

# Dengue infection

Maria G. Guzman<sup>1</sup>, Duane J. Gubler<sup>2</sup>, Alienys Izquierdo<sup>1</sup>, Eric Martinez<sup>1</sup> and Scott B. Halstead<sup>3</sup>

**Abstract** | Dengue is widespread throughout the tropics and local spatial variation in dengue virus transmission is strongly influenced by rainfall, temperature, urbanization and distribution of the principal mosquito vector *Aedes aegypti*. Currently, endemic dengue virus transmission is reported in the Eastern Mediterranean, American, South-East Asian, Western Pacific and African regions, whereas sporadic local transmission has been reported in Europe and the United States as the result of virus introduction to areas where *Ae. aegypti* and *Aedes albopictus*, a secondary vector, occur. The global burden of the disease is not well known, but its epidemiological patterns are alarming for both human health and the global economy. Dengue has been identified as a disease of the future owing to trends toward increased urbanization, scarce water supplies and, possibly, environmental change. According to the WHO, dengue control is technically feasible with coordinated international technical and financial support for national programmes. This Primer provides a general overview on dengue, covering epidemiology, control, disease mechanisms, diagnosis, treatment and research priorities.

Dengue is currently one of the world's most important neglected tropical diseases<sup>1</sup> and its incidence has increased >30-fold in recent decades alongside the geographical expansion of the *Aedes* vector mosquitoes and dengue viruses (DENVs)<sup>2,3</sup>. Transmission of DENVs occurs in Eastern Mediterranean, American, South-East Asian, Western Pacific and African regions, with new cases occurring and spreading to non-endemic areas in the United States and Europe. Dengue epidemics impose high costs to health services, to families and to the economic systems of affected countries<sup>1</sup>.

The term 'dengue viruses' groups four genetically and antigenically related viruses that are known as serotypes 1–4, each of them grouped into genotypes. Infection by any of the four serotypes can result in a range of clinical manifestations for which the timing or sequence of infections can be an important determinant of disease severity and course<sup>4</sup>. Dengue illness is clinically classified as either dengue with or without warning signs or severe dengue<sup>2</sup> (FIG. 1). This classification was launched by the WHO in 2009 for the purpose of improving clinical management. The assessment of warning signs is designed to permit the early identification of patients with more-severe disease manifestations who require supportive therapy. Dengue illness can also be divided into three separate phases: the acute (febrile) phase, the critical (plasma leakage) phase and the convalescent or reabsorption phase (BOX 1). The 2009 classification replaced the previous 1997 WHO system that addressed and underscored the two pathological phenomena associated with the disease: plasma leakage and abnormal haemostasis. Under this classification,

patients were designated as having either dengue fever — a nonspecific febrile illness and the most common manifestation of DENV infection — or dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS) — a combination of plasma leakage and coagulopathy, sometimes accompanied by bleeding that can lead to a rapid fall in blood pressure and consequently to circulatory shock and organ impairment<sup>3</sup> (BOX 1).

This Primer provides special emphasis on the epidemiology, diagnosis, clinical management, pathogenic mechanisms and control of dengue. Research priorities are also discussed.

## Epidemiology Transmission

DENVs are maintained in an endemic–epidemic cycle involving humans and mosquitoes in crowded tropical urban centres. These viruses are fully adapted to humans, and the highly domesticated principal vector mosquito *Aedes aegypti* emerged long ago from sylvatic cycles involving non-human primates and canopy-dwelling *Aedes* mosquitoes in the rainforests of Asia and Africa<sup>4</sup>. Although these cycles still exist, their public health importance is uncertain. *Ae. aegypti* was introduced to the Americas during the slave trade in the 1600s and spread worldwide as the shipping industry expanded. This species lives in intimate association with and feeds on humans, rests in their homes and lays its eggs in man-made water containers. The average female mosquito lives for approximately 1 week, but some females can live for ≥2 weeks<sup>5</sup>.

Correspondence to M.G.G. Institute of Tropical Medicine 'Pedro Kouri', PAHO/WHO Collaborating Center for the Study of Dengue and its Vector, Autopista Novia del Mediodía, Km 6 1/2, Havana 11400, Cuba. [lupe@ipk.sld.cu](mailto:lupe@ipk.sld.cu)

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**Author addresses**

<sup>1</sup>Institute of Tropical Medicine 'Pedro Kouri', PAHO/WHO Collaborating Center for the Study of Dengue and its Vector, Autopista Novia del Mediodia, Km 6 1/2, Havana 11400, Cuba.

<sup>2</sup>Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore.

<sup>3</sup>Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

The female mosquito becomes infected when she takes a blood meal during the acute febrile and viraemic phase of illness. During the extrinsic incubation period, the virus first infects midgut cells and then disseminates to replicate in numerous mosquito tissues, ultimately infecting the salivary glands 5–12 (generally 8–10) days later, a process that is influenced by ambient temperature, the viral strain and the competence of the mosquito. Once the salivary glands are infected, the mosquito is infective and can transmit the virus to another person during blood-feeding<sup>4</sup>. The mosquito remains infective for life and can infect every person it subsequently feeds on or probes while trying to feed. The time from infection to onset of illness (the intrinsic incubation period) in humans ranges from 3 to 14 days, with an average of 4–7 days<sup>4,6</sup> (FIG. 2). Vertical transmission can occur when the infected female mosquito transmits the virus through the eggs to her offspring, but the epidemiological importance of this mode of transmission is uncertain<sup>4</sup>.

**Global burden of disease**

Although DENVs achieved distribution throughout the tropics in the eighteenth and nineteenth centuries, during the twentieth and twenty-first centuries, globalization enabled their more-rapid spread and the introduction of multiple viral serotypes into permissive areas, resulting in most tropical regions becoming hyperendemic (that is, with multiple viral serotypes co-circulating). This rapid spread began with a pandemic of dengue in South-East Asia in the 1950s that was associated with regional economic and urban growth after World War II. Epidemic activity dramatically accelerated in the 1970s and 1980s, leading to a global geographical expansion of viruses and mosquito vectors, and the consequent widespread DENV transmission across the tropics and subtropical areas<sup>4,7</sup> (FIG. 3). This geographical expansion resulted in increased frequency and magnitude of epidemics and increased frequency of severe disease (FIG. 4). The principal drivers of this twentieth century pandemic were global trends, such as human population growth, urbanization, modern transportation, global trade and the absence of effective mosquito control in endemic countries<sup>4,7–11</sup>. The geographical spread and increased epidemic activity in the 1970s coincided with the jet airplane becoming a principal mode of human travel<sup>4,8</sup>. This development led to more-frequent epidemics followed by clinically silent or undetected transmission

during inter-epidemic periods. Large cities tend to be hyperendemic, with co-circulation of all four serotypes. Epidemics might occur when herd immunity to one of the four serotypes wanes and/or when a new epidemic strain of virus emerges or is introduced. Although not documented, an increase or change in vector competence of the mosquito population might also influence epidemic transmission.

Recent best estimates of dengue disease burden suggest that over half of the world's population (3.6 billion people) live in areas that place them at risk of DENV infection<sup>12</sup>, with 390 million overall DENV infections, 96 million symptomatic infections<sup>10</sup>, 2 million cases of severe disease<sup>12</sup> and 21,000 deaths per year<sup>13</sup>. The highest incidence of DENV infection occurs in Asia where children between 5 and 15 years of age are primarily infected, followed by the American tropics where the modal age of infection is 19–40 years, depending on the country<sup>8</sup>. Dengue rates in Africa are unknown<sup>14</sup> because many outbreaks and cases might be misattributed to malaria<sup>15</sup>. During the past 40 years, a steady increase in dengue epidemic activity in Africa has been noted, as well as in the isolated islands of the Pacific and Indian Oceans<sup>4,8,14</sup>.

As with disease burden, the economic consequences of dengue are not well studied. However, estimates of disability-adjusted life years have shown dengue to be in the same order of magnitude or higher as most of the major infectious diseases, such as upper respiratory infections and hepatitis B virus infection<sup>16</sup>. Recent estimates of direct and indirect costs resulting from DENV infections are considerable, averaging US\$2.2 billion per year in the Americas between 2000 and 2007, US\$1.2 billion in South-East Asia per year between 2001 and 2010 and US\$76 million in Africa per year<sup>17,18</sup>. A recent study estimates the annual global cost of dengue at US\$8.9 billion<sup>19</sup>. These are conservative estimates and are subject to many uncertainties.

**Mechanisms/pathophysiology****Dengue viruses**

**The viral genome.** DENVs belong to the genus *Flavivirus* of the *Flaviviridae* family. The four serotypes are enveloped, spherical viral particles with a diameter of approximately 500 Å<sup>20</sup>. The genome of each serotype comprises approximately 11 kb of positive-sense, single-stranded RNA that encodes ten proteins. The three structural proteins encoded by the genome are the membrane (M) protein, envelope (E) protein and capsid (C) protein; the non-structural (NS) proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

**Structure and function of the E protein and M protein.**

The delivery of the DENV genome into the host cytoplasm is a multistep process that begins with fusion of the viral membrane with the host plasma membrane, followed by endocytosis of the virus into an endosome and then pH-dependent fusion of the viral and endosomal membranes (FIG. 5). The inside of the virus particle is formed by RNA complexed with capsid proteins and is surrounded by a lipid bilayer membrane

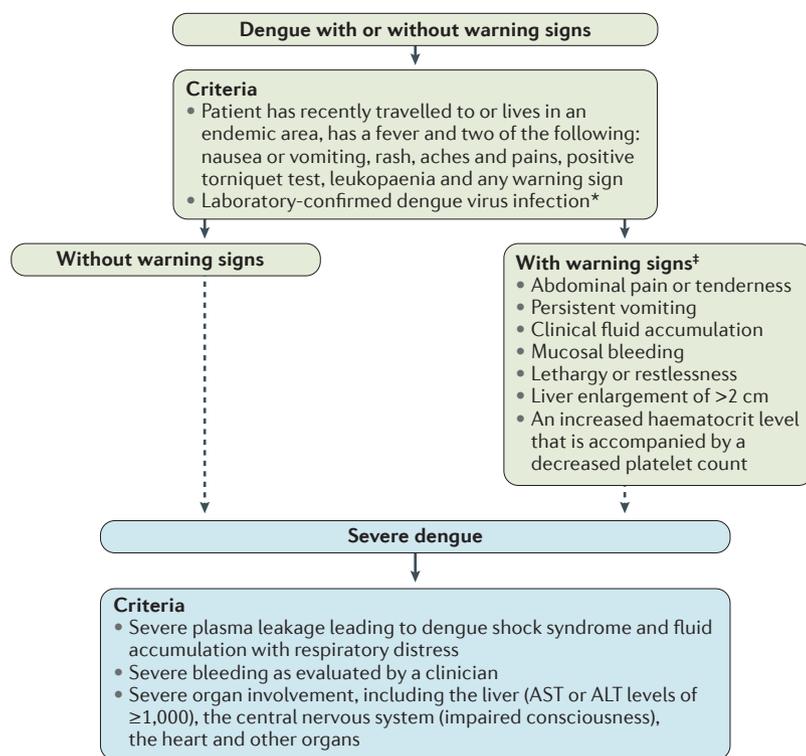


Figure 1 | **The 2009 WHO dengue clinical classification.** The criteria for dengue with or without warning signs and for severe dengue. Dashed arrows indicate that not all patients progress to severe dengue. \*Important when there is no sign of plasma leakage. †Requires strict observation and medical intervention. ALT, alanine aminotransferase; AST, aspartate aminotransferase. From REF. 2. Adapted with permission, from WHO/TDR, Dengue Guidelines for Diagnosis, Treatment, Prevention and Control — New Edition, World Health Organization and Special Programme for Research and Training in Tropical Diseases, WHO Press, Figure 1.4, 2009.

that contains externally anchored M protein and E protein, which together orchestrate host–viral interactions during entry. The E protein is arranged on the surface of DENVs as 90 tightly packed monomers that lie flat against the membrane and facilitates viral entry into host cells by binding to cellular receptors and mediating the fusion of viral and cellular membranes<sup>21,22</sup>. Of its three domains (DI–DIII), DIII of the E protein is responsible for binding to host receptors and several mutations have been identified in this domain that affect receptor binding<sup>21–23</sup>. The hinge that connects DI to DII is highly flexible and is used to manoeuvre DII in the low pH environment of the endosome, leading to the exposure of the DII fusion loop. This fusion loop then interacts with the endosomal membrane to facilitate fusion of the virus with the endosomal membrane and release of viral RNA into the host cell<sup>24</sup>. Upon release from infected cells, new DENV particles can either contain processed surface M protein, and therefore be infective or ‘mature’, or retain the uncleaved precursor form of the M (prM) protein on its surface and thereby remain in an ‘immature’ form. Some viral particles can have a mixture of M protein and prM protein on their surface and these particles may or may not be infective.

**Structure and function of the NS proteins.** The NS proteins are involved in viral replication and packaging, processes that are closely linked to host endoplasmic reticulum (ER) and secretory pathway function (FIG. 5). NS1 is a 46 kDa glycoprotein that exists in three forms: the ER-resident form; the membrane-anchored form; and the secreted form. NS1 is initially synthesized as a soluble monomer and becomes associated with the membrane after dimerization in the lumen of the ER<sup>25</sup>. The crystal structure of NS1 has recently been determined and revealed exposed hydrophobic domains in the dimer that probably mediate this membrane association<sup>26</sup>. Intracellular NS1 participates in early viral RNA replication and is found in virus-induced vesicular compartments that house the viral replication complexes<sup>27</sup>. NS1 is also transported to the cell surface, where it either remains associated with the cell membrane or is secreted (sNS1) as a soluble, lipid-associated hexameric species. sNS1 can be detected in the blood of infected patients from the first day of symptoms and circulates at levels in the ng per ml to mg per ml range during the acute phase of infection<sup>28</sup>, and blood levels of sNS1 correlate with peak viraemia and disease severity in secondary DENV infection<sup>28</sup>. Several studies have suggested that sNS1 is a key mediator of dengue pathogenesis. For instance, highly purified recombinant NS1 (rNS1) devoid of bacterial endotoxin activity directly activates mouse macrophages and human peripheral blood mononuclear cells via Toll-like receptor 4 (TLR4), leading to the induction and release of pro-inflammatory cytokines and chemokines. In addition, in *in vitro* and *in vivo* models of vascular leakage, exposure to NS1 resulted in the disruption of endothelial cell monolayer integrity<sup>29,30</sup>. The key features of NS1 and of the other DENV NS proteins are detailed in TABLE 1.

