DENGUE: AN OVERVIEW

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Abstract. Dengue affects millions of people annually, and it is a re-emerging disease in the tropical world. The increasing number of dengue cases over the last decades has been explained by association with unplanned urbanization and lack of efficient health facilities, demographic transition, travel/commercial development and limit efficacy of the vector control efforts. The clinical presentations of dengue range from mild illness to the life-threatening severe forms of the disease associated with plasma leakage, shock, severe bleeding, or multi-organ failure, which may be fatal. Although shock and plasma leakage seem to be more prevalent as age decreases, the frequency of severe bleeding or internal hemorrhage augments as age increases. Increases in liver enzymes, unlike conventional viral hepatitis, indicate liver involvement during dengue infections. Fatal cases were found to have significant frequencies of shock, altered consciousness, massive gastrointestinal bleeding, renal/hepatic failure, and concurrent bacteremia. The early recognition of dengue infection, bleeding tendency, and signs of circulatory collapse would reduce mortality rate in patients with dengue infection. The implementations of effectively sustainable vector control and effective dengue vaccines are keys to success for prevention and control of this disease.

Keywords: dengue, clinical manifestation, diagnosis, pathogen, pathogenesis, prevention, treatment

INTRODUCTION

Dengue is one of the most common mosquito-borne viral infections. The dengue virus is a single-stranded RNA virus and is transmitted to humans by the *Aedes aegypti* and *Aedes albopictus* mosquito species. This mosquito-borne arboviral infection is endemic in more than 140 countries with a geographic distribution in Asia, the Americas, the Eastern Mediterranean, and Africa. The World Health Organization (WHO) has reported that more than 2.5 billion people are at risk of dengue infection by one or more dengue viruses, and this risk is mainly in children living in tropical and subtropical countries. Estimates of the disease burden suggest nearly 100 million symptomatic dengue infections worldwide every year with the majority (75%) occurring in Asia and the Western Pacific region (Bhatt *et al*, 2013). In recent decades, outbreaks of increasing severity of dengue infection have been reported worldwide.

Changing factors in ecology and demographics are thought to contribute to the emergence of dengue infection in the last half-century. Contributory factors associated with the geographical expansion of dengue into new countries and urban settings include the increasing geographical range of *Ae. aegypti*, population growth, urbanization, slum growth, human population migration, movement of dengue virus by infected travelers, trade development, and improved diagnostic capabilities in medical practice (Kyle and Harris, 2008; Cummings *et al,* 2009).

Urban areas in the Tropics have experienced increased transmission of dengue virus. This trend has been caused by unplanned urbanization that leads to poor quality housing and high urban

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population density, and crowding along with poor quality water, sewer, and waste management systems (Barbazan *et al,* 2002; Guzman and Kouri, 2003; Nakhapakorn and Tripathi, 2005; Anyamba *et al,* 2006). Thus, an expansion of its geographical distribution and an increasing burden of health care resources caused by dengue are predicted in future decades.

Effective vector control management is the only method to reduce dengue infection in endemic areas. Nevertheless, historic vector control management efforts have had limited success in reducing transmission rates. Therefore, an effective dengue vaccine used in the target population and historic preventative measures such as raising public awareness may effectively control dengue in endemic areas (Pang *et al,* 2015).

Hyperendemic dengue is a major public health problem in many countries in South and Southeast Asia. In these regions, *Ae. aegypti* and *Ae. albopictus* are commonly found in urban and rural areas, and multiple dengue serotypes are circulating. A trend of general increase in the number of dengue cases has been seen in these regions. In particular, the disease is a leading cause of hospitalization and death in children. Within a population, the extent of hyperendemicity and timing of the introductions of differing serotypes mainly determine serotype-specific immunity. This serotype-specific immunity then determines the age distribution of clinically detectable dengue infections.

