedures for the management of severe dengue, morbidity and chronic sequelae in the adult population remain poorly studied. Despite the overall low mortality associated with dengue, the high morbidity and consequent economic and resource burden on health services in endemic settings is substantial and increasing.

Progress towards development of effective therapeutics has been slow, despite notable advances in our understanding of disease pathogenesis and considerable investment in antiviral drug discovery. For decades, ADE has been widely used to explain dengue pathogenesis, but it was only recently demonstrated in vivo and in clinical studies. It is now clear that the risk of ADE is not universal among all secondary DENV infections, and that progression to severe dengue requires appropriate antibody-to-virus ratios.

Technologies for identifying people at risk of developing severe dengue are desperately needed. Given the heterogeneous nature of the disease, development of a single, robust clinical algorithm for risk prediction that is broadly applicable across all age groups and in different locations is likely to prove difficult without inclusion of highly discriminating biomarkers.

At present, the mainstay of management for most symptomatic dengue cases remains careful observation and prompt but judicious use of intravenous hydration therapy for those with substantial vascular leakage. Increasing efforts are being made to develop truly evidence-based guidelines for supportive care across many medical disciplines. However, although there is evidence supporting the use of isotonic crystalloid solutions for initial resuscitation of DSS, formal evaluation of the efficacy and safety of the synthetic colloid regimens in regular use for severe DSS should be considered. We suggest that development of a collaborative regional platform, comprising well established centres across dengue endemic countries with the necessary infrastructure and clinical expertise to contribute to a multicentre fluid resuscitation trial, would be an invaluable resource. This platform might also prove useful for trials of other interventions, including antiviral drugs.

Various promising technologies for detection of DENVs are currently in the pipeline. New point-of-care sample-in, answer-out nucleic acid amplification technologies are being developed to improve performance, in some cases with the potential to test for multiple pathogens using a single specimen. Connectivity solutions linking data from diagnostic laboratories and point-of-care test readers or devices could also provide opportunities for automated surveillance systems. The rate-limiting bottleneck in bringing new diagnostics to market, however, is the access to well characterised samples for assay performance evaluation. We suggest the establishment of an international reference laboratory network to facilitate sharing reference materials and development of standardised protocols.

Several important lessons can be learnt from the CYD-TDV trials. First, to enable long-term efficacy and safety estimates by serostatus, baseline blood samples need to be taken for all trial participants,[171] and this procedure is currently being implemented in the phase 3 trials of Butantan and Takeda. Vaccine trial designs should account for the known period of cross-protection between serotypes, and the period when more enhanced disease is seen; therefore, they must include active surveillance of trial participants, ideally up to 5 years.[171] Second, there is an urgent need to identify better correlates of both protection and enhancement. Third, the standard plaque reduction neutralisation tests used currently to measure immunogenicity cannot distinguish between neutralising serotype-specific antibodies and non-neutralising cross-reactive antibodies. Thus, an assay that distinguishes between serotype-specific and cross-reactive antibodies would accelerate vaccine development.

Finally, all dengue endemic countries need a more effective surveillance system to guide disease prevention and control efforts. There is a remarkable scarcity of reliable evidence for the effectiveness of any dengue vector control method. Reduction in disease incidence, rather than entomological indices alone should be studied as an endpoint. A critical assessment of vector control tools should guide a research agenda for determining the most effective interventions and how to best combine state-of-the-art vector control with vaccination.[172] There is an urgent need for integration of dengue research within a broader arbovirus research agenda.

Contributors

AW-S coordinated the writing of the manuscript and wrote the first and final drafts. E-EO was responsible for the sections on pathogenesis and virology, OH on vector control measures and surveillance, and BW on clinical manifestations and clinical management. E-EO created the figure, BW created the panels and table. All authors contributed to and approved the final manuscript.

Declaration of interests
AWS serves and OH has served as a paid consultant to WHO. BW reports personal fees from Takeda Pharmaceuticals for membership of the data monitoring committee of their dengue vaccine clinical trials (reference numbers DEN-203, DEN-204, DEN-205, DEN-301, DEN-313), outside the area of work discussed here. E-EO was a paid Scientific Advisory Board member for the dengue vaccine trials for Sanofi Pasteur (2014–16) and is a paid member of the Scientific Advisory Board on dengue for Janssen Pharmaceuticals in 2018. The views expressed in this Seminar are those of the authors and do not necessarily represent the decisions or policies of WHO.

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