### Locus of DENV infection in vivo

**Overview of infection.** During mosquito feeding, DENV is inoculated into the dermis and epidermis, and some virus is also injected directly into the bloodstream. In the skin, this delivery results in the infection of macrophages, dendritic cells and Langerhans cells. These infected cells can migrate from the initial site of infection to lymph nodes, which triggers the recruitment of monocytes and macrophages that then become subsequent targets of DENV infection. As a result, the number and variety of cells infected with DENV increases and the infection can become disseminated throughout the lymphatic system with the infection of cells of the mononuclear lineage, including blood-derived monocytes, myeloid dendritic cells and splenic and liver macrophages<sup>31,32</sup>.

**Determination of infected cells in vivo.** The identification of sites of DENV infection *in vivo* is problematic because many of the stains that are used to visualize dengue viral antigens do not discriminate between intracellular antigens that have been phagocytosed and those that are indicative of active viral invasion and replication. For this reason, to identify sites of DENV

infection, it is crucial to use probes for DENV-specific RNA, negative-sense RNA or for NS proteins, which are produced at sites of viral replication or assembly. The few studies that have been published using these markers have detected DENV infection in monocytes that are present in the blood and macrophages from the liver, skin, spleen and thymus<sup>33,34</sup>. In addition, sites of infection can also be identified through the detection of viral particles. Peripheral blood monocytes from patients with dengue have been found to harbour DENVs<sup>35</sup>, and immature skin dendritic cells support the growth of DENVs<sup>36</sup>.

Although dengue viral particles or antigens have been shown to localize to neurons, microglia and endothelial cells in the human central nervous system (CNS)<sup>37</sup>, no evidence of DENV replication was found in the CNS when a sensitive viral RNA amplification method was applied to samples from patients who had died as a result of DENV infection<sup>38</sup>. Moreover, no DENV antigens were detected in CNS tissue from 13 children who had died from DHF/DSS<sup>33</sup>.

DENV can also infect the liver, resulting in apoptosis of hepatocytes<sup>39</sup>, and when apoptotic DENV-infected hepatocytes are engulfed by Kupffer cells, they form Councilman bodies — the classic histopathological finding in the livers of those with dengue and yellow fever. Results from studies that attempted to

culture DENVs in explanted human tissue, primary human cells and human cell lines suggest that DENV1 is unable to replicate in mature Kupffer cells. By contrast, primary hepatocytes and hepatocyte cell lines were successfully infected but underwent apoptosis shortly after<sup>40,41</sup>. Although DENV infection imparts considerable damage to the liver, infection of this organ might not make a considerable contribution to the distribution or maintenance of acute infection<sup>33</sup>.

**Host receptors of DENV entry.** DENVs are capable of infecting many different cell types *in vitro*, including epithelial cells, endothelial cells, hepatocytes, muscle cells, dendritic cells, monocytes, bone marrow cells and mast cells. Despite the ability to infect these cells with DENVs in a laboratory setting, their roles in dengue pathogenesis and the cellular receptors involved in their infection remain unknown. Although candidate receptors — such as heparan sulfate, dendritic cell-specific ICAM3-grabbing non-integrin, macrophage mannose receptor 1, heat shock protein 70 (HSP70) and HSP90 — have been described for *in vitro* systems, their contribution to infection in humans is not established<sup>42</sup>.

#### Infection and response

Four main factors control DENV disease response along a response continuum: immune status, virus strain, genetic status and age (FIG. 6).

**Immune status.** Infection with any DENV serotype results in long-term homotypic immunity (that is, immunity against the serotype causing the infection) with a short period of heterotypic immunity (that is, immunity against another serotype)<sup>43,44</sup>. Only a small fraction of circulating antibodies in monotypic-immune individuals (that is, those only immune to one viral serotype) neutralize homologous DENV. Polyclonal antibodies are directed against several epitopes; some are directed against quaternary epitopes located at the hinge region between DI and DII of the E protein on the surface of intact virions<sup>45,46</sup>. Immediately after an individual's first DENV infection, antibodies might neutralize heterotypic DENVs in *in vitro* assays. Over the next few months, antibodies become increasingly specific to the DENV serotype that is causing the infection<sup>47</sup>. These *in vitro* changes in neutralizing antibody specificity correlate with *in vivo* observations. Monotypic-immune humans demonstrate an initial short period of cross-protection against infection with heterotypic DENVs (approximately 2 months) and a longer period of protection against severe disease (approximately 2 years) caused by heterotypic DENV infections<sup>47</sup>. Recently, a class of strong broadly cross-neutralizing antibodies was recovered and characterized<sup>48,49</sup>. Whether these antibodies are preferentially selected during second heterotypic DENV infections and contribute to pan-DENV protection are unknown. Neutralizing antibodies circulate for a lifetime and are thought to explain the observed long-lasting protection against re-infection with homotypic DENVs<sup>50,51</sup>.

#### Box 1 | Key clinical terms

##### Acute phase

- Characterized by high fever that is driven by high viral loads (viraemia)

##### Critical phase

- Characterized by plasma leakage into the abdominal and pleural cavities, which becomes evident at the end of the febrile (acute) stage of illness (days 3–6)
- Warning signs that announce shock are usually present

##### Convalescent phase

- Involves both cessation of plasma leakage and reabsorption of leaked fluids

##### Dengue or dengue fever

- A nonspecific febrile illness that is characterized by fever and the presence of two or more other symptoms, such as headache, rash, retro-orbital or ocular pain and myalgia
- Most patients have a satisfactory resolution without signs of severity or warning signs (referred to as dengue without warning signs according to the 2009 WHO classification)

##### Dengue haemorrhagic fever

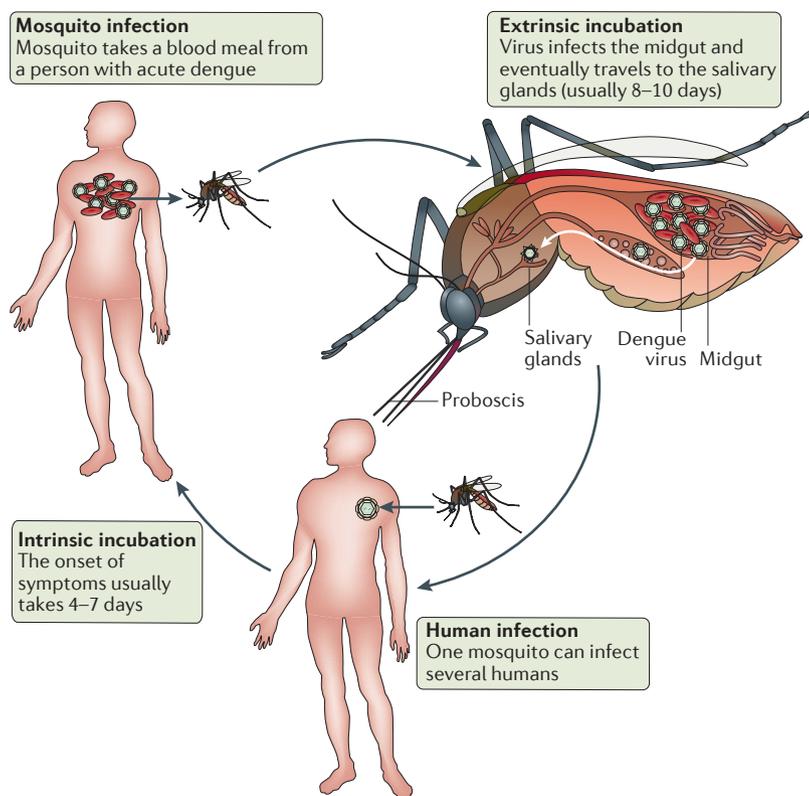
- Characterized by increased vascular permeability, plasma leakage, bleeding, thrombocytopenia and fever (according to the 1997 WHO classification)
- The term and concept are not included in the revised 2009 WHO classification nor are they recommended for triage of patients because it is not necessarily associated with severity, among other reasons

##### Dengue with warning signs

- At the end of the febrile period, some patients have signs or symptoms that are suggestive of important fluid loss associated with capillary leakage (for example, severe abdominal pain), announcing the imminence of shock and indicating that fluid replacement is urgently required (according to the 2009 WHO classification)

##### Severe dengue

- Circulatory shock or respiratory distress associated with severe plasma leakage, severe bleeding or severe organ involvement (frequently myocarditis, encephalitis and severe hepatitis) with or without shock or bleeding (according to the 2009 WHO classification)



**Figure 2 | The urban dengue virus cycle in humans and mosquitoes.** An *Aedes aegypti* mosquito can become infected by feeding on a person in the viraemic phase of infection. During the extrinsic phase of the cycle, dengue viruses first infect mosquito midgut cells and other tissues before disseminating to the salivary glands. An infected mosquito can then transmit the dengue virus to several humans as it feeds and 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.

The majority of circulating antibodies are non-neutralizing and are directed against various antigens on the E protein and the prM protein. In the absence of blocking by type-specific neutralizing antibodies, non-neutralizing antibodies usually enhance the entry of any DENV into Fc receptor-bearing cells. This phenomenon is called antibody-dependent enhancement (ADE)<sup>52,53</sup> and makes DENVs unique among human viral infections, in that pre-infection partial immunity to one or more DENV (so-called sensitization) upgrades disease severity. As such, severe dengue and DHF/DSS occur most often in individuals with monotypic immunity during a second heterotypic DENV infection. Severe cases also occur (infrequently) in primary infections. By contrast, third and fourth DENV infections are typically mild or asymptomatic<sup>54,55</sup>.

In dengue-endemic countries, DHF/DSS also occurs during primary infections in infants born to mothers who are immune to DENV. These mothers circulate antibodies from two or more lifetime DENV infections that occurred before pregnancy<sup>56–58</sup>. As discussed above, these individuals develop broad neutralizing antibodies and are usually protected against severe disease with heterotypic DENV strains<sup>55,59</sup>. Epidemiological data

from many countries indicate that these multitypic antibodies protect infants from dengue illness for several months after birth<sup>60,61</sup>. Then, the concentration of these neutralizing maternal antibodies eventually falls below a protective threshold, with most antibodies having a half-life of approximately 40 days. For a short period, if the infant is infected with DENV, severe disease mediated by ADE may occur owing to the presence of maternal non-neutralizing antibodies.

In addition to serotype-specific immunity, the interval between an individual's first DENV infection and subsequent infections might be an important determinant of disease severity. For example, in a comparison of groups of identical age, DHF occurred at an eightfold higher rate in those whose secondary DENV2 infections were separated by 20 years than those whose secondary infection was 4 years after their first. A possible reason why severe disease rates increase with longer intervals between infections might be related to the steady decline in heterotypic neutralization of DENV2 by DENV1 antibodies<sup>62</sup>. A second phenomenon sometimes observed in dengue is that infection sequences that were previously benign can suddenly become pathogenic. For example, in Tahiti in 2001, DHF occurred in children 4–13 years of age who contracted DENV1 infections, even though they had previously been infected with DENV2 4–5 years earlier<sup>63</sup>. By contrast, during the 1980 Rayong (Thailand) epidemic, no severe disease accompanied secondary DENV1 infections, even though they comprised 37.5% of all secondary DENV infections<sup>64</sup>, indicating that, although infection sequence is important, a secondary infection with DENV1 does not necessarily cause severe disease.

The relative contribution of antibodies versus T cells to protection against dengue is not well understood, although there is growing evidence that both components are required to prevent infection, overt disease and severe disease<sup>65–67</sup>. For example, recent studies on naturally infected humans and infection of humanized mice indicate that T cells contribute to protection against severe disease associated with heterotypic secondary DENV infections<sup>68</sup>. Effective CD8<sup>+</sup> T cell immunity is largely mediated by epitopes on NS proteins, their respective contribution varying between different DENV serotypes<sup>69</sup>.

**Viral serotypes.** Although all four DENV serotypes are transmitted by *Aedes* mosquitoes and, in principle, cause the same clinical manifestations and show similar patterns of systemic dissemination, there are some biological differences between them<sup>60,70</sup>. Indeed, associations between particular serotypes or genotypes and disease severity, epidemic potential and the efficiency of transmission have been described, but these associations could be influenced by factors other than intrinsic viral characteristics, such as host immunity, the ability of the mosquito vector to become infected and to transmit the virus to humans, and the conditions (which are not well known) that support the displacement of one genotype by another<sup>71–73</sup>.