Past age distributions of indigenous dengue cases in South and Southeast Asia and the Americas have differed. Dengue syndromes in South and Southeast Asia occurred mainly in children, whereas they occurred in all age groups, including the elderly in the Americas. Recently, several Asian countries have reported an epidemic shift in age groups of dengue from mainly children to adolescents and young adults with increased disease severity (Charoensook *et al,* 1999; Pancharoen *et al,* 2002; Pongsumpun *et al,* 2002; Kulanatne *et al,* 2005). Of dengue virus infection cases in Thailand, adults aged over 15 year are estimated to be 30-40% of cases (Patumanond *et al,* 2003).

Children and adults with dengue show some differences in clinical presentations, laboratory findings, and severe complications (Kittigul *et al,* 2007; Hanafusa *et al,* 2008; Namvongsa *et al,* 2013). The incidence of dengue amongst travelers has been reported to be increasing (1.0-6.7%), which has been suggested to be a potential hazard for international travelers returning from endemic areas (Jelinek, 2000; Brien *et al,* 2001; Stephen *et al,* 2002; Pongsumpun *et al,* 2004). Recent reports have indicated that adult travelers returning from Asia to Western countries are more likely to have dengue than malaria, resulting in a greater probability that healthcare providers in Western countries will be confronted with travel-acquired dengue infections (Schwartz *et al,* 2008; Burdino *et al,* 2011; Wilder-Smith, 2012; Leder *et al,* 2013).

In travelers returning with fever, clinical manifestations of dengue infection are comparable with observations in the endemic area where dengue may go unnoticed. This situation highlights that surveillance should be maintained in non-endemic countries, and that febrile travelers returning from countries that are dengue endemic areas should be properly evaluated and followed up (Freedman *et al,* 2006; Massed and Wilder-Smith, 2009). Travelers should be encouraged to protect themselves from mosquito bites to avoid infections and onward transmission of dengue in new areas where *Ae. aegypti* is established.

PATHOGEN AND PATHOGENESIS

Dengue virus is a single-stranded RNA virus of the genus *Flavivirus* in the family Flaviviridae. It is the etiological agent of dengue infection. The four serotypes of dengue virus are DEN-1, DEN-2, DEN-3, and DEN-4, all of which are transmitted by the *Aedes aegypti* and *Ae. albopictus* species of mosquito.

The rainy season is the time of peak transmission of dengue virus in hyperendemic and endemic areas, and high temperatures also contribute to transmission. Rare cases of dengue transmission by needlestick, receipt of infected blood component, tissue or organ transplantation, and transplacental infection have been documented, although

the vast majority of cases are transmitted by mosquitoes (Chen and Wilson, 2004; Wagner *et al,* 2004; Tan *et al,* 2005; Mohammed *et al,* 2008; Tambyah *et al,* 2008; Wilder-Smith *et al,* 2009; Tangnararatchakit *et al,* 2012; Costa *et al,* 2015).

Any dengue virus has an incubation period of 4-8 days, after which there is an dengue virus infection that may manifest as a wide spectrum of illness ranging from asymptomatic or subclinical infection, undifferentiated fever, dengue fever (DF), and severe forms of the disease associated with plasma leakage, including dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), severe bleeding, encephalopathy, and multi-organ failure (WHO, 2009). DHF is characterized by rapid onset of capillary leakage accompanied by thrombocytopenia, hemoconcentration, vascular collapse, abdominal pain, and hemorrhagic manifestations (WHO, 1997). DF and DHF have been classified as distinct clinical entities, yet they are probably a continuum of the same pathophysiology with divergent outcomes in vascular integrity dysfunction.

Asymptomatic cases of dengue occur more frequently than symptomatic cases at a variable ratio (0.9:1, respectively to 18:1, respectively), and the ratio depends on geographical area, epidemiological context and the immunologic characteristics of an individual (Hadinegoro *et al,* 2016; Olivera-Botello *et al,* 2016). However, patients with asymptomatic infection may be the reservoir for dengue virus transmission to mosquitoes and subsequently to humans and should be considered in estimating disease burden.

