Dengue

Summary

- Up to 390 million infections annually, 500 thousand cases of severe dengue, with an estimated 36,000 deaths
- Vector: mosquitoes, Aedes species
- Flavivirus, 4 main serotypes (DEN 1-4)
- Infection with one serotype produces lifelong immunity against this serotype, but only short-lasting cross-protection against other serotypes.
- Main clinical presentations: fever, arthralgia-rash, hemorrhagic syndrome (FD, AR, HS)
- Plasma leakage is the hallmark of severe dengue
- WHO 2009 classification: Dengue and Severe Dengue (D/SD); Warning signs (WS) help clinicians identify cases in need of closer surveillance (dengue with warning signs [D +/- WS])
- WHO 1997 classification: Dengue fever, dengue hemorrhagic fever and dengue shock syndrome (DF/DHF/DSS).
- No antiviral treatment is available at present, but mortality is greatly reduced by appropriate supportive treatment
- 2 Dengue vaccines are licensed: CYD-TDV (Dengvaxia®) should only be administrated to seronegative people, TAK-003 (Qdenga®) may be used in seropositive and seronegative individuals. Future efficacy and safety monitoring is warranted

Virus

Dengue viruses belong to the family of Flaviviridae (yellow viruses), genus *Flavivirus*. The virus has a positive sense single-stranded RNA genome. It is translated into a large precursor protein, which is then cleaved by host-cell and viral proteases into three structural and seven non-structural proteins (See Figure 5).
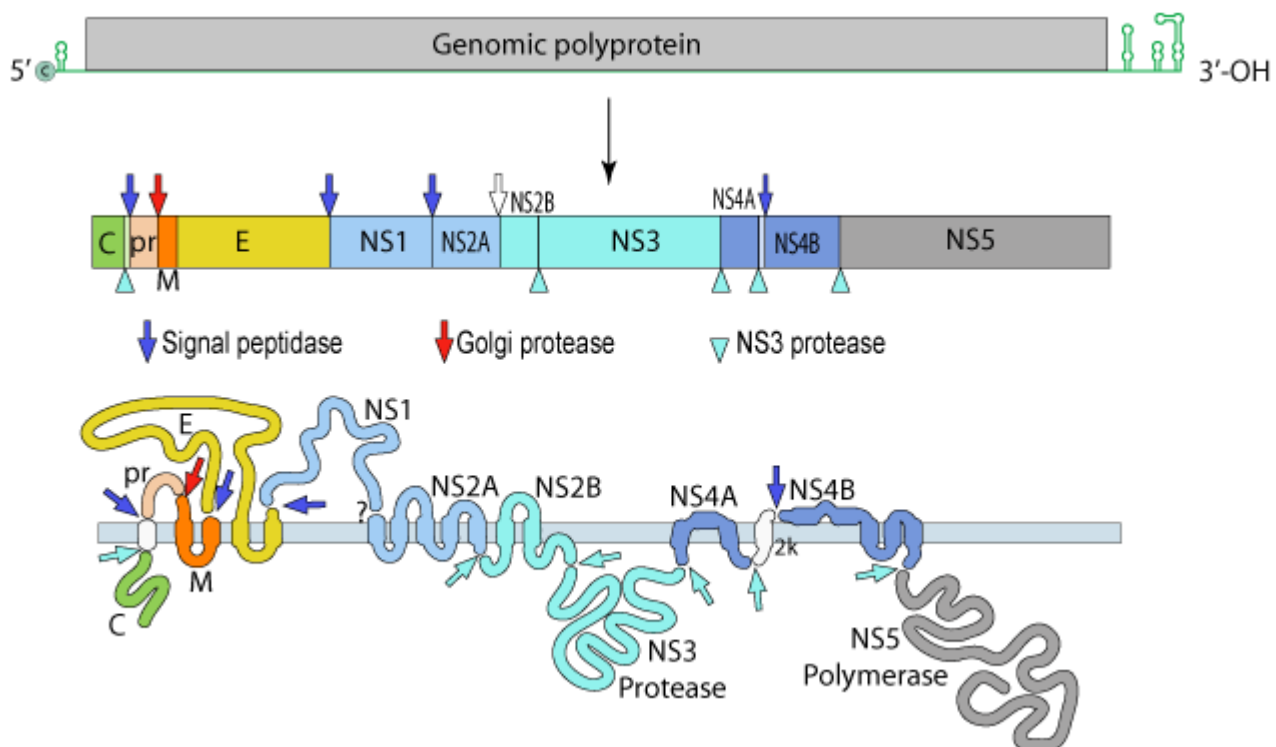
Figure 5: Dengue ssRNA genome and proteins (viralzone.expasy.org)