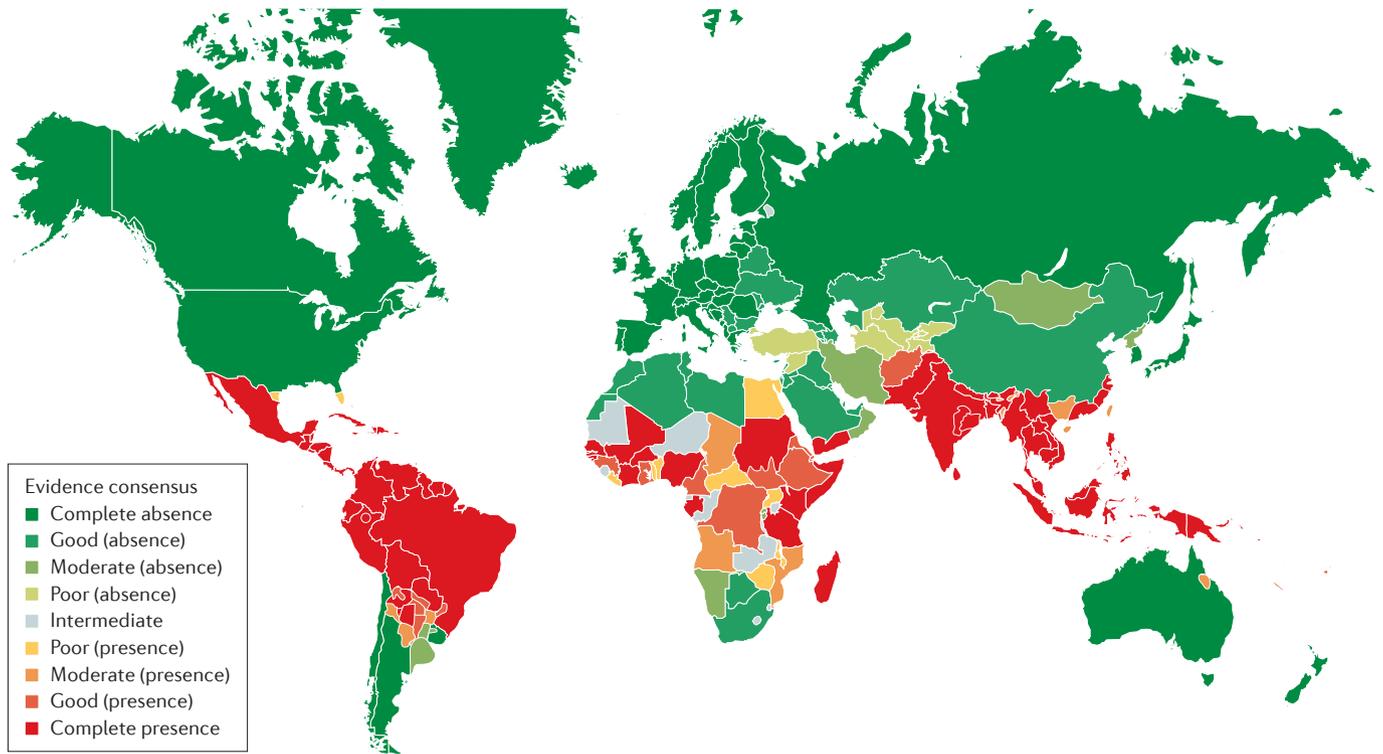


Figure 3 | **The suitability of different regions for dengue virus transmission.** The global evidence consensus, risk and burden of dengue is shown with evidence consensus on complete absence (dark green) through to complete presence (dark red) of dengue. Adapted from REF. 10, Nature Publishing Group.

The hypothesis that some DENVs have greater ‘virulence’ and epidemic potential than others was introduced in the 1970s<sup>61,74</sup>. The DENV polyprotein demonstrates 30% divergence between the four serotypes, and several genotypes within each serotype show different geographical distributions<sup>71</sup>. Some data indicate that genetic changes in DENVs might directly affect transmission potential in mosquitoes or disease expression in infected humans. For example, some studies have shown that DENV2 of Asian origin replicates to higher titres in human dendritic cells, infects *Ae. aegypti* mosquitoes more efficiently and is transmitted at a higher rate than American DENV2 strains<sup>75,76</sup>. In addition, some strains of DENV3 replicate at a higher rate in mosquitoes than other DENV3 strains, leading to the capacity to displace established DENV3 strains<sup>68,77</sup>. Similarly, when DENV4 was introduced to Puerto Rico, it caused three major epidemics in 1982, 1986 and 1998; the latter two epidemics were each associated with a clade (that is, a monophyletic group of the same genotype) change in the circulating virus<sup>78,79</sup>. Similar clade changes associated with endemic and epidemic transmission have been observed with DENV2 in the South Pacific<sup>74,80</sup> and DENV3 in Sri Lanka<sup>81,82</sup>. To date, most of the genetic changes associated with epidemic potential have resulted in amino acid changes in NS proteins.

Several pathogenesis studies have been performed on patients with dengue who were clinically classified as having either dengue fever or DHF/DSS<sup>3</sup>. Although both

dengue fever and DHF/DSS can be associated with any serotype, some sequences of infection have been associated with severe disease at a higher frequency than others<sup>83</sup>. In addition, some serotypes may be associated with DHF/DSS during a secondary infection but result predominantly in mild or asymptomatic infections during primary infections. Furthermore, there is no evidence that severe dengue regularly accompanies primary infections of susceptible individuals. In this context, DENVs are not inherently ‘virulent’ but are instead conditionally virulent. That condition is usually the presence of pre-infection circulating DENV antibodies, as discussed above<sup>73</sup>. For example, in the very ‘clean’ epidemiological setting of the Santiago de Cuba DENV2 epidemic of 1997, susceptible individuals of all ages who were infected by the DENV2 Asian genotype primarily developed subclinical disease. By contrast, individuals who contracted a secondary infection with DENV2 after first having a DENV1 infection almost always experienced overt disease (the overt to subclinical disease ratio was nearly 1). Similar observations have been reported for primary DENV4 infections<sup>84</sup>.

Of interest is the possibility that, during the course of epidemics, there is a rapid selection of DENV neutralization escape mutants or of mutations that affect the ability of certain DENVs to cause infections that involve different degrees of ADE<sup>85</sup>. In support of this possibility, in three Cuban epidemics, month-to-month increases were observed in the proportion of severe secondary

DENV cases compared with mild cases as well as in case fatality rates. For example, in the 1997 DENV2 outbreak in Santiago de Cuba, there was an emergence of severe disease that was accompanied by a stable amino acid switch in NS1 (REF. 86). Somewhat similar increases in severity of secondary DENV2 infections have been described in Taiwan and Nicaragua; these were associated with several mutations, including changes in the structure of the DENV 3' untranslated region (UTR)<sup>87,88</sup>. Although the precise mechanisms of the rapid acquisition of increased fitness of DENVs are unknown, suspicions increasingly point to improved viral survival during interactions with the human innate immune system<sup>89</sup>.

**Host genetics.** As with most infectious diseases, host factors, many of which are measured by proxy genetic markers, can control the outcome of infection through mechanisms that are not yet fully understood. Some host factors that affect the outcome of DENV infection have been well documented. For example, an unidentified gene that is present in black individuals moderates the clinical severity of secondary DENV infections, and studies have shown that the rates of DHF/DSS were lower in infected black patients than in white patients with the same secondary DENV2 infection experience<sup>90,91</sup>. TABLE 2 includes some human leukocyte antigen (HLA) and non-HLA genes that are linked with increased susceptibility or resistance to dengue<sup>92-102</sup>.

**Patient age and sex.** Age affects dengue disease expression in a contradictory manner. The disease severity that accompanies a first DENV infection is directly related to age. In susceptible young children, first DENV infections are usually occult or mild, whereas adults experiencing a first DENV infection often develop dengue fever. More complicated outcomes are observed in the elderly or in those with chronic diseases, such as diabetes mellitus, chronic obstructive pulmonary disease or cardiovascular disease<sup>2</sup>. Bleeding phenomena are common in both the elderly and those with comorbidities. Menorrhagia has been observed in adult women during primary DENV infections and gastrointestinal bleeding has been observed in individuals with peptic ulcer disease. The risk of progressing to DHF/DSS in sensitized individuals varies inversely with age. When an entire population (people  $\geq 3$  years of age) was exposed to identical rates of secondary DENV2 infection, DHF rates were more than fivefold higher in children than in adults<sup>103</sup>. This observation can probably be explained by intrinsic host susceptibility to vascular permeability that accompanies a secondary DENV infection<sup>104,105</sup>. Indeed, healthy children have been reported to have a higher capillary fragility than adults. The greater density and surface area of growing microvessels in childhood could be the reason for why children have this higher microvascular permeability<sup>106,107</sup>. The outcome of secondary DENV infections is also controlled by sex, with girls  $>4$  years of age having higher rates of DSS than boys of any age<sup>59,86</sup>.

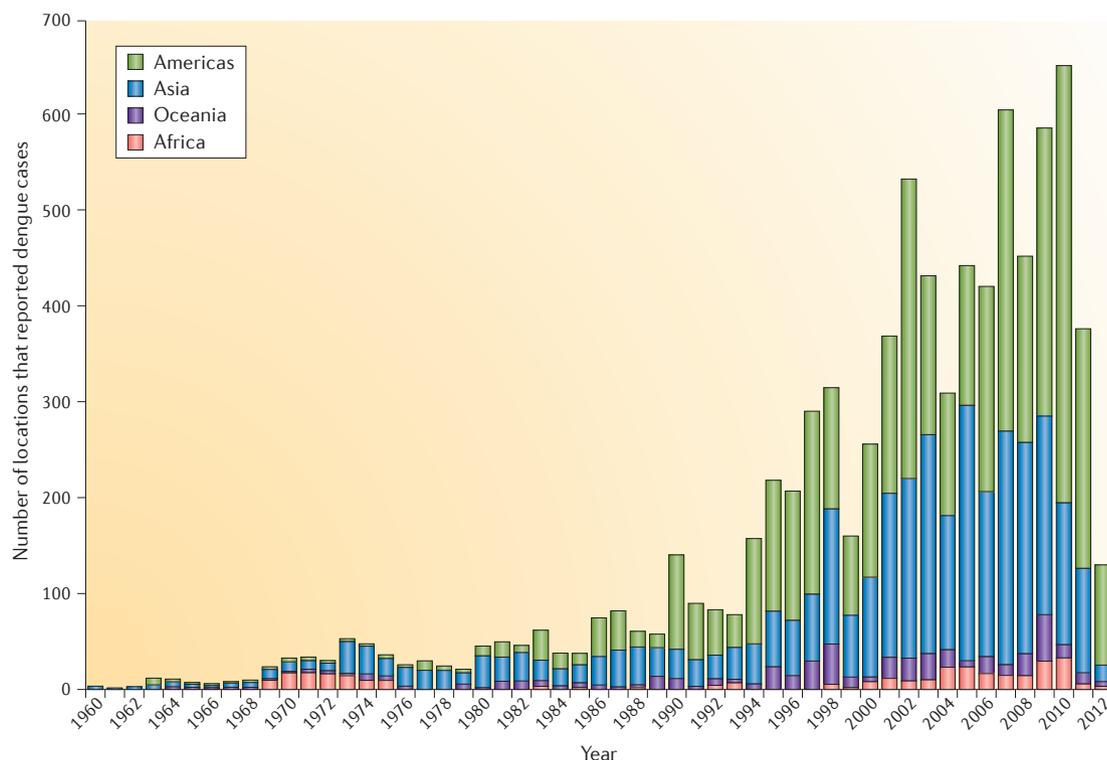
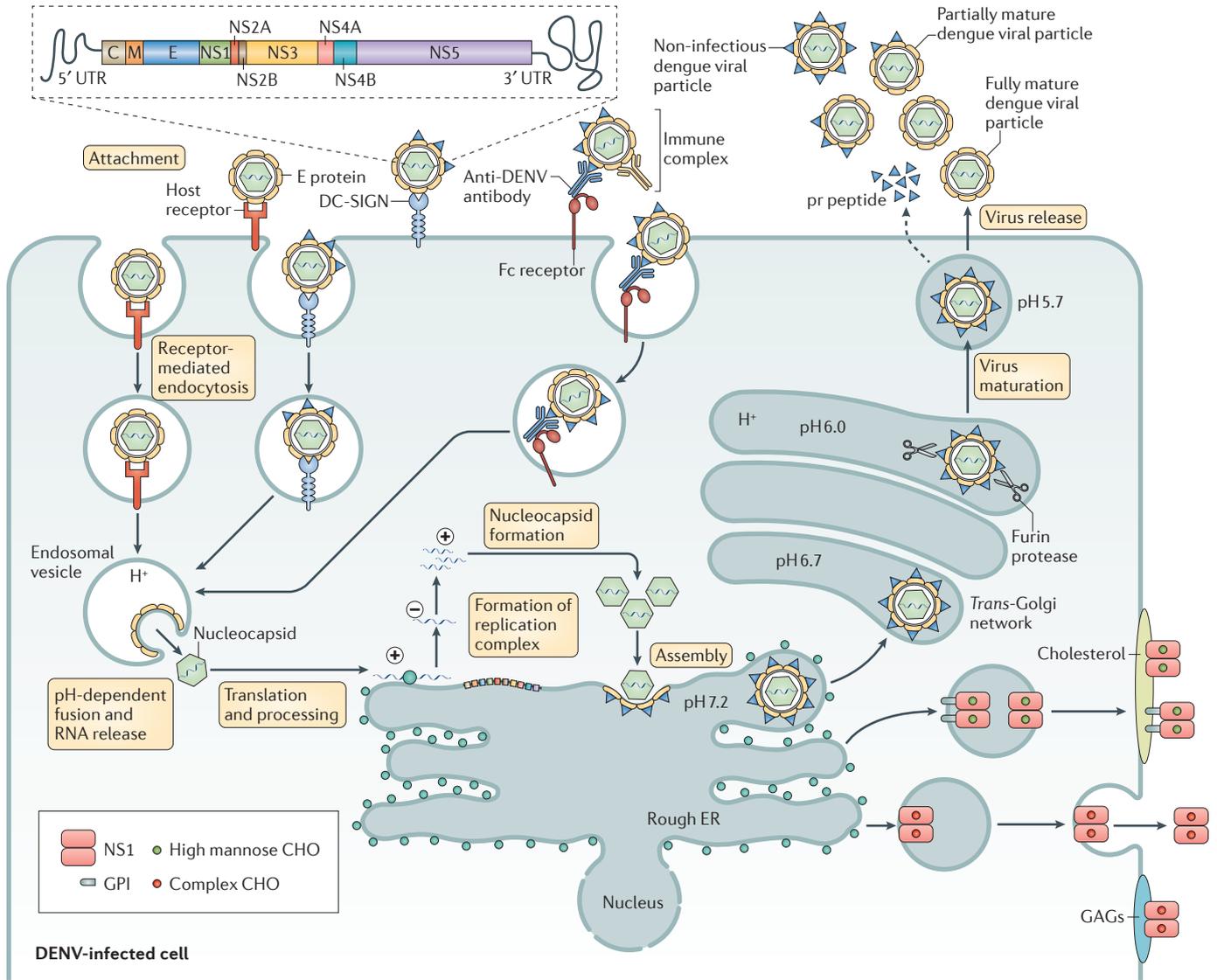


Figure 4 | **The changing epidemiology of dengue.** The number of locations that reported dengue between 1960 and 2012 are shown. An increasing trend is apparent in the number of locations reporting dengue, with higher figures in American and Asian regions. The figure shows the number of occurrence points by date recorded in mapping exercises; it is neither a prevalence nor an incidence metric, but an audit of data sources per year. Adapted from REF. 301, Nature Publishing Group.