Although recovery from dengue infection with one serotype confers lifelong homologous immunity, protection against other serotypes is short-term. Thus, a secondary infection can occur with other dengue serotypes. Previous epidemiologic data reveals that secondary heterotypic dengue virus infection is a risk factor to develop severe DHF/DSS, mediated most likely by antibody-dependent enhancement (ADE) of infection. Pre-existing homotypic antibodies bind to heterotypic dengue virions (virus-antibody complexes) and enable Fcγ receptor-mediated uptake by target Fcγ receptor-bearing cells (*eg*,monocyte/macrophage) resulting in increased viral replication and viremia (Hadinegoro *et al,* 2016). Changing inflammatory cytokine production (such as TNF∝, interleukin-1, interleukin-2 interleukin-6, interleukin 12, macrophage migration inhibitory factor, and HMGB1, MCP-1) produced by T-lymphocytes, monocytes/macrophages, and endothelial cell is observed in dengue patients who have increased vascular permeability, thrombocytopenia, and activation of coagulation and fibrinolysis (Green and Rothman, 2006; Guzman and Harris, 2015). In addition, secreted NS1 protein, anti-NS1 antibodies and increased complement activation (C3a, C5a) might be involved in increased production of inflammatory cytokines, causing intravascular coagulopathy and virus-induced vascular leakage by implicated local and systemic effects. Thrombocytopenia caused by bone marrow suppression, shortened platelet survival and increased platelet consumption due to platelet adhesion occurs during the dengue infection and reaches nadir during the day of defeverescence (toxic stage) (Chuansumrit and Chaiyaratana, 2014; Guzman and Haris, 2015).

Although secondary dengue infection remains the strongest known risk factor for DHF/DSS, viral genetics, serotype sequence, host factors, and time interval between primary and secondary infections can modulate severity of illness (Gamble *et al,* 2000; Guzman *et al,* 2002; Hammond *et al,* 2005; Anderson *et al,* 2014; Guzman and Harris, 2015).

CLINICAL MANIFESTATION

Dengue infection should be suspected if the patient in a dengue epidemic or endemic area has a fever of 10 days or less with myalgia, headache, flushing, anorexia, nausea or vomiting, arthralgia, bone pain, and peri-orbital pain with no apparent signs or symptoms of respiratory tract infection or organ-specific signs of other infectious diseases.

The clinical spectrum of dengue infection ranges from mild illness (undifferentiated fever, non-severe DF) to the life-threatening severe forms of the disease associated with plasma leakage (DHF/DSS), severe bleeding or multi-organ failure, which may be fatal.

Classical DF is a non-fatal febrile illness with a duration of around 5-7 days that is associated with sudden onset, anorexia, myalgia, headache, and occasional rash.

DHF is characterized by a high continuous fever of 2-7 days and a rapid onset of capillary leakage with thrombocytopenia, hemoconcentration, vascular collapse, abdominal pain, and hemorrhagic manifestations.

Shock (DSS) results from severe loss of plasma volume into serous spaces (*eg*, the pleural space or peritoneal cavity) or severe internal hemorrhage. Clinical symptoms of DSS in the acute febrile phase, which usually lasts 3-8 days, are mostly similar to those of DF and severe dengue (DHF), including fever, nausea/vomiting, headache, rash, and myalgia, but abdominal pain and severe or widespread bleeding are more frequent in DSS and less frequent in DF.

Dengue patients sometimes experience minor hemorrhagic manifestations including petechiae, epistaxis, gingival bleeding, and menorrhagia, but DF is rarely associated with severe hemorrhage leading to shock.

The reasons for age-related differences in dengue severity are not well understood. The clinical course has been observed to differ between children and adults. Children experience plasma leakage (DHF) and DSS more frequently compared with adults. This may reflect age-dependent differences in intrinsic vascular permeability. However, bleeding manifestations, in particular severe internal hemorrhage and hepatic dysfunction, have been reported to be more common in adults and older age groups than in children (Guzman *et al,* 2002; Hammond *et al,* 2005; Wichmann *et al,* 2005; Kittigul *et al,* 2007; Guilarde *et al,* 2008; Tantawichien, 2012; Namvongsa *et al,* 2013; Souza *et al,* 2013; Chuansumrit and Chaiyaratana, 2014; Tantawichien, 2015).