Dengue virus has 4 main serotypes. Infection with one serotype results in lifelong immunity to subsequent infection with that particular serotype (homologous immunity). There is no lasting cross-protection between the serotypes (heterologous immunity).

In 2013, a 5th serotype (DEN-5) was described, of which the clinical significance is not yet understood. Contrary to DEN 1-4, it has a sylvatic transmission cycle, which may hamper current dengue control efforts.

Epidemiology and transmission

Dengue prevalence increased over 15-fold over the last two decades, attributable to three principal drivers: urbanization, globalization and lack of effective mosquito control. Dengue viruses have fully adapted to a human-*Aedes aegypti*-human transmission cycle in large, crowded urban centers in the tropics. In rapidly developing suburbs, running tap water is often lacking, and people depend on fetching water in small reservoirs. Sewage systems are often open and are ideal breeding sites for mosquitoes. Increased mobilization with more car

tires, together with a surge in the use of plastics, contributes to mosquito propagation since water containing mosquito larvae is co-transported. International travel can transport the virus to new regions with little mosquito control. Transported rubber car tires and lucky bamboo plants have been shown to carry *Aedes spp.* larvae.

Dengue virus infects an estimated 300-530 million cases annually, of which almost 100 million manifest clinically. The estimated annual death rate of 36.000 deaths due to dengue virus is relatively low, but high numbers of less sick dengue patients can overburden health structures. Dengue occurs in 129 countries and 70 percent of the burden is in Asia. As with other arboviruses, the geographic distribution of dengue is determined by the distribution of its vectors (See Figure 6). The main reservoir of the dengue serotypes 1-4 is probably man.
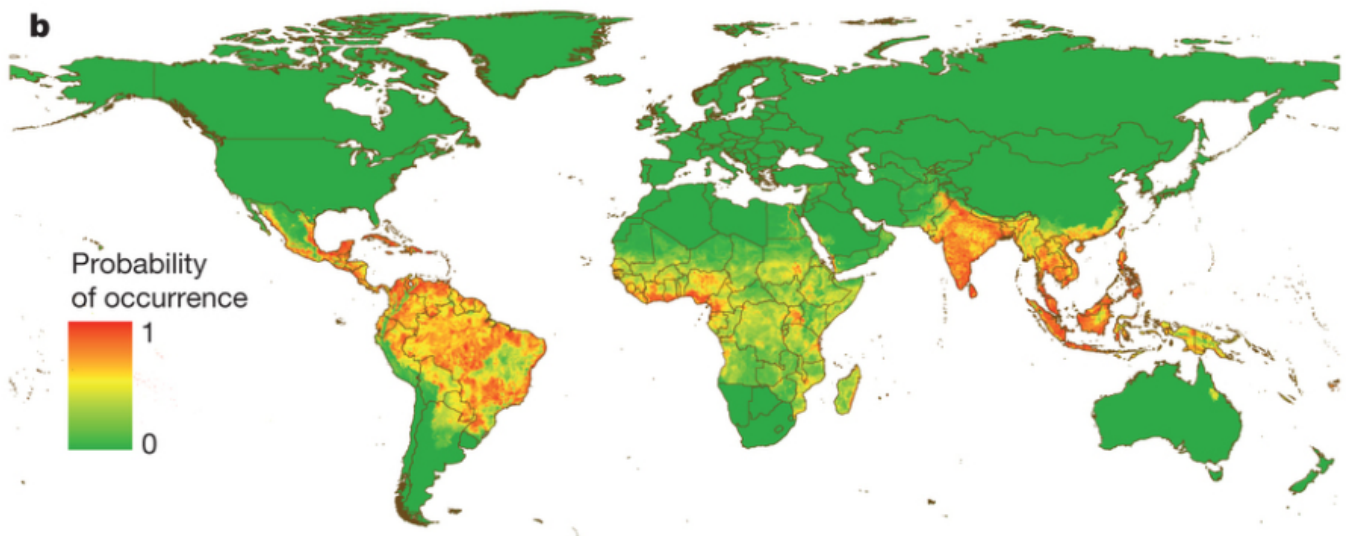
Figure 6: Probability of dengue occurrence (Bhatt et al, Nature)