**Pathophysiology**

Acute DENV infections are expressed along a continuum from inapparent to undifferentiated fever, to an acute febrile viral exanthema and finally to a complex of physiological abnormalities that affects multiple systems, including the liver, blood coagulation, complement, haematopoiesis and the vascular systems.

**Vascular permeability.** Dengue vascular permeability syndrome, which was historically known as DHF/DSS, includes the abnormalities that affect the vascular system. Several studies have shown that there is a range of capillary permeability and plasma leakage affecting most individuals with overt dengue illnesses, rather than distinct pathological mechanisms underlying



**Figure 5 | Dengue virus life cycle.** Mature viral particles attach to host cells by the binding of the envelope (E) protein to unknown receptors. Viral entry is achieved through receptor-mediated endocytosis. Inside the host cell, pH-dependent rearrangement of the E protein facilitates fusion of the viral and endosomal membranes to release the nucleocapsid, which disassembles to release the capped viral genomic RNA. The genomic RNA is then translated into a long polyprotein, which is autocatalytically cleaved by the non-structural 2B (NS2B) or NS3 viral protease and host proteases into individual proteins. The released NS proteins are targeted to the site of replication on endoplasmic reticulum (ER)-derived vesicle packets to initiate transcription. NS1 is initially expressed in association with the ER; the monomer is modified by the addition of high mannose carbohydrate (CHO) moieties, resulting in membrane association. A subset of dengue virus (DENV) NS1 acquires glycosyl-phosphatidylinositol (GPI). Both membrane-bound NS1 and GPI-anchored NS1 are trafficked to the cell surface via an unknown pathway, where they have been

shown to associate with lipids such as cholesterol. Some of the cell surface-associated NS1 can be previously secreted NS1 that has bound directly to cell surface glycosaminoglycans (GAGs). Some NS1 can also be secreted from host cells. Meanwhile, the precursor form of the membrane (M) protein (prM) and the E protein are embedded into the ER membrane and enclose the newly formed nucleocapsid as it buds into the ER lumen to form an immature particle. This particle is trafficked via the secretory pathway, in which the low pH of the *trans*-Golgi network causes substantial rearrangement of the prM and E proteins that permits the cleavage of prM by furin protease to form the mature M protein. The virion is released from the host cell with the release of the pr peptide (dashed arrow). A subset of viral particles are released with prM still intact and are unable to infect new host cells. + and - signs indicate positive-sense and negative-sense RNA, respectively. C, capsid; DC-SIGN, dendritic cell-specific ICAM3-grabbing non-integrin; UTR, untranslated region. Adapted from REF. 302, Nature Publishing Group.

Table 1 | Main features of dengue virus non-structural proteins

Protein	Size (kDa)	Key features	Main functions	Refs
NS1	46	• Can be endoplasmic reticulum-anchored, membrane-associated or secreted (sNS1)	• Intracellular NS1 is involved in early viral RNA replication • sNS1 activates the innate immune system and is implicated in vascular leakage	25,27, 39,40
NS2A	22	• Hydrophobic integral membrane protein	• Involved in RNA replication	303
NS2B	14	• Small hydrophobic protein	• Co-factor for NS3	303
NS3	69	• Multifunctional protein with several catalytic domains	• Involved in nucleoside triphosphatase and helicase functions during RNA synthesis	304
NS4A	16	• Hydrophobic integral membrane protein	• Required for the formation of replication vesicles	304
NS4B	30	• Small hydrophobic protein	• Suppresses IFN $\beta$ and IFN $\gamma$ signalling	304
NS5	105	• Largest and most highly conserved dengue virus protein	• Involved in RNA synthesis • Involved in blockade of the IFN system	305

IFN, interferon; NS, non-structural.

DHF/DSS. For example, endothelial cell damage by infection or extensive cell death does not seem to be responsible for the increase in vascular permeability associated with dengue<sup>33,108</sup>. Indeed, patients who are recovering from DHF regain normal endothelial function relatively quickly, implying that whatever causes vascular permeability is more reversible than endothelial damage, and might include one or more soluble mediator<sup>109</sup>. In addition, increased microvascular permeability has been reported in patients with DHF/DSS at or around the time they experience defervescence (a decrease in increased temperature)<sup>109,110</sup>, indicating that vascular permeability is not directly correlated with peak viraemia that usually occurs on the first day after the onset of fever. A mild increase in microvascular permeability has been observed in infected volunteers experiencing dengue fever, indicating that dengue disease severity occurs across a continuum<sup>111,112</sup>.

Recent studies on myeloid cells, both *in vitro* and in mice, indicate that NS1 induces vascular leakage and activation of TLR4, resulting in the production of inflammatory cytokines<sup>29,30</sup>. These studies suggest that circulating DENV NS1 triggers endothelial barrier dysfunction, which causes increased permeability of human endothelial cells *in vitro*. These findings open a new window of opportunity for dengue drug and vaccine development<sup>29,30</sup>.

**Thrombocytopenia.** Thrombocytopenia results from transient bone marrow suppression and increased peripheral destruction of platelets during the febrile and early convalescent phases of the disease, and results in platelet counts as low as 5,000 per ml (compared with approximately 200,000 platelets per ml in healthy individuals)<sup>113,114</sup>. Whereas thrombocytopenia occurs commonly across a wide range of infectious diseases, severe thrombocytopenia accompanies clinically significant vascular permeability during acute DENV infections. Remarkably, in the bone marrow, early suppression of the production of all blood cell types occurs

during the early febrile phase of DENV infection<sup>115,116</sup>. A possible explanation for this suppression comes from studies on lymphocytic choriomeningitis virus infection. Here, in infected laboratory animals, bone marrow suppression was mediated by interferon- $\alpha$  (IFN $\alpha$ ) production<sup>117</sup>. Towards the end of the febrile period of a DENV infection, the bone marrow cells recover to normal density and diversity, leaving only a residual megakaryocyte arrest that can be observed in autopsy studies<sup>118</sup>.

**Coagulopathy.** The impaired haemostasis that accompanies DHF/DSS involves a series of alterations in the coagulation system that disrupts the regulation of clot formation. For example, an increase in activated partial thromboplastin time (APTT; which measures time to clot formation) and a reduction in the level of fibrinogen (a factor that promotes clot formation) are fairly consistent findings in DHF/DSS<sup>119–121</sup>. The evidence that haemorrhage in dengue is caused by classic disseminated intravascular coagulation is under debate<sup>120</sup>. The concentration of procoagulant markers is increased in some DHF/DSS cases, but this increase is usually mild and is accompanied by a considerable reduction in the concentration of anticoagulant proteins.

Factors that might contribute to these alterations include secreted viral effectors. Recent reports have shown that NS1 binds to thrombin *in vivo* to form NS1–thrombin complexes. In addition, *in vitro*, rNS1 inhibits prothrombin activation and prolongs APTT in human platelet-deficient plasma<sup>122</sup>. Release of heparan sulfate or chondroitin sulfate (molecules similar in structure to heparin that mimic its function as an anticoagulant), which are possibly sheared off by NS1 from the glycocalyx, can also contribute to altered haemostasis<sup>112</sup>.

In most patients, coagulopathy is relatively minor and resolves within a few days. In some children with severe shock, these minor derangements are compounded by the effects of prolonged hypotension and tissue hypoxia. Major bleeding occurs by erythrocyte extravasation in the gastrointestinal tract.

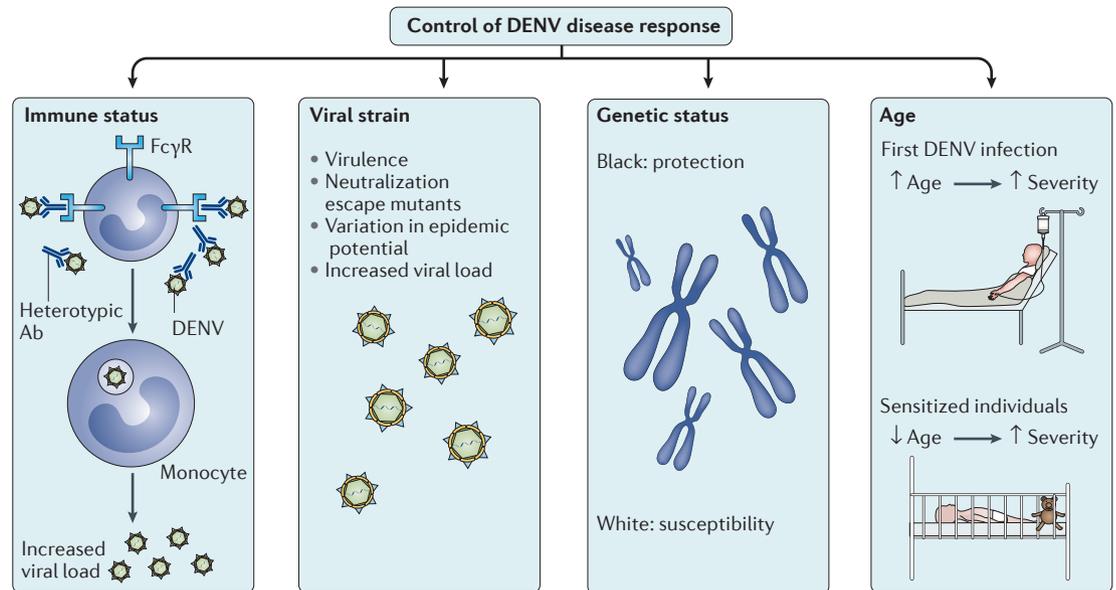


Figure 6 | **Factors involved in the control of dengue virus disease response.** Four main factors control dengue virus (DENV) disease response along a response continuum: immune status, virus strain, genetic status and age. Individuals at risk of a secondary infection are at a higher risk of severe disease through the antibody-dependent enhancement phenomenon, with a higher production of virus. Genetic dengue susceptibility favours disease severity. For example, white individuals have been found to have a higher chance of developing severe disease than black individuals. Younger age is associated with disease severity in individuals experiencing a secondary infection. The association of each serotype and genotype to disease severity, epidemic potential and efficiency transmission could be influenced by the differences among them, but also by other conditions such as host immunity, the ability of the vector to become infected and to disseminate the virus to humans, among others. FcγR, Fcγ receptor.

**Complement activation.** The complement system becomes activated to control DENV infection, and this activation contributes to pathogenesis through interaction with the coagulation system. In classic studies on complement in children with DHF/DSS, temporal and peak production of complement split products correlated with blood fibrinogen levels and thrombocytopenia<sup>123</sup>. Complement activation has been described in detail in a 6-month-old infant with DSS during a primary DENV infection<sup>124</sup>. Most studies on complement activation in dengue have centred on patients with secondary DENV infections and have led to the conclusion that complement activation was mediated via the classical pathway by circulating immune complexes. By contrast, studies on complement activation in infants experiencing a primary DENV infection have implicated the alternative pathway in activating complement during infection. Indeed, in children experiencing a secondary DENV infection, NS1 might be responsible for activating complement by the alternative pathway<sup>122</sup>.

**Liver enlargement.** Liver enlargement and dysfunction are common during DENV infection, with liver enlargement having a significantly stronger association with DHF/DSS than with dengue fever (55% compared with 18%;  $P < 0.01$ )<sup>125</sup>. For instance, one study in Thailand showed that hepatomegaly was observed in a high percentage of all children admitted with serologically confirmed severe dengue<sup>126–128</sup>. Liver enlargement seems to occur for two reasons: generalized oedema due to vascular permeability and an inflammatory

response that occurs after infection of hepatocytes by DENVs. However, almost no cellular inflammatory response was observed in livers from patients who died as a result of DENV infection, reinforcing the observation that liver damage might be caused by apoptosis, as discussed above.

Despite the prevalence of liver enlargement, jaundice in dengue illness, even in DSS, is rare<sup>125,129</sup>. By contrast, changes in liver enzyme levels, which are markers of liver dysfunction, are common. Aspartate aminotransferase and alanine aminotransferase blood levels are increased in 60–90% of children with DHF. The increase in the levels of the transaminases was shown to be mild to moderate in one study, but a small group of patients (7–10%) had transaminase levels that were tenfold higher than the upper limit of normal. In this study, co-infection with hepatitis B virus or hepatitis C virus was not related to liver enzyme changes, and liver enzyme levels were significantly higher in patients with prolonged shock<sup>130</sup>. Finally, this study also demonstrated that the levels of serum bilirubin, alkaline phosphatase and gamma-glutamyl transpeptidase were raised in 7%, 16% and 83% of patients, respectively.

**Models of vascular permeability in dengue.** Many researchers attribute vascular permeability to a lethal ‘cytokine storm’ caused by overactive T cells, heterologous T cell responses or defective T cell responses (original antigenic sin)<sup>131–133</sup>. Other hypotheses to explain this phenomenon posit that circulating immune complexes activate complement or that immune responses to

DENV proteins cross-react with host systems to generate short-lived immune or autoimmune DHF/DSS<sup>134–136</sup>. However, none of these explanations can account for why infants who have never previously experienced DENV infection develop severe dengue disease. Alternatively, capillary leakage might be caused by factors produced in target cells that are directly related to DENV infection<sup>137–142</sup>. However, this hypothesis does not fit with the observation that, although target cells are infected throughout the course of infection, the onset of vascular permeability is delayed to the time of defervescence.