The symptoms of dengue generally last for 3-7 days before the fever subsides and symptoms remit. During the convalescence stage, the patients with dengue infection, even in DSS, may have rapidly increasing appetite, convalescence rash on lower extremities (a confluent rash with characteristic, scattered, round areas on pale skin), and sinus bradycardia. Most dengue infections are self-limiting with normalization of all abnormal hemostasis occurring in the convalescent stage or within 1-2 weeks after defervescence.

The apparent increased prevalence in the complications of dengue in the adolescent, adult, and elderly have resulted from the emergence of severe bleeding, fulminant hepatic failure, and encephalopathy in DF and DHF cases (Tsai *et al,* 1991; Anuradha *et al,* 1998; Agarwal *et al,* 1999; Wichmann *et al,* 2005; Pungjitprapai and Tantawichien, 2008; Tantawichien, 2012; Tantawichien, 2015). High mortality rates have previously been reported in elderly patients with dengue infection because of medical co-morbidity and waning of host immunity (Rigau-Perez and Laufer, 2006; Kuo *et al,* 2007; Lee *et al,* 2008; Gautret *et al,* 2012; Pang *et al,* 2012).

The prognosis of dengue infection largely depends on early diagnosis, recognition of plasma leakage, and treatment with immediate replacement of fluid along with intensive supportive care. Severity classification is important because the practice of the physician in patient observation, place of management, intensity of management (intravenous fluids, blood or plasma transfusion, and medicines) are likely to depend on the classification system used. WHO released a new classification in 2009, which is dengue with or without warning signs and severe dengue because the previous WHO 1997 classification (DF, DHF, and DSS) has had some issues. Its classification system has poor classification of disease severity and difficulties in usage in the clinical setting as well as in triage during outbreaks, and it could have led to inaccurate reporting of severities of dengue worldwide because of the difficulty for reporting clinicians in using it (WHO, 1997; WHO, 2009; Srikiatkhachorn *et al,* 2010; Hadinegoro, 2012).

Clinicians should monitor the progression of a dengue infection if the following warning signs manifest: persistent or severe vomiting, abdominal

pain or tenderness, liver enlargement, drowsiness or alteration of consciousness, fluid accumulation with respiratory distress, epitaxis, gum bleeding, gastrointestinal bleeding, retinal hemorrhage, oliguria, and hemoconcentration with severe thrombocytopenia. Physicians should note any of these warning signs, as they may lead to severe dengue (Leo *et al,* 2011; Horstick *et al,* 2012; Prasad *et al,* 2013). Severe dengue is defined by one or more of the following: plasma leakage (DHF) possibly resulting in shock (DSS), severe bleeding, and severe organ impairment such as hepatic failure, acute renal failure, and encephalopathy (Gamble *et al,* 2000; Guzman *et al,* 2002; Hammond *et al,* 2005; Wichmann *et al,* 2005; Green and Rothman, 2006; Kittigul *et al,* 2007; Guilarde *et al,* 2008; WHO, 2009; Anderson *et al,* 2014; Chuansumrit and Chaiyaratana, 2014; Guzman and Harris, 2015). Without treatment, mortality may be as high as 20%, but proper management, including intravenous rehydration, can dramatically reduce mortality to less than 1%. Viral factors such as serotypes, structural and nonstructural proteins of dengue virus, and viral load as well as host factors such as age, gender differences, genetic, nutritional status, immune reaction, and co-existing medical conditions may contribute towards the severity of dengue infection.

Dengue hemorrhagic fever and dengue shock syndrome

Typically, DHF resembles DF in many clinical respects, but it is characterized by high continuous fever 2-7 days, hemorrhagic diathesis, hepato-megaly, and circulatory disturbance (DSS).