The bite of infected female *Aedes* mosquitoes transmits dengue. The virus develops a life-long non-cytocidal infection in the mosquito. It may infect the mosquito ovaries and offspring (transovarial transmission). *Aedes* eggs can withstand dehydration for several months, and eggs of some *Aedes* species survive for several years. This cycle can be repeated for multiple generations and drive new outbreaks. It takes at least one week from the egg's hatching to the mosquito's adult stage. This is essential information for understanding the "dry day" principle (see below). Infection of humans occurs when dengue virus is introduced into the

skin via the insect's saliva during a bite of female mosquitoes. *Aedes albopictus* is a less competent vector for dengue virus but survives in a more temperate climate. Global warming might therefore increase the population at risk for dengue.

Dengue transmission follows two patterns that are not mutually exclusive. "Epidemic" dengue occurs when a single virus strain is introduced into a new region. Adults and children are affected, but dengue hemorrhagic fever is rare. In "hyperendemic" dengue, there is continuous circulation of multiple dengue serotypes. Seasonal variation is common and urban areas are particularly affected. Children are more at risk than adults, with a higher risk of dengue hemorrhagic fever.

Clinical aspects

Three-quarters of the estimated 390 million dengue virus (DENV) infections annually are clinically unapparent. These asymptomatic cases have the potential to contribute significantly more to virus transmission to mosquitoes than previously recognized, as high levels of viremia have been detected in infected people who do not have an interruption of their daily routine and who continue to have exposure to the virus' vectors.

Symptomatic dengue infection begins with a sudden onset of a flu-like syndrome. The febrile phase lasts 2-7 days, and the fever is biphasic (saddleback fever) in 5 percent of cases. Skin rash, headache, myalgia and arthralgia are frequent symptoms. The rash may have a dengue-specific appearance of "white isles in a red sea" (Figure), but this finding has low sensitivity (up to 20%).

Figure: Dengue skin rash "white isles in a red sea" (Photograph Dr. R. Huits, ITM)

There may be marked muscle pain (breakbone fever), especially in the back and in the extraocular eye muscles (pain around and behind the eyes when looking sideways).

According to the 2009 WHO guidelines for diagnosis, treatment, prevention and control of dengue, a positive tourniquet test (aka. Rumpel-Leede or capillary fragility test) increases the probability of dengue in acute febrile illness. The sphygmomanometer is inflated around the upper arm to mid-systolic blood pressure. After the cuff is left in place for 5 minutes, more than 20 petechiae in a 3 cm diameter circle in the crease of the elbow indicate a positive test. Recent literature suggests that the tourniquet test is more effective in detecting true negative than true positive cases, and the test should not be used for diagnosing dengue.

Severe dengue

Severe dengue may be rapidly fatal and usually results from a second dengue infection more than 18 months after a resolved first infection. An estimated 500 000 people with severe dengue require hospitalization each year, a large proportion of whom are children.

Complications may develop after 3 to 5 days when the first fever subsides (defervescence), and endothelial dysfunction may lead to hemoconcentration. Patients may develop hemorrhage, ranging from petechiae, ecchymosis and purpura to overt bleeding from mucosal surfaces (epistaxis, melena), injection sites and cerebrovascular accidents. They may develop shock with plasma leakage; pleural or pericardial effusion or ascites can be observed by ultrasonography. Detection of an oedematous gallbladder wall by serial ultrasonography identifies patients at risk for developing severe dengue.