The onset of thrombocytopenia, altered haemostasis, complement activation, liver damage and detectable vascular permeability before defervescence suggest that DHF/DSS might result from a factor or factors that circulate throughout the acute illness, which exceeds a threshold at defervescence. DENV virions and NS1 circulate throughout the acute phase. As discussed above, NS1 interacts with the complement system, can extend APTT and several lines of evidence support the notion that NS1 contributes to vascular permeability. Indeed, APTT values are the strongest correlate of vascular permeability in patients with dengue illness<sup>143</sup>. Moreover, it has been known for several decades that antibodies against DENV NS1 protect against lethal dengue disease in animal models<sup>144</sup>. Perhaps then, terminal T cell-mediated cytolysis of DENV-infected cells releases a bolus of cell-bound NS1 to surpass a threshold resulting in vascular permeability<sup>145</sup>. Finally, the mechanisms that underlie peak vascular permeability during defervescence in dengue remain unclear, but intensified research efforts might uncover these in the future.

## Diagnosis, screening and prevention

### Signs and symptoms

Dengue is a dynamic illness, despite its short duration (no more than 1 week in nearly 90% of cases). Its clinical expression can change as the days go by and can also worsen suddenly. Dengue illness can evolve into three phases: the acute febrile phase — observed in most of the patients — and the critical and the recovery (convalescent) phases<sup>2</sup> (BOX 1).

Fever occurs during the acute febrile stage and is generally the first clinical manifestation of illness with a variable intensity. It is associated with headache and vomiting, as well as body pains. In children, fever is frequently the only clinical manifestation or is associated with rash and/or unspecific digestive symptoms. The pharynx can become reddened, but other signs and symptoms of the respiratory system are not frequent or clinically significant. Slight abdominal pain and diarrhoea can occur; diarrhoea more frequently occurs in patients who are <2 years of age and in adults. In general, compared with children, adolescents and adults show a ‘flu-like syndrome’ (including malaise, headache and body pains) with more prominent digestive symptoms than respiratory symptoms, if any. During the febrile stage, leukocyte counts are usually decreased. Petechiae (small spots on the skin caused by broken capillaries) or ecchymosis (large subcutaneous bleeding spots) can be present, with or without thrombocytopenia. After 2–5 days, these symptoms can be followed by rapid clinical deterioration. Most patients with dengue recover after defervescence; however, the clinical state of some patients worsens when the fever drops. Thus, the period

Table 2 | Examples of gene variants associated with susceptibility to or protection from dengue illness

Gene variants	Populations	Association	Refs
<i>HLA-A*0203</i>	Thailand	Susceptibility to DF	92
<i>HLA-DQB1*01</i>	Mexico	Susceptibility to DF	93
<i>HLA-DQ1</i>	Brazil	Susceptibility to DF	94
<i>HLA-A*0207</i>	Thailand	Susceptibility to DHF	92
<i>HLA-A*24</i>	Vietnam	Susceptibility to DHF	96
<i>HLA-A*03</i>	Malaysia, China and India	Susceptibility to DHF	95
<i>HLA-DRB1*0901</i>	Vietnam	Protection from DHF	98
<i>HLA-A*33</i>	Vietnam	Protection from DHF	96
<i>HLA-B*18</i>	Malaysia, China and India	Protection from DHF	95
Combination of <i>IL10</i> (–1082/–819/–592) ACC or ATA haplotype and <i>TNF</i> –308A allele	Cuba	Susceptibility to DSS	99
<i>IL10</i> (–1082/–819/–592) ACC or ATA haplotype	Cuba	Susceptibility to DSS	99
<i>DCSIGN1</i> (also known as <i>CD209</i> )–336G allele	India	Susceptibility to DHF	100
<i>FCGR2A</i> (H or H131)	Cuba	Susceptibility to DF or DHF	101
Combination of <i>TNF</i> –308 AA or AG and <i>IL10</i> 1082 AA genotypes	Venezuela	Susceptibility to DHF	102
<i>VDR</i> 352C allele	Vietnam	Resistance to DSS	97
<i>FCGR2A</i> (R or R131)	Cuba	Resistance to DHF and clinical outcome	101

*DCSIGN1*, dendritic cell-specific ICAM3-grabbing non-integrin 1; DF, dengue fever; DHF, dengue haemorrhagic fever; DSS, dengue shock syndrome; *FCGR2A*, Fc fragment of IgG receptor IIA; *HLA*, human leukocyte antigen; *TNF*, tumour necrosis factor; *VDR*, vitamin D3 receptor.

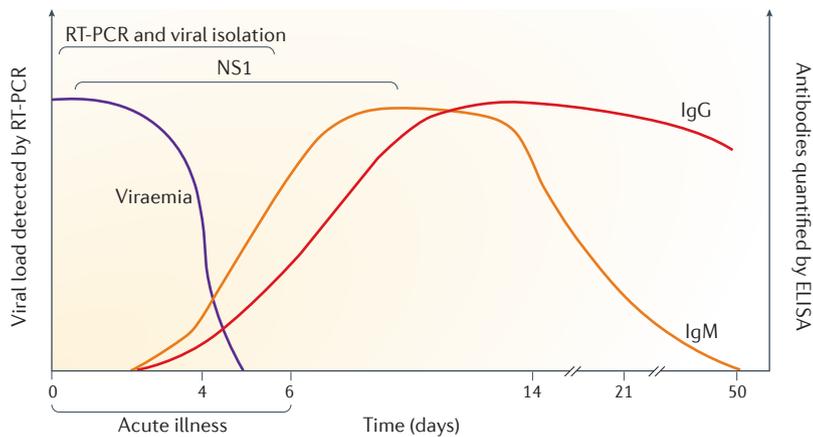


Figure 7 | **Dengue diagnosis.** Viraemia, non-structural 1 (NS1) antigen and antibodies change over time; thus, different diagnostic tests will be appropriate depending on the stage of infection. ELISA, enzyme-linked immunosorbent assay; RT, reverse transcription. Adapted from REF. 153, Nature Publishing Group.

during which the fever subsides indicates the beginning of the critical phase.

The critical phase coincides with the leakage of plasma that can lead to shock, which is characterized by coldness in the teguments, weak pulse, delayed capillary filling, tachycardia, oliguria and hypotension. Shock is caused by low blood volume (hypovolaemia). At the beginning, not all clinical signs of shock are observed, and, in this setting, shock can be detected by a narrowing of the differential arterial tension or pulse pressure (a difference of  $\leq 20$  mmHg between the maximum or systolic arterial tension and the minimum or diastolic arterial tension). At this stage, patients usually have a flushed face, a warm trunk, cold and clammy extremities, diaphoresis (sweating), slow venous filling, restlessness, irritability, pain in the upper and middle abdomen and decreased urinary output. In addition, patients might also exhibit signs of impaired haemostasis, including scattered petechiae on the forehead and extremities, spontaneous ecchymoses, easy bruising and bleeding at venipuncture sites, and circumoral and peripheral cyanosis (blue skin discolouration). Gastrointestinal bleeding occurs in  $<10\%$  of patients and usually follows a period of uncorrected hypotensive shock. Patients with shock also experience rapid and potentially laboured breathing, a weak pulse and have a rapid heartbeat that sounds 'thready'. Finally, their livers are usually firm, tender and can become enlarged to 4–6 cm below the costal margin, the haematocrit level is increased and the platelets — which were decreasing progressively — reach their lowest count. In those who recover, this critical phase lasts for 24–36 hours and is followed by a rapid convalescence.

Convalescence can involve complications, such as encephalopathy, bradycardia, ventricular extrasystoles and, rarely, myocarditis and encephalitis.

According to the 2009 WHO clinical classification, a patient can have dengue with or without warning signs or severe dengue (FIG. 1), highlighting that severity is considered as the second step of the same disease. In other words, dengue can be considered to be a single disease entity that is both systemic and dynamic.

### Diagnostic approaches

**Detection of viraemia.** DENV viraemia is detectable 24–48 hours before fever onset and continues for 5–6 days (FIG. 7). During this period, infective virus, its specific RNA and the NS1 protein can be detected in patient blood, serum and plasma, and also in tissues from fatal cases<sup>146</sup>. Virological, molecular and serological methods are used to confirm DENV infection for epidemiological surveillance and clinical diagnosis.

Anti-DENV IgM antibody detection is the most widely used test in routine practice<sup>147</sup>. Anti-DENV IgM titres in sera from patients in the acute phase of disease are measured to serologically confirm infection, whereas patients in convalescence are identified through IgM and IgG seroconversion by comparing antibody titres in paired acute and convalescent sera<sup>146,148</sup>. For patients who are suspected of having dengue, a presumptive diagnosis can be made by the detection of anti-IgM antibodies in samples collected at day 6 of acute symptoms. Commercial kits for IgM or IgG detection in enzyme-linked immunosorbent assay (ELISA) and less-sensitive rapid test formats are available<sup>149,150</sup>.

Reverse transcription PCR (RT-PCR), real-time RT-PCR, DENV isolation in mosquito cell lines and by mosquito inoculation facilitate confirmation and identification of the agent virologically. Although virus isolation and identification is highly specific, it has a relatively low sensitivity and is resource-consuming and time-consuming. By contrast, DENV RNA detection provides a rapid, sensitive and specific method for virological diagnostic confirmation. NS1 protein detection provides a window of opportunity for early aetiological diagnosis. The sensitivity and specificity of DENV NS1 detection depend on the infecting serotype, the timing of sample collection and the parity of DENV infection (primary versus secondary), as well as the format of the test<sup>151,152</sup>.

BOX 2 shows the interpretation of dengue tests and TABLE 3 summarizes the WHO recommended diagnostic tests according to the laboratory surveillance level<sup>2,153</sup>. Whereas all of these methods can be used to establish aetiological diagnosis, bedside rapid tests for antigen, antibody or simultaneous antigen and antibody detection are preferable if they are of satisfactory sensitivity and specificity.

The recent introduction and extension of two new arboviruses in dengue-endemic countries of the American region — chikungunya (an alphavirus detected at the end of 2013 in the Caribbean island of St Martin) and Zika (a flavivirus detected in May 2015 in Brazil) — impose new challenges for the diagnosis of dengue and the arbovirus in general. The diagnosis of any of these viruses is based on RNA and/or IgM detection. However, the duration of viraemia is different between these infections, antibody cross-reaction is observed between DENV and Zika virus (which belong to the same viral family: *Flaviviridae*) and commercial, adequately evaluated kits for serology are needed for chikungunya virus and Zika virus infections. To face this emergency, the network of Arbovirus National Laboratories of the American region (RELDAs; formerly

the Dengue Laboratory Network of the Americas) conducted by the Pan American Health Organization (PAHO), recommended a new diagnostic algorithm for DENV, Zika and chikungunya viruses. In the first step, DENV, chikungunya and Zika viral RNA is detected by real-time PCR in acute samples. If available, IgM serology on serum samples collected from individuals with clinically suspected DENV infection or Zika virus infection should be tested by IgM Capture ELISA to both DENV and Zika virus. If positive to both viruses, a secondary flavivirus infection should be considered<sup>154</sup>.

Considering this new epidemiological situation, it is expected that dengue and flavivirus diagnostic guidelines will change with new algorithms according to the epidemiological situation and more-sensitive and specific as well as better evaluated commercial kits for serology.

#### **Identification of patients at risk of severe disease.**

As DENV infections can result in severe and life-threatening illness, identifying which patients are at risk of an outcome that requires supportive interventions is important. Differentiating this group from the thousands of mild cases during outbreaks is a major medical challenge; simple and inexpensive strategies are urgently needed. The 2009 WHO classification system for the identification of patients at risk of severe disease is summarized in FIG. 1 (REF. 155).

Biomarkers for dengue prognosis that are under evaluation include a high level of viraemia and NS1 protein, the level of microparticles that are produced as a consequence of apoptotic cell death and cellular activation, the level of some immune-response mediators, such as IL-1 receptor-like 1 (IL1RL1; also known as ST2), tumour necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL), and some biochemical alterations, but to date none have been approved for routine practice<sup>156,157</sup>. However, as part as the routine laboratory follow-up of a suspected dengue case, a full blood count should be done at the first patient visit. A decreasing white blood cell count makes a diagnosis of dengue very likely, whereas plasma leakage is suggested by a rapid decrease in platelet count, mainly if it is associated with a rising haematocrit level. Fluid accumulation, which can be detected by X-ray or ultrasonography, is a conclusive warning sign of plasma leakage. In addition, laboratory findings during the critical stage of illness that vary according to the severity of vascular permeability include prolonged bleeding time, increased APTT, thrombocytopenia, increased levels of liver enzymes, activated complement with high levels of C3a and C5a, fibrin split products and low levels of fibrinogen. Chest X-ray is the best method for detecting pleural effusions, and abdominal sonograms can detect gallbladder wall thickening and ascites<sup>2,158</sup>. Patients should also be closely monitored for signs of shock.

Daily monitoring of the clinical warning signs to detect early progression from mild to severe illness remains the most useful method to prevent fatal disease<sup>2</sup>, and serial ultrasonographic studies could be better than existing markers, such as the haematocrit

level, to identify patients who are at risk of developing severe dengue who merit intensive monitoring<sup>159</sup>. Clinical algorithms have also been proposed for both dengue case identification and dengue prognosis, but none are in routine use<sup>160–162</sup>.