The critical stage associated with plasma leakage (20% increase in hematocrit over baseline) and marked thrombocytopenia ($<100x10^9$/l) associated with bleeding frequently occurs at the end of febrile phase of illness (WHO, 1997).

Right-sided pleural fluid detected by chest roentgenogram or free fluid in the peritoneal cavity and thickening of gall bladder wall detected by ultrasonography has been interpreted as evidence of plasma leakage, and this is usually only clinically detectable after intravenous fluid therapy unless plasma leakage is significant (Setcawan *et al,* 1995; Srikiatkhachorn *et al,* 2007; Wang *et al,* 2007). The right-sided or bilateral pleural effusion is generally not prominent, but becomes increasingly more so after excessive intravenous fluid administration.

In mild DHF cases, the changes in blood pressure and pulse may be minimal and transient with patients recovering shortly after treatment. In more severe DHF cases, the classification of DSS is established by a rapid and weak pulse, narrowing of the pulse pressure to less than 20 mmHg, or an unobtainable blood pressure (WHO, 1997). Clinical indicators of impending DSS, include severe abdominal pain, change from fever to hypothermia, restlessness, sweating, prostration, and tender hepatomegaly. When continuous loss of plasma that becomes excessive occurs, the patient may progress rapidly to profound shock.

Prolonged shock is often complicated metabolic acidosis, severe gastrointestinal bleeding, and disseminated intravascular coagulopathy (DIC). DSS was an independent risk factor (odds ratio 220) for development of acute renal failure in adult patients with DHF (Lee *et al,* 2009). In a few patients, cardiac involvement ranging from abnormality of electrocardiogram, mild elevation of cardiac biomarkers to myocarditis and/or pericarditis was observed, and some of these patients died (Miranda *et al,* 2013). Acute respiratory failure is a rare complication but has a high mortality rate (Wang *et al,* 2007a).

Although children have a greater likelihood of developing hypovolemic shock in DHF characterized by increased microvascular permeability compared to adults, adults and elderly with dengue infection still have a high mortality rate (Agarwal *et al,* 1999; Gamble *et al,* 2000; Rigau-Perez and Laufer, 2006; Kuo *et al,* 2007; Lee *et al,* 2008; Gautret *et al,* 2012; Pang *et al,* 2012; Tantawichien, 2012). High fatality rates of dengue in adults were significantly associated with pre-existing co-morbid medical illnesses such as cardiac diseases and renal diseases (Kuo *et al,* 2007; Lee *et al,* 2008; Leo *et al,* 2011; Gautret *et al,* 2012; Pang *et al,* 2012; Tantawichien, 2015).

Because the altered vascular permeability is short-lived and spontaneously converts to normal level, the period of clinically significant plasma leakage usually lasts 24-48 hours after deferverscence. Diuresis ensues as plasma leakage terminates, convalescent rash, transient hypertension and sinus bradycardia have been described during convalescence in patients with DHF/DSS.

Hemorrhage associated with dengue infection

Hemorrhage often occurs between 5-to-8 days after the onset of illness and is a contributory factor to morbidity and mortality, particularly during the severe thrombocytopenia (Chuansumrit and Chaiyaratana, 2014).

The pathogenesis of abnormal bleeding in dengue is multifactorial and encompasses severe thrombocytopenia, platelet dysfunction, blood coagulation defects, and vasculopathy.

Typically seen coagulopathies are increased aPTT and low fibrinogen levels, but the likely major causes of clinical hemorrhage are severe thrombocytopenia and platelet dysfunction.

Variable degree of hemorrhage may occur at any sites, most commonly petechiae, epistaxis, and gingiva or vaginal bleeding, and usually on days 5-to-8 of the illness. Bleeding from the nose, gums, and upper gastrointestinal tract are not uncommon in patients with dengue infection.

Vaginal bleeding (menorrhagia) was a common site of bleeding (24.6% in adults with dengue infection) and hormonal therapies, such as premarin and primulute N, are suggested for patients exhibiting excessive vaginal bleeding (Tantawichien, 2012).