Prediction of severe dengue remains challenging, mainly because the determinants of a complicated course of dengue virus infection are poorly understood. Severe dengue was observed to occur more frequently in secondary dengue infections. In 1977, this led to the development of the concept of 'Antibody-Dependent Enhancement (ADE). Secondary dengue infections were found to be correlated with higher levels of viremia. A molecular model to support the ADE hypothesis was described by Dejnirattisai et al. Briefly: Dengue infection leads to the development of homologous neutralizing and protective antibodies. Upon subsequent infection with a different serotype, these antibodies may enhance the replication of even immature virus particles. This results in higher levels of viremia (replication of both mature and immature virions), thereby increasing the release of pro-inflammatory cytokines and, thus, the severity of the disease.

The prevailing dengue serotype may be a determinant of severe dengue. This should probably also be evaluated against existing population immunity to previous dengue serotypes. In a recent meta-analysis, Soo et al. compared the percentage of severe cases of both primary and secondary infections with different serotypes of dengue virus. They found that the presence of certain serotypes, including primary infection with DENV-3 from the SEA region and secondary infection with DENV-2, DENV-3, and DENV-4 also from the SEA region, as well as DENV-2 and DENV-3 from non-SEA regions, increased the risk of severe dengue infections.

Apart from the fever, rash, arthralgia, hemorrhage and symptoms related to the plasma leakage syndrome, additional manifestations of dengue infection are described:

- Liver failure, which is caused by hypoperfusion or hypoxia rather than direct viral liver damage
- Neurological symptoms such as encephalopathy and seizures
- Cardiac manifestations, including myocarditis, arrhythmias and heart failure
- Secondary hemophagocytic lymphohistiocytosis

There is no specific treatment for dengue or severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. To facilitate the clinical management of patients with dengue virus infections, a new classification system was introduced by the WHO in 2009.

WHO dengue classification

Recognizing severe dengue remains a challenge for the clinician. In 2009, WHO adopted a new classification of symptomatic dengue infections i.e.. dengue with or without warning signs (WS +/-). While the performance of the triage based on the presence of warning signs (WS) need further validation across different clinical settings, this practical classification helps clinicians identify those patients in need of closer surveillance and/or hospitalization. Dengue warning signs include spontaneous or provoked bleeding, severe abdominal pain, persistent vomiting, painful hepatomegaly, dyspnoea, lethargy and effusions (see Figure 8). Severe dengue is defined by the occurrence of plasma leakage and or fluid accumulation leading to shock or respiratory distress, and/or severe bleeding, and/or severe organ impairment.
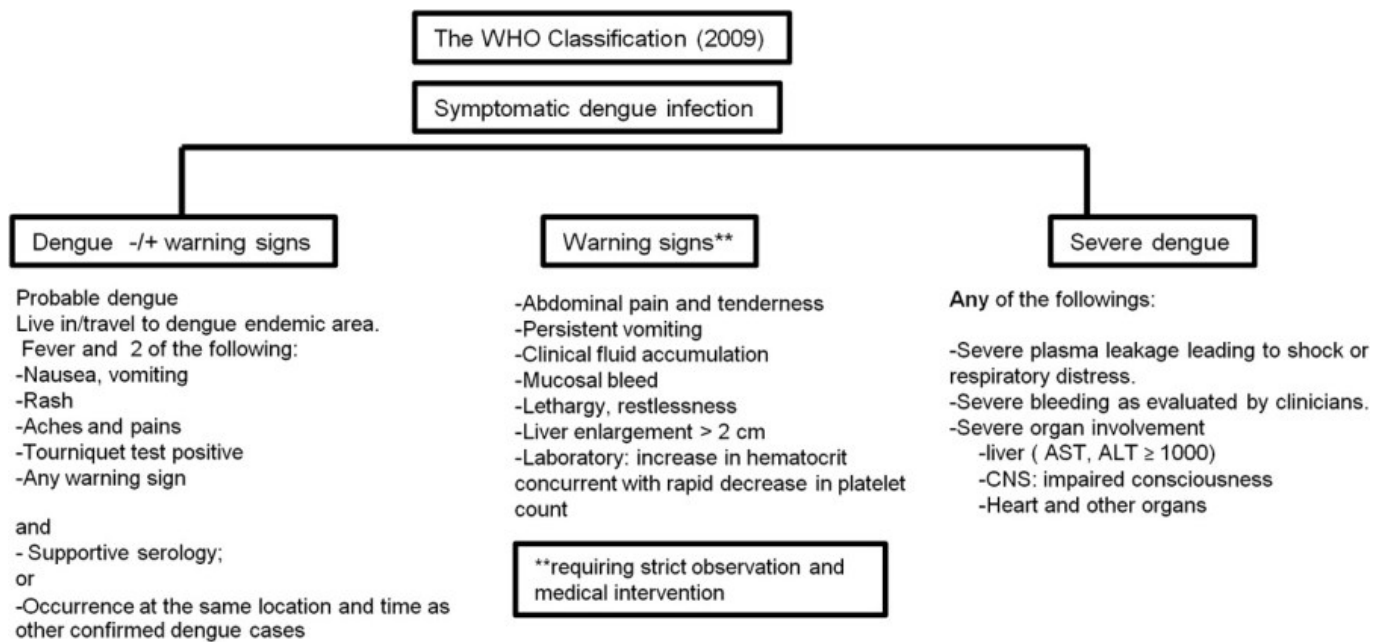
Figure: WHO dengue Classification 2009