**Classification systems.** There are somewhat competing views in the field as to the optimal approach for the clinical classification of patients with dengue and the identification of warning signs of severe disease, and several reviews and position papers regarding the usefulness of the 2009 WHO system compared with the 1997 WHO system have been published<sup>163,164</sup>. Prospective clinical studies developed in Asian and Latin-American countries have concluded that the 2009 WHO dengue classification system may be better at detecting severe DENV infection cases compared to the previous WHO classification system<sup>165–167</sup>. Others have argued that the revised 2009 WHO classification has a high sensitivity for identifying severe dengue and is easy to apply<sup>168</sup>; some consider the 2009 system to be promising from both research and clinical perspectives<sup>169</sup>. Indeed, the 2009 classification system has greater discriminatory power for detecting patients who are at risk of progression to severe disease and those who need hospitalization than the 1997 classification<sup>170</sup>. Furthermore, the 2009 system is simple to use for triage and case management according to disease severity, even in primary care settings<sup>171</sup>, and for disease surveillance. It also reflects the natural course of dengue illness from mild to severe disease and covers all clinical manifestations<sup>172</sup>. A formal

#### **Box 2 | Interpretation of dengue diagnostic tests**

Dengue virus infection is confirmed if any of the following test results are produced:

- RT-PCR or real-time RT-PCR are positive
- Positive virus culture
- Positive NS1 test
- IgM seroconversion\*
- IgG seroconversion

A presumptive dengue diagnosis is made if either of the following test results are produced:

- Positive IgM in a single serum sample\*
- High titre of IgG in a single serum sample tested by haemagglutination inhibition assay or ELISA ( $\geq 1,280$ )\*

ELISA, enzyme-linked immunosorbent assay; NS1, non-structural 1 protein; RT, reverse transcription. \*The recent introduction and expansion of Zika virus infection into dengue-endemic countries impose a further diagnostic challenge. IgM antibodies in patients with secondary dengue virus infection can cross-react with Zika virus. For this reason, the Pan American Health Organization recommends IgM detection to both dengue virus and Zika virus antigens. Samples that are IgM positive against dengue virus or Zika virus antigens individually are considered a primary infection of dengue virus or Zika virus, respectively, whereas samples that are positive against both viruses are considered a presumptive flavivirus infection. Similarly, specific IgG cross-reaction can be observed with Zika virus antigens. For a more specific serological diagnosis, neutralization assays to both viruses are recommended<sup>154</sup>.

expert consensus was reached in La Habana, Cuba, in 2013 with dengue experts from the Americas<sup>173</sup>, where a decrease in disease lethality after the introduction of the revised classification was evident<sup>174</sup>.

That said, through the analysis of retrospective data, some investigators have found that warning signs are not as useful in adults as they are in children<sup>175</sup>, and have argued that the current recommended predictors of severe dengue are, therefore, limited<sup>176</sup>. Others have put forward that there is a need for a more precise definition of warning signs to enable optimal triaging for accurate identification of patients who require hospitalization<sup>177</sup>. In addition to these critiques, one study described that both the 1997 and the 2009 WHO classification systems show high sensitivity but lack specificity<sup>178</sup>, and that the 2009 system requires refined definitions of severe bleeding and organ impairment to improve its clinical relevance<sup>179</sup>. A major ongoing clinical study, coordinated by one of the three large European Union-funded consortia that are currently working on dengue research themes, might address some of these issues<sup>180</sup>. Finally, since the introduction of the revised criteria, a high number of patients have been admitted to hospital or placed under clinical observation during dengue epidemics. This increase is probably owing more to traditional hospital-based methods of managing patients with dengue than to the 2009 WHO classification system, a conclusion that is supported by the fact that this increase in clinical intervention can be alleviated through the participation of trained primary care health units, which the WHO is trying to facilitate<sup>181</sup>.

Although the 2009 WHO classification is more applicable to clinical and epidemiological purposes than the 1997 classification, debate continues regarding its usefulness for pathogenesis research<sup>182</sup>. In particular, some have argued that the dengue fever, DHF and DSS classifications were more capable of correctly identifying cases of plasma leakage than the 2009 system, and that this identification served as a useful predictor of disease severity that was directly related to the main underlying model of pathogenesis. However, in a separate study, the same authors concluded that the 1997 system misclassified a substantial proportion of

patients<sup>183</sup>. Specifically, only 68% of patients who were in need of clinical intervention were classified as having DHF and, therefore, in using this system, it could be inferred that 32% of severe cases would be missed. One of these studies has been analysed by a group of experts<sup>184</sup>, who concluded that the revised classification reflects clinical severity in real time, which is something that clinicians have wanted for some time, and with its simplified structure will facilitate effective triage and patient management and also allow collection of improved comparative surveillance data.

### Vaccine-based prevention

The mechanisms of protective dengue immunity are not well understood. Neutralizing antibodies against viruses serve as the most commonly used correlate of protection. As discussed above, antibodies produced during an infection provide lifelong protection to the homologous virus but short-lived protection against the other three serotypes<sup>185</sup>. Most neutralizing antibodies recognize the E protein, and high DENV neutralizing antibody titres in mice and monkeys have been correlated with protection<sup>186</sup>. However, there is no proof that protection is always associated with neutralizing antibodies, as evidenced by the absence of protection against DENV2 in some vaccinated individuals who have appreciable levels of circulating neutralizing antibody<sup>187,188</sup>. Different antibody responses, such as antibody-dependent cell-mediated cytotoxicity and complement fixation, might also correlate with antibody-mediated protection against DENVs<sup>189,190</sup>. Moreover, T cell-mediated functions might correlate with protection *in vivo*. In general, CD4<sup>+</sup> T cells can control viral infection through various mechanisms, including the production of antiviral and inflammatory cytokines, cytotoxic killing of infected cells, the enhancement of CD8<sup>+</sup> T cell and B cell responses and the promotion of immune memory responses. Similarly, CD8<sup>+</sup> T cells also act through the production of pro-inflammatory cytokines, such as TNF and IFN $\gamma$ , and can be directly cytotoxic to viral infected cells<sup>191,192</sup>.

The development of safe and fully protective dengue vaccines faces several challenges: ideally, the vaccine should protect against the four serotypes; long-term protection is required otherwise an individual might become susceptible to breakthrough infection and enhanced disease owing to waning and non-protective immunity (that is, a dengue vaccine could lead to DHF/DSS through ADE if immunity is not sustained or is partial); there is no animal model that exactly replicates human dengue disease; although DENV neutralizing antibodies protect in some circumstances, the full correlates of protection are not known; and vaccine candidates need to be evaluated in the context of changing patterns of transmission intensity and circulating viruses.

Several DENV vaccines are currently under development, including some in phase III safety and efficacy testing. One that has completed phase III efficacy testing is under registration in several countries (TABLE 4). These vaccines are outlined below.

Table 3 | Proposed model for organization of dengue laboratory services

Tests	Primary health centre	Regional centres	Reference centre
Virus isolation	–	–	+
RT-PCR and real-time RT-PCR	–	–	+
Antigen detection using ELISA	–	+	+
Antigen detection using a rapid test	+	+	+
Serology using ELISA	–	+	+
Serology using a rapid test	+	+	+
Serology using PRNT and HI	–	+	+

+, test should be performed; –, test should not be performed; ELISA, enzyme-linked immunosorbent assay; HI, haemagglutination inhibition assay; PRNT plaque reduction neutralization test; RT, reverse transcription. Adapted with permission, from WHO/TDR, Dengue Guidelines for Diagnosis, Treatment, Prevention and Control — New Edition, World Health Organization & Special Programme for Research and Training in Tropical Diseases, WHO Press, Table 4.4, 2009.

Table 4 | Dengue vaccine candidates by type and their stages of development

Vaccine type	Developer	Approach	Current status
Live attenuated	Mahidol University	Live attenuated tetravalent virus isolates	Not being tested
	WRAIR and GSK	Live attenuated tetravalent virus isolates	On hold
	US NIAID, US NIH and Instituto Butantan	Recombinant live attenuated, directed mutagenesis and DENV–DENV chimeras	Phase II
	Acambis and Sanofi Pasteur	Live attenuated tetravalent chimeric YFV–DENV (CYD–TDV (also known as ChimeriVax))	Registration in several countries
	US CDC, Inviragen and Takeda	Live attenuated chimeric DENV2–DENV (DENVax)	Phase II
Subunit	Hawaii Biotech and Merck	Truncated recombinant envelope protein (DEN–80 <sup>F</sup> )	Phase I
	IPK and CIGB	Tetravalent recombinant subunit vaccine comprising domain III of the envelope protein fused to the capsid protein (DIII–C)	Preclinical (NHP)
Inactivated	WRAIR and GSK	Purified inactivated virus	Phase I

CDC, Center for Disease Control and Prevention; CIGB, Cuban Center for Genetic Engineering and Biotechnology; CYD–TDV, chimeric yellow fever virus–DENV tetravalent dengue vaccine; DENV, dengue virus; GSK, GlaxoSmithKline; IPK, Tropical Medicine Institute Pedro Kouri; NHP, non-human primate; NIAID, National Institute of Allergy and Infectious Diseases; WRAIR, Walter Reed Army Institute of Research; YFV, yellow fever.

**Live attenuated vaccines.** Live attenuated vaccines have numerous advantages, including the ability to induce an immune response that mimics the response to natural infection, the induction of robust B cell and T cell responses and the ability to confer lifelong immune memory. Live attenuated vaccines can be produced at relatively low cost and might be effective after one dose<sup>186</sup>. Early dengue vaccine efforts focused on passaging wild-type DENV strains through various types of primary cells or cell lines, including primary dog kidney (PDK) and African green monkey kidney (GMK) cells. Passaging of DENV *in vitro* renders it less virulent in humans and was investigated in two series of work.

In the first series, vaccine strains from each serotype obtained by passage through PDK cells or primary GMK cells were selected and tested in monovalent, bivalent, trivalent and tetravalent vaccinations in Thai adults<sup>193</sup>. Of the tetravalent recipients, only one of ten seroconverted to all four serotypes, and neutralizing antibody responses were directed primarily against DENV3. Subsequently, several tetravalent vaccine formulations were tested and the dominant neutralizing antibody response was still against DENV3 (REFS 194, 195). Following on from these studies, the DENV3 vaccine strain was re-derived genetically, grown in Vero cells and tested in volunteers<sup>196</sup>. All recipients had adverse reactions and the trial was halted<sup>186</sup>.

In the second series, different formulations of the tetravalent vaccine were tested in monkeys and flavivirus-naïve adults and children<sup>197,198</sup>. The formulations were improved to reduce the reactogenicity and increase the immunogenicity<sup>199,200</sup>. These new formulations were safe and moderately effective, and the authors recommend that studies in a larger number of adults and then in children are warranted<sup>186</sup>.

Another attenuation strategy is the targeted mutagenesis of 3' UTR regions of DENV RNA<sup>201</sup>. The viral 3' UTR is approximately 450 nucleotides long and comprises four defined domains: domain A; domains A2 and A3, which seem to work as enhancers for viral

RNA replication; and domain A4 and the 3' stem loop, which are essential elements for viral replication<sup>202</sup>. The deletion was created by the removal of nucleotides 172–143 from the 3' UTR. This deletion, designated Δ30, has been shown to attenuate DENV1 and DENV4 in rhesus monkeys and to inhibit dissemination of DENVs in mosquitoes<sup>203,204</sup>. Monovalent and tetravalent preparations have been given to human volunteers and produced good immune responses<sup>205</sup>. A phase I trial investigated a single dose of four different formulations of a live tetravalent vaccine in flavivirus-naïve volunteers. The vaccines were well tolerated, produced no severe adverse events and only one dose induced a good neutralizing antibody response in 75–90% of the individuals<sup>206</sup>. One of these tetravalent DENV vaccines was licensed to several vaccine developers<sup>207</sup> and entered large-scale phase III efficacy trials in Brazil following a small human challenge trial conducted in the United States. A single dose of the dengue vaccine TV003 fully protected 21 vaccinated volunteers against infection in a virus challenge study, whereas 20 unvaccinated controls all developed an infection<sup>208</sup>.

In addition, a candidate tetravalent dengue vaccine (called CYD–TDV) has been developed, via the insertion of the prM and E genes of the four DENV serotypes into the genetic backbone of the 17D yellow fever vaccine virus<sup>209</sup>. Two ChimeriVax phase III trials were conducted in >30,000 children in five Asian and five American countries. Overall efficacy in the Asian trial was 56.5% and 60.8% in the American trial<sup>187,188</sup>. In addition, a reduction in severe complications was reported with a vaccine efficacy of >80% against DHF. These vaccines seem to boost immune responses and protect individuals who have had one previous DENV infection and are, therefore, at risk of ADE. However, these vaccines failed to protect seronegative individuals against clinical infection with all four DENV serotypes, and a group of young vaccinated children had higher rates of hospitalized breakthrough DENV infections than controls<sup>210</sup>. Children who were ≤5 years of age when vaccinated

experienced a DENV disease resulting in hospitalization at five times the rate of controls. The aetiology of disease in placebo and vaccinated children that results in hospitalization during a DENV infection, while clinically similar, are of different origin. The implications of the observed mixture of DENV protection and enhanced disease in CYD vaccinees is under study<sup>211</sup>. CYD-TDV seems to protect people who have been infected once and, accordingly, are at risk of severe disease. But, conversely, it puts people who were susceptible to a first infection at risk of severe disease. Even so, the vaccine is approved in Mexico, the Philippines and Brazil.

Another vaccine construct has been developed by substituting the prM and E genes of DENV2 PDK-53 with those of wild-type DENV1, DENV3 or DENV4 (REF. 212). Three different formulations of these tetravalent vaccine (DENVax) were tested in monkeys, and all vaccinated monkeys developed neutralizing antibodies against all four serotypes after one or two doses<sup>213</sup>. On the basis of these results, phase I and phase II trials were carried out to evaluate different vaccination regimens, formulations and alternative routes of immunization<sup>214</sup>. The vaccine was well tolerated in children and adults 1.5–45 years of age, irrespective of prior dengue exposure; mild injection-site symptoms were the most common adverse events. DENVax induced a neutralizing antibody response and seroconversion to the four DENVs, as well as cross-reactive T cell-mediated responses that could be necessary for a broad protection against dengue illness<sup>215</sup>. Currently, phase III trials of the vaccine have been initiated in several Asian countries.