Of the dengue patients with plasma leakage (DHF), severity of bleeding varied markedly with spontaneous petechiae, hematemesis, melena, menorrhagia, and epistaxis. Risk factors of severe bleeding are platelets ≤20,000/mm^3 ($20x10^9$/l), high aspartate aminotransferase (AST), or alanine aminotransferase (ALT), prolonged prothrombin time (PT), severe plasma leakage (DSS), patients with DIC, or fulminant hepatic failure (Chamnanchanunt *et al,* 2012).

Pre-existing peptic ulcer or hemorrhagic gastritis in adults with DF or DHF may result in massive hematemesis, and this kind of hemorrhaging may be not associated with profound shock in adults dissimilar to previous reports in children. The literature contains few reports of endoscopic findings in dengue-infected adults with upper gastrointestinal bleeding, and those findings reported hemorrhagic gastritis most commonly (40.9-58.5%), followed by gastric ulcer, and duodenal ulcer (Tsai *et al,* 1991; Chiu *et al,* 2005). However, the utility of endoscopic therapy in upper gastrointestinal bleeding in dengue patients has not been established (Wung *et al*, 1990).

Subcapsular splenic hemorrhaging and ruptures are rare and life-threatening internal hemorrhaging that may occur spontaneously or due to trauma and may not be noticed. Splenectomy is still the favored treatment for splenic rupture, but favorable outcomes with conservative treatment have been recently and numerously reported (Imbert *et al,* 1993; Pungjitprapai and Tantawichien, 2008).

Averting a fatal outcome in the dengue patient with severe hemorrhaging requires early diagnosis, intensive supportive care and replacement therapy.

Reports about pregnant women with DF or severe dengue in Asia have emphasized the concept that young women in hyperendemic and endemic area are susceptible to dengue infection (Thaithumyanon *et al,* 1994; Bunyavejchevin *et al,* 1997; Corles *et al,* 1999).

Obstetricians must be alert to the risk of dengue infection in pregnant women and should take history and order laboratory test relevant to dengue infection. Dengue during pregnancy is of particular importance in pregnant women who are traveling from non-endemic countries to endemic ones (Carroll *et al,* 2007).

Spontaneous abortion and severe postpartum bleeding were reported to be caused by uterine hemorrhage in dengue-infected pregnant women (Thaithumyanon *et al,* 1994). Unexpected hemorrhage that is challenging to control in post-operative period may be caused by surgical

procedures, including cesarean sections that are performed on patients with dengue infection, may reveal previously undetected dengue-induced hemostatic defects (Adam *et al,* 2010).

Vertical transmission of dengue from mother to fetus has been reported that caused a full-blown illness in the neonate similar in manifestations to those seen in children and adults (Bunyavejchevin *et al,* 1997). The effects of dengue infection on pregnant women and their fetuses or newborns are unclear. Nevertheless, recent studies have shown that dengue infection may have been the culprit for fetal deaths and morbidity in pregnant women but did not appear to cause any infant abnormalities (Basurko *et al,* 2009; Pouliot *et al,* 2010; Chitra and Panicker, 2011).

Severe organ impairment and unusual manifestations

Hepatomegaly and increased levels of AST and ALT were more commonly found in patients with dengue infection, especially DHF (Kuo *et al,* 1992; Kalayanarooj *et al,* 1997; Souza *et al,* 2004; Trung *et al,* 2010; Kittitrakul *et al,* 2015; Treeprasertsuk and Kittitrakul, 2015). Due to these reports that certain liver enzymes may be elevated in dengue infections, the clinician should include dengue infection in the differential diagnosis of acute viral hepatitis in Asia. Dissimilar to conventional viral hepatitis, the dengue patient has levels of AST that are higher than that of ALT possibly owing to excessive release of AST from damaged myocytes during dengue infections, and these liver enzymes increase to maximum levels at 