The former WHO classification (1975, revised in 1997) was derived from a pediatric population. It identified Dengue fever, dengue hemorrhagic fever and dengue shock syndrome (DF/ DHF/ DSS). It was used to classify disease severity for surveillance purposes. The main criticisms are summarized below:

1. poorly related to disease severity
2. misdirecting clinicians in identifying severe disease
3. difficult to use (tests required are often not available/difficult to apply)
4. does not help for triage in outbreaks
5. leads to different reporting globally due to the difficulties in using the classification for reporting clinicians.

Further comparison of the usefulness of the 1997 and 2009 WHO Dengue Case Classifications can be found in recent publications.

Diagnosis

(see also the section: Laboratory diagnosis of arboviral infections).

Common hematological abnormalities include leukopenia and thrombocytopenia. Both are poor predictors of disease severity. Increased hematocrit (≥20% increase) indicates severe disease since it can point towards plasma leakage syndrome and evolution to shock syndrome.

Biochemical abnormalities correlate with disease severity and organ failure. Increased transaminase levels and hypoproteinemia are observed in severe dengue. Proteinuria, where proteins as large as albumin are lost, occurs and is consistent with disruption in the function of the endothelial glycocalyx layer. Hyperferritinemia in dengue-infected patients is associated with immune activation and coagulation disturbances and may reflect macrophage activation.