Following on from live attenuated vaccines, another generation of vaccine candidates, including subunit vaccines, inactivated vaccines, DNA vaccines and viral vector vaccines, is being launched.

**Subunit vaccines.** The advantages of protein vaccines compared with live attenuated vaccines are that they are safe, the induction of a balanced immune response to the four DENV serotypes should be feasible and the immunization schedule can be accelerated, reducing the risk of incomplete immunity and the potential for ADE. However, these vaccines require the use of adjuvants and multiple doses to achieve optimal immunogenicity, and they may not be as efficient as live attenuated vaccines at inducing long-lasting immunity<sup>186</sup>.

The protein target of subunit vaccine development for dengue has been the E glycoprotein, as the majority of neutralizing epitopes on the DENV virion are located in this protein. Recombinant E protein has been produced using *Escherichia coli*, baculovirus and insect cells, yeast and mammalian cells<sup>216–219</sup>. Truncated recombinant E protein subunits (80E) of each serotype were obtained in a *Drosophila melanogaster* Schneider 2 cell expression system and were found to induce neutralizing antibody responses in mice and in non-human primates<sup>220</sup>. A phase I trial of the DENV1-80E vaccine candidate has been completed<sup>221</sup> and a phase I trial of a tetravalent formulation began in 2012 (REF. 222). The subunit vaccine might be an important component in a prime–boost vaccine regimen.

Domain III-capsid (DIII-C) is a novel candidate vaccine containing viral fragments that might potentially induce neutralizing antibodies and cell-mediated immunity. DIII-C has been evaluated in Balb/c mice and Vervet monkeys<sup>223,224</sup>. In animal models, DIII-C has been shown to induce a serotype-specific immune response in terms of both antiviral antibodies and cellular immune response with partial protective efficacy<sup>225</sup>. This candidate is at an advanced stage of preclinical development.

**Inactivated vaccines.** Vaccination with inactivated vaccines ideally should induce a balanced immune response without the viral interference (wherein the replication of one virus can inhibit the generation of a balanced immune response against all four serotypes as it can interfere with the replication of the other serotypes) that can occur with live attenuated vaccines. In addition, there is no risk of viral replication or reversion to wild-type virus. Inactivated vaccines are less effective in inducing long-lasting immunity than live attenuated vaccines, so multiple doses and adjuvants are needed for optimal immunogenicity in unprimed individuals. A dengue inactivated vaccine might be useful as part of a heterologous prime–boost vaccine regimen<sup>186</sup>.

A dengue purified formalin-inactivated vaccine (DPiV) is being developed and has been shown to be immunogenic in rhesus macaques. A phase I trial began in 2011, and two phase I trials of a tetravalent candidate began in 2012 in a dengue-primed population and in a non-endemic area<sup>186</sup>.

**DNA vaccines.** DNA vaccination results in antigen expression by both major histocompatibility complex (MHC) class I and MHC class II, leading to the activation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as well as an antibody response. In addition, DNA vaccines are non-replicating and, therefore, safer than live attenuated vaccines, with low reactogenicity. Other advantages include low cost, ease of production and temperature stability<sup>186</sup>. Most of the DNA vaccine-based approaches in dengue have focused on eliciting immune responses to the prM protein and the E protein in mice and monkeys<sup>186</sup>. DNA vaccines based on the NS1 protein have also been tested in mice<sup>226,227</sup>, and another DNA vaccine, based on the expression of DENV1 prM, E and NS1 proteins, induced better protection than a DNA vaccine without NS1 (REF. 228). Further advances in DNA vaccination may lead to a successful DENV vaccine.

**Viral vector vaccines.** Several viral vector platforms, including vaccinia virus, adenovirus and alphavirus vectors, have been explored as delivery vehicles for DENV antigens. Viral vector dengue vaccine candidates are focused on eliciting and evaluating anti-E protein antibody responses. No viral vector vaccine has advanced to clinical phase I testing<sup>186</sup>.

#### Vector control-based prevention

As the twentieth century dengue pandemic expanded over the past 40 years, prevention and control of the disease relied solely on mosquito control, as there was no

licensed vaccine. However, as evidenced by the increasing global disease burden and expanding geographical distribution of both the viruses and the mosquito vectors, it is clear that mosquito control, as used in most countries, has failed to control dengue<sup>229–231</sup> (FIG. 3). The reasons for this failure are complex and a detailed discussion is beyond the scope of this Primer. Briefly, after the successes of the American hemispheric *Ae. aegypti* and global malaria eradication programmes in the 1950s and 1960s<sup>232</sup>, there was widespread complacency about vector-borne diseases in general, and dengue in particular<sup>229</sup>. Moreover, dengue was not considered a major public health problem by policy makers who controlled budgets because epidemics were intermittent and mortality was low. This led to a redirection of resources and a lack of commitment to dengue control on the part of permissive countries and to deteriorating public health infrastructure<sup>233</sup>. Finally, limitations placed on the use of effective insecticides, such as dichlorodiphenyltrichloroethane (DDT), and the improper use of mosquito control tools that were available were contributing factors to the failure.

There were two countries that, temporarily, were exceptions to this general failure: Singapore and Cuba. Singapore was one of the first countries in Asia to experience DHF in the 1960s<sup>234</sup>. A highly successful *Ae. aegypti* control programme, which prevented epidemic dengue in Singapore for nearly 20 years, was implemented in 1968. The programme had three main pillars: legislation that levied fines on individuals whose premises were found to be infested by *Ae. aegypti* mosquitoes; larval source reduction and control; and community outreach and education<sup>235</sup>. Although this programme is still functioning and effectively controlling *Ae. aegypti*, it has failed to prevent the re-emergence of epidemic DENV transmission in the past 20 years<sup>236</sup>. The reasons for this re-emergence are not fully understood, but are thought to be a combination of low herd immunity, high frequency introduction of DENVs from neighbouring endemic or epidemic countries that had not controlled the disease and a highly dense human population<sup>236,237</sup>.

The Cuban programme was initiated in 1981 during the first large epidemic of DHF in the Americas<sup>237,238</sup>. This programme was based on the same three pillars used in Singapore, but added a fourth pillar: extensive use of space spraying of pyrethroid and organophosphate insecticides to kill adult mosquitoes using ultra-low volume and thermal fogging machines<sup>238</sup>. Epidemic dengue was controlled in Cuba for almost 30 years, but this programme also ultimately failed because of economic problems and, as with Singapore, the introduction of DENVs from neighbouring endemic or epidemic countries that had not controlled dengue have occurred.

There are several important lessons to be learned from these experiences. First, sustainable dengue control cannot be achieved by individual countries or communities when they are surrounded by areas with endemic or epidemic dengue. Thus, effective sustainable programmes must be developed on a regional basis as clearly demonstrated by the American hemispheric eradication programme<sup>231,239</sup>. Second, sustainable control

requires long-term commitment by endemic countries. Those countries must use their own resources instead of relying on international agencies whose funds might not be relied on with certainty<sup>229,231</sup>. Last, to be effective, mosquito control tools must be used properly by trained personnel<sup>240</sup>. Otherwise, dengue control efforts become a waste of time and money.

Fortunately, the future for dengue control using vector control looks brighter as there are numerous new tools in the development pipeline. A new organization, the Partnership for Dengue Control, has recently been formed to facilitate an integrated approach to dengue control<sup>241</sup>. As a global alliance of partners and stakeholders interested in controlling dengue, it will bring together the leading expertise in dengue and public health to design new strategies for dengue control by integrating new and existing mosquito control tools with vaccination. Recent expert consensus workshops have reviewed currently available mosquito control methods as well as those in the development pipeline that might become available in the next 5 years. Briefly, the reviews concluded that, for currently available tools, targeted indoor residual spraying with synthetic pyrethroid insecticides combined with larval control were the most likely to provide effective *Ae. aegypti* control, provided the methods were used properly. The new tools in the pipeline include new non-pyrethroid residual insecticides that can be used for the control of dengue as well as new uses for those insecticides. Thus, in addition to targeted indoor and outdoor spraying, these compounds, which may have a residual activity of  $\geq 6$  months, can also be used as spatial repellents, to treat curtains and other materials hanging in mosquito resting areas and in lethal ovitraps (devices that mimic natural mosquito breeding sites).

Other tools in the pipeline include biological (*Wolbachia*) and genetic (sterile males) control<sup>241–243</sup>. A strain of *Wolbachia*, a natural bacterial parasite of insects, has been adapted to *Ae. aegypti*. When infected, the female *Ae. aegypti* has a reduced lifespan and has increased resistance to infection with DENVs, both of which can decrease transmission. When released into a natural population of *Ae. aegypti*, the *Wolbachia* spreads via normal mating, ultimately infecting most individuals in that population. A major advantage of this method is that it provides sustainable control. The sterile male method uses a dominant lethal gene carried by male *Ae. aegypti*, which are released into the natural mosquito population. When the males carrying the lethal gene mate with wild-type female *Ae. aegypti*, the progeny die as larvae, therefore, reducing the population. The advantage of this method is rapid reduction of the mosquito population, but the disadvantage is that it is not sustainable. Both of these approaches are in advanced field trials in several countries in Asia, South America and Central America, with promising results<sup>241</sup>.

Unfortunately, none of these mosquito control methods are likely to be completely effective in controlling dengue if used alone, but if used in an integrated control programme with other synergistic mosquito control tools and vaccines, effective control might be achieved<sup>241</sup>.

### Management

Central health policy-making institutions of each country should have programmes aimed at avoiding dengue-related fatalities. Permanent capacity building is necessary<sup>244</sup> to ensure the adequate classification and supportive care of patients<sup>245</sup>. Moreover, judicious fluid management during the critical phase coupled with continuous monitoring<sup>246</sup>, reorganization of sanitary services during epidemics<sup>247</sup> and dengue research are all vital to improve outcomes for patients<sup>248</sup>. A very comprehensive review on case treatment and management can be found in the WHO Dengue Guidelines for Diagnosis, Treatment, Prevention and Control<sup>2</sup>. In general, more histopathological and virological studies are needed to define the causes and pathogenesis of all of the complications that can accompany dengue illness.

### General approaches

No specific treatment for dengue is currently available; consequently, patients are provided with supportive care. As management decisions need to be made before a confirmed dengue diagnosis by serology or other tests, clinicians must rely on clinical and epidemiological data to decide whether to classify a patient as a suspected dengue case<sup>249</sup>. In the absence of an effective antiviral drug, the prescription of bed rest and abundant liquids by mouth can be pivotal in determining the outcomes of patients. Indeed, fluid intake during the 24 hours before being seen by a clinician has been significantly associated with decreased risk for hospitalization of patients with dengue fever<sup>250</sup>. Analgesic and antipyretic drugs, such as paracetamol (neither aspirin nor NSAIDs should be taken), can be prescribed at the usual dosage for children and adults. Bed nets or repellents to avoid mosquito bites should be used to prevent other cases at home and in the neighbourhood.

Fluid therapy is key to dengue management and is applied based on disease severity. In mild dengue, oral fluid therapy can be as effective as intravenous fluid replacement and there is no need for hospitalization. Nevertheless, the requirement for hospital admission must be made according to the analysis of each clinical case, and hospitalization might be required, for instance, when a patient has diabetes mellitus or other comorbidities or characteristics, such as in the case of pregnant women, newborns or the elderly<sup>251,252</sup>. Old age and comorbidities, such as cardiovascular disease, stroke, respiratory disease and renal disease, might contribute to the development of severe dengue<sup>253</sup>.

### Severe dengue

**Capillary leakage and shock.** Capillary leakage becomes evident at the end of the febrile stage (days 3–6) and increases during the next 24–48 hours. At defervescence, the identification of warning signs (FIG. 1) has a greater discriminatory power for detecting patients who are at risk of progression to severe disease and those who need hospitalization<sup>254</sup>, as the 2009 WHO guidelines recommend<sup>2,163</sup>. Families also must be educated to identify the so-called warning signs of dengue shock when fever subsides<sup>171</sup> and, when available, patients should be monitored clinically for markers of severe disease risk.

Warning signs are the clinical heralds that announce the imminence of shock. In this situation, the fluid lost from the circulatory system owing to capillary leakage must be replaced by immediately initiating intravenous fluid therapy, of which crystalloid solutions (volume expanding fluid replacements such as lactate Ringer solution) or physiological (normal) saline solution are recommended<sup>255</sup>. Therapy with fluid support should be continued, according to the clinical situation of the patient and their fluid balance. As a rule, most patients with dengue recover if they are stratified and managed according to WHO recommendations<sup>167,173</sup>, which are guidelines that are supported by recognized experts<sup>9</sup>.

Some patients have a poor clinical evolution and present with pronounced frank hypotension, mental confusion and worsening of the other signs<sup>256</sup>. In these cases, intravenous administration of crystalloid solutions should be continued according to the WHO recommendations<sup>257,258</sup>. If appropriate management is continued, most patients will recover and go on to require maintenance fluid therapy, which is calculated according to the clinical situation of the patient. For those who do not recover, colloids (volume expanding solutions that contain human albumin, gelatin or starch) can be administered as an alternative to crystalloid solutions.

**Impaired haemostasis.** The haemorrhages that can accompany dengue illness are multifactorial. Although haemorrhages can worsen outcomes for patients who experience DSS, they are not necessarily associated with capillary leakage and can occur at any moment of the critical phase or during the convalescent phase<sup>259</sup>. Nevertheless, haemorrhages are frequent complications of prolonged shock rather than a general dengue complication (such as disseminated intravascular coagulation) and, therefore, the best way to prevent major haemorrhages is to prevent shock or treat it quickly and appropriately. Risk factors for severe haemorrhage include the duration of shock and low-to-normal levels of haematocrit at shock onset, whereas platelet count is not predictive of bleeding and does not correlate with its severity<sup>260</sup>. A sudden decrease in the levels of haematocrit and haemoglobin without an improvement of the patient is a sign of silent haemorrhage. Bleeding most commonly occurs in the gastrointestinal system where it can manifest as haematemesis (the vomiting of blood) and/or melaena (black, tar-like faeces). Treatment of gastrointestinal bleeding involves transfusion with packed red blood cells. Intracranial or lung bleeding can also occur and these are associated with a poor prognosis that can sometimes involve multiple organ system failure<sup>261</sup>. Finally, thrombocytopenia is frequently observed in the course of dengue illness, but there is currently no evidence to support the practice of prophylactic platelet transfusions, which are costly and sometimes harmful<sup>262</sup>.

**Other complications of severe dengue.** Cardiac, hepatic, respiratory and neurological complications can all accompany dengue illness. DENV infection has been shown to cause cardiac disease with clinical manifestations ranging from a mild increase of disease biomarkers to

myocarditis and/or pericarditis, and patients with severe dengue might show evidence of systolic and diastolic cardiac impairment that predominantly affects the septum and right ventricular walls<sup>263</sup>. Patients with dengue and clinical cardiovascular manifestations have been tested for abnormalities in the biomarkers troponin I and the amino-terminal fragment of brain natriuretic peptide, and these patients have also been studied using echocardiography and cardiac MRI<sup>264</sup>. These studies have shown that DENV infection can induce the destruction of cardiac fibres, the absence of myocyte nuclei and the loss of striations<sup>265</sup>. Dengue myocarditis or myocardiopathy can occur alone, can be associated with other organ dysfunctions or can be a complication of DSS. As such, cardiac function should be carefully monitored and, where appropriate, the patient should be admitted to an intensive care unit and given inotrope therapy (such as dopamine and dobutamine), according to local guidelines<sup>266</sup>.

The capillary leakage involved in severe dengue can have consequences for the respiratory system. These can include pleural effusion and respiratory distress associated with pulmonary oedema, which can be worsened by over-hydration during fluid therapy<sup>267</sup>. To avoid respiratory distress, during fluid therapy, the balance of fluids must be monitored<sup>246</sup>, oxygen therapy is mandatory and in some cases mechanical ventilation should be initiated.

In cases of acute dengue hepatitis, patients might require liver support and a hepatic pre-coma regimen (in attempt to avoid hepatic encephalopathy). In addition, patients with dengue hepatitis might also require management of other associated complications, such as bleeding (using transfusion of blood rather than platelets or fresh frozen plasma). Metabolic complications, such as acidosis, hypoglycaemia and hypocalcaemia, could be important components of dengue severity, although not necessarily associated with severe liver dysfunction. Pancreatitis is an infrequent complication of DENV infection<sup>268,269</sup>.

### Emerging therapies

A major unmet need in dengue management is a safe and effective antiviral drug<sup>270</sup>. Although some antiviral candidates have been tested in randomized controlled trials, the results have been poor<sup>271</sup>. In addition, a trial of chloroquine — which has had *in vitro* antiviral effects against DENV infection and has additional anti-inflammatory properties — did not show any significant effect on the duration of viraemia or on the clinical course of illness<sup>272</sup>.

An alternative approach to using antivirals to treat dengue is to modulate the immune system, as cytokines have been implicated in contributing to capillary leakage. Although corticosteroids can suppress the immune system and decrease inflammation, a study in patients with dengue demonstrated no effect of steroid use on clinical signs or symptoms. Similarly, statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) were unable to produce anti-inflammatory or endothelial-stabilizing effects in those with dengue<sup>273</sup>. Modern bioinformatics and high-throughput screening approaches are being used to try to identify new antiviral molecules and drugs that could stabilize the endothelium and, therefore, prevent or reverse capillary leakage in dengue illness.

## Quality of life

### Effects of acute illness

Patients with dengue usually visit the physician with a main complaint of an unexplained high fever and malaise, sometimes with rash, petechiae or other skin and mucosal bleeding and vomiting. As a rule, these patients cannot attend school or go to work for  $\geq 1$  week. Dengue (also named 'breakbone disease') is frequently a very painful disease and mainly produces abdominal pain, backache, headache and myalgias<sup>274</sup>. By contrast, myositis (muscle inflammation) and muscle weakness are distinctly uncommon manifestations of DENV infection. Patients with dengue who have myositis have high levels of creatine phosphokinase and could have respiratory muscle involvement, which sometimes requires mechanical ventilation<sup>275</sup>. Guillain–Barré syndrome should be considered in patients who present with myalgias and general weakness or progressive limb weakness after the acute illness<sup>276</sup>.

### Long-term effects of infection

Although fever and acute illness usually resolve within 1 week (mainly in children but also in some adults), most adult patients take longer to recover. In some patients, some dengue symptoms last for 6 months after the acute phase. The most frequent symptoms that are present at 6 months after acute illness are weakness (27.6% of patients), headache (14.8% of patients) and arthralgias (10.6% of patients). One study showed that these symptoms had an irregular appearance, were inconsistent and were mostly related to physical or mental exertion<sup>277</sup>. The persistence of some dengue symptoms has even been demonstrated 2 years after the acute illness<sup>278</sup>. Liver enzymes can normalize 1 month after acute illness<sup>279</sup> or continue to be raised for a much longer duration as a marker of liver cytolysis<sup>280</sup>. Fatigue post-DENV infection can result in clinical disease beyond the acute phase of infection, which may be explained by the immune alterations that are triggered by the DENV<sup>281</sup>. Some immunocompetent patients who had experienced severe dengue among other infectious diseases have had an increase in lymphocyte count preceding clinical deterioration that included interstitial pneumonitis, airway obstruction, CNS disturbances and systemic capillary leak syndrome, all of which were thought to represent immunopathological tissue damage<sup>282</sup>.

### Neurological, renal and haematological outcomes

Neurological features have had an increased frequency in patients with dengue who have been admitted to hospital. These can be categorized into dengue encephalopathy (which is caused by hepatic failure or metabolic disorders), encephalitis (which is caused by direct virus invasion), neuromuscular complications (such as Guillain–Barré syndrome or transient muscle dysfunctions) and neuro-ophthalmic manifestations. Other neurological symptoms, such as transitory post-dengue Parkinsonism have been reported following serologically confirmed DENV infection. Transient and permanent visual disturbances have also been reported<sup>283</sup>. Impaired vision caused by dengue-associated ocular inflammation

is an emerging ophthalmic condition and often involves the posterior segment (dengue-related maculopathy). The prognosis for these patients is variable and, although they usually regain good vision, they can retain persistent partially altered vision (which is defined as partially altered vision even up to 2 years after the clinical resolution of the acute disease)<sup>284</sup>. The aetiology and mechanisms of these complications deserve careful study.

Renal manifestations are uncommon and include acute kidney injury, proteinuria, glomerulonephritis and haemolytic uraemic syndrome<sup>285</sup>.

Among haematological alterations, haemophagocytic lymphohistiocytosis (an inflammatory condition characterized by increased proliferation of activated lymphocytes and macrophages that are capable of phagocytosing red blood cells leading to severe anaemia) has been recognized as a dengue-associated condition<sup>286</sup>.

### Outlook

There have been major advances in vaccines, specific antiviral drug development and vector control activities in the past 10 years. Used effectively, these new tools will provide new opportunities to control the disease. New molecular diagnostic and antigen detection tests and a better understanding of pathogenic mechanisms will enable earlier diagnosis and more-effective clinical management. In addition, a better understanding of virus–vector interactions and the dynamics of DENV transmission along with the new vector control tools that are being developed should lead to more-effective prevention and control of the disease.

Several vaccine candidates are currently in clinical trials, with live attenuated vaccines being the most successful developed to date<sup>287</sup>. The impact of the vaccination should be estimated by also considering vaccine coverage, the epidemiological situation of dengue, genetic polymorphisms within populations, dengue immunity of the target population and vector control activities<sup>288</sup>. The introduction of a licensed dengue vaccine into national programmes represents a challenge. According to the epidemiological situation, countries should decide whether high-risk groups or the total population should be vaccinated, and in which age groups and regions where the vaccine should be introduced. Mathematical models could become a very useful tool for the understanding of population-wide dengue strategies<sup>289</sup>.

As of April 2016, Sanofi Pasteur completed phase III testing and received approval from the WHO's Scientific Advisory Group of Experts on Immunization. The company has licensed their three-dose tetravalent live attenuated CYD vaccine (Dengvaxia) in five countries (Brazil, El Salvador, Mexico, Paraguay and the Philippines) for use in individuals 9–45 years of age, 70% of whom should be circulating DENV antibodies<sup>188,210,290,291</sup>.

Despite these advances, dengue research is still a priority. In 2006, the Special Programme for Research and Training in Tropical Diseases and the WHO convened a Dengue Scientific Working Group with experts from 20 countries who reviewed existing knowledge on dengue and established the research priorities, which were organized into four main streams: research related to reducing dengue fatality rates and disease severity; research on transmission control through improved vector management; research related to primary and secondary prevention; and health policy research aimed at contributing to an adequate public health response. The objective was to provide information for policy makers and foster the development of more cost-effective strategies to reverse the epidemiological trend of dengue. Unfortunately, owing to the current complex global dengue epidemiology, after almost 10 years,

### Box 3 | Dengue research priorities topics

#### Reduce disease severity and fatality rates

- Evaluate the 2009 WHO classification in clinical practice
- Identify and validate prognostic markers (warning signs and risk factors) for severe disease
- Prepare guidelines for clinical management of severe cases
- Study dengue and comorbidities, pregnancy and in the elderly
- Develop new diagnostic methods for early clinical diagnosis and clinical differentiation to chikungunya virus and Zika virus infections, tests for dengue confirmatory diagnosis, evaluation of commercial kits and quality assurance for diagnosis
- Implement training programmes in case management at the different levels of the health care system
- Define the molecular mechanisms of dengue and severe dengue with special emphasis on plasma leakage, bleeding and dengue in infants
- Investigate the mechanisms of virus–human and virus–mosquito cell interactions, including host genetic associations with dengue virus (DENV)
- Study the protective and pathogenic immune responses in DENV infection and the mechanisms of antibody-dependent enhancement, as well as the relationship with Zika immunity
- Investigate the association between genetic changes in DENV and phenotypic expression

#### Reduce transmission

- Study of vector biology and ecology
- Develop and evaluate new vector control tools
- Introduce and evaluate a strategy for integrated vector management
- Implement training programmes in vector control to strengthen capacity
- Study temporal, virological and immunological variables associated with transmission dynamics in different epidemiological settings
- Apply mathematical modelling and geographical information systems for evaluating transmission dynamics, predicting transmission patterns and developing control measures
- Define and evaluate better vector indices for surveillance and epidemic responses
- Improve methods to study insecticide resistance
- Develop early warning indicators of dengue outbreaks and epidemics and effective response systems

#### Prevention

- Develop new vaccine candidates via research on various areas
- Apply mathematical modelling to estimate the effect of vaccination and the interaction between vaccination and vector control
- Define indicators for post-vaccination surveillance
- Integrate vaccine introduction with effective mosquito control

#### Health policy research

- Define the burden and cost of illness, including in Africa
- Identify strategies for multi-sectorial and multi-country collaboration for addressing dengue, integrating all factors and efforts to diminish transmission

this approach is yet to stop the spread and emergence of dengue<sup>292,293</sup>.

Today, there are many promising areas for research. Interdisciplinary studies of the interactions between the virus, the human and the mosquito vector are required for a better understanding of the illness pathogenesis, DENV transmission dynamics, vaccine and drug development, and better diagnostic tools. The integration of clinical and epidemiological data with basic research, genomic studies and the application of advanced technology (such as 'omics', nanotechnology, biosensors and molecular modelling, among others) should be supported. No less important are studies to define the social, environmental and other risk factors for DENV transmission. BOX 3 summarizes the main research priorities and topics.

Recognizing the importance of dengue, several international initiatives are ongoing. As a result of these efforts, the WHO published the Global Strategy for Dengue Prevention and Control in 2012 with three main targets: to reduce mortality to 50% by 2020; to reduce morbidity to 25% by 2020; and to better study disease burden. This strategy relies on five technical elements: diagnosis and case management; integrated surveillance and outbreak preparedness; sustainable vector control; future vaccine implementation; and research<sup>13</sup>.

At present, some of the WHO efforts are to identify early warning signs of outbreaks, to implement a strategy for integrated vector management and to analyse issues related to dengue vaccine and vaccination<sup>2,294–298</sup>. Other international initiatives include the Dengue Vaccine Initiative, the Partnership for Dengue Control and the multi-country research projects International Research Consortium on Dengue Risk Assessment, Management and Surveillance, Dengue Research Framework for Resisting Epidemics in Europe, and Innovative Tools and Strategies for Surveillance and Control of Dengue, which are supported by the European commission, among others<sup>241,299,300</sup>.

Overall, the international multi-sector response, as outlined in the 2012 WHO Global Strategy<sup>13</sup> and the Partnership for Dengue Control, will be crucial to reversing the global dengue trend as well as addressing other emerging vector-borne diseases, such as chikungunya, Zika and yellow fever virus infections. This strategy should include improved vector control through strategies such as community-based interventions to reduce breeding sites, an increase in the scope and breadth of dengue surveillance, improved case management to reduce the case fatality rate, a vaccination strategy and, potentially, novel entomological approaches to reduce transmission by altering mosquito ecology or genetics.

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#### Author contributions

Introduction (M.G.G.); Epidemiology (D.J.G.); Mechanisms/pathophysiology (S.B.H.); Diagnosis, screening and prevention (A.I., D.J.G., E.M. and M.G.G.); Management (E.M.); Quality of life (E.M.); Outlook (M.G.G.); Overview of Primer (M.G.G.).

#### Competing interests

D.J.G. is a patent holder on the Takeda vaccine and a stock holder in the company. He has consulted for Takeda and Sanofi, and was on the Scientific Advisory Board of Novartis Institute for Tropical Diseases from 2003 to 2012. All other authors declare no competing interests.