Patients with dengue or other febrile illness usually seek medical attention within several days of fever onset. Documenting the day of symptom onset (day 0) is essential to choose a single specimen diagnostic approach. DENV viremia occurs 3–5 days before fever onset and continues for approximately 5 days into the febrile illness. Viremia can be detected by molecular assays targeting DENV RNA (such as RT-PCR) or by immunoassays targeting DENV nonstructural protein 1 (NS1) antigen. An anti-DENV IgM response becomes detectable by IgM-capture immunoassays (Enzyme-Linked Immuno Sorbent Assay (ELISA) or Immune Fluorescence Assays (IFA)) 3–5 days after onset of fever. IgM levels peak 6–10 days after fever onset and may persist for up to 90 days. IgG antibodies can be detected from day 7 onwards and may persist for life. Anti-dengue IgG-antibodies may increase sooner in the event of secondary dengue infection. In view of these kinetics, laboratory diagnostic tests in a patient with suspected dengue infection should consider the day of symptom onset (Figure 9).
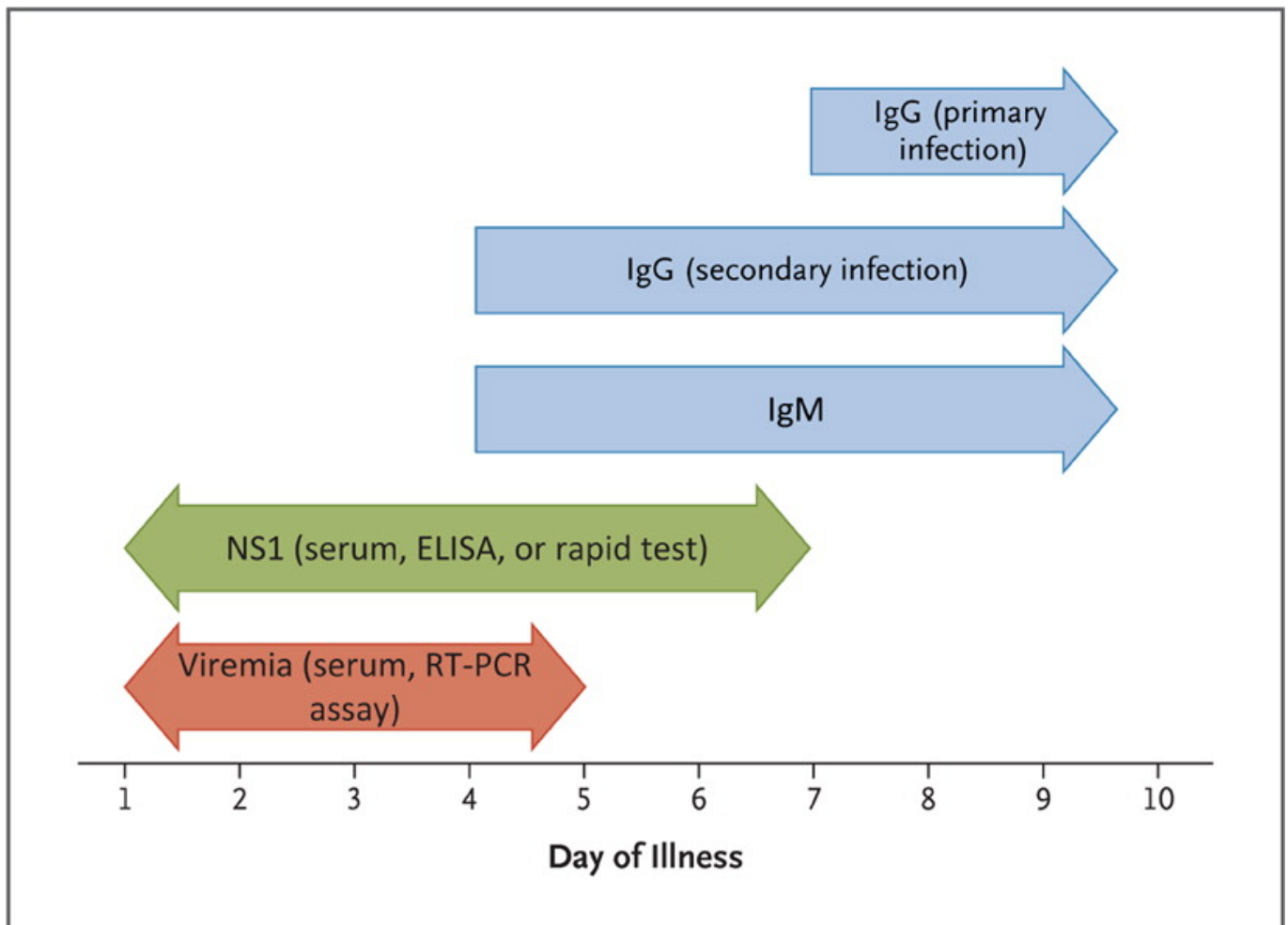
Figure 9: Laboratory Diagnostic Options in a Patient with Suspected Dengue Infection (Simmons et al, NEJM)

Flaviviruses share antigenic epitopes, which elicit cross-reacting antibodies. This cross-reactivity may result in false positive test results. To identify false positive test results or confirm true positives, virus neutralization tests can be performed. Because of the costs and technical expertise required, the use of these tests is mainly restricted to reference laboratories.

Treatment

No antiviral compounds are available for the treatment of dengue virus infections.

Corticosteroids are not effective.

Most cases can be treated on an outpatient basis. Symptomatic treatment should avoid aspirin and NSAIDs (risk of bleeding), but paracetamol can be used. The patient or the parents of the sick child should be counseled on dengue complications. In-patient care is required if warning signs appear as these may predict severe dengue to occur on days 4-7 after symptom onset.

In the case of warning signs, isotonic crystalloid fluids such as Ringer's lactate should be used to restore circulating blood volume. Fluid resuscitation requires observation in intensive care units. When the endothelial function recovers, fluid overload may cause iatrogenic complications. In patients with severe dengue infection, adjuvant therapy, including vasopressor and inotropic therapies, renal-replacement therapy and further treatment of organ impairment may be necessary.

Blood transfusion and fresh frozen plasma are sometimes required to treat severe bleeding. In case of massive bleeding, platelet transfusion may be needed in addition to packet cell transfusion. Platelet transfusion is warranted in patients with a platelet count <10.000/µl and active bleeding, but there is no benefit in prophylactic platelet transfusion without active bleeding.

Prevention

Personal protection

Contact with *Aedes* mosquitoes can be reduced using insect repellents. Sleeping at night under a bed net does not give any protection against *Aedes* sp. that bite during the day but can be useful for e. g., children sleeping during the day.

Vaccination

Immunity to dengue virus infections is complex, as is the development of dengue vaccines. As discussed under the section 'Severe dengue', dengue infection with one serotype leads to the development of lasting homologous neutralizing and protective antibodies, but it induces

only short-term immunity against other (heterologous) serotypes. Because of antibody-dependent enhancement (ADE), infection with a second serotype may lead to more severe illness. Hence there is concern over increasing the risk of severe dengue by vaccination. After infection with 2 different serotypes, broad immunity is observed.

Chimeric Yellow Fever-Dengue–Tetravalent Dengue Vaccine or CYD-TDV (Denvaxia®) is a tetravalent, live attenuated, chimeric vaccine and combines four chimeric yellow fever 17D-dengue vaccine viruses, where the premembrane and envelope proteins from each of the four DENV types replace the same proteins in a yellow fever 17D backbone virus. Three doses at months 0, 6 and 12 are administered. CYD-TDV is now used in about 20 countries in Latin America and Southeast Asia as part of their dengue control program after a study had shown an 80.3% efficacy against hospitalization and a 56,5 – 60,8% efficacy in contracting dengue disease in children. An additional analysis with retrospective determination of serostatus at the time of vaccination showed that children that were seronegative at the time of the first vaccination had a higher risk of developing severe dengue. Vaccination is therefore limited to people living in endemic areas ranging from 9-45 years of age who have had at least 1 documented dengue virus infection previously. This pre-vaccination screening for past dengue disease complicates the rollout of this vaccine in many low-resource settings.

TAK-003 (Qdenga®) is a tetravalent live attenuated DENV-2 virus with chimeras replacing the premembrane- and envelop genes of the DENV-2 with those from wild-type DENV-1, DENV-3 and DENV-4 strains. Two doses at months 0 and 3 are administered. The overall vaccine efficacy in children and adolescents 4 to 16 years of age was 80.9 % and 73,3 % at 12 and 18 months of follow-up, respectively. There was a 90.4 % efficacy against hospitalization for dengue. The vaccine efficacy was slightly higher among the baseline seropositive than baseline seronegative, without increased risk of severe dengue. Since DENV-2 was the backbone of TAK-003, efficacy was highest against DENV-2. There was no efficacy against DENV-3, and data were insufficient to evaluate efficacy against DENV-4.

Vector control

See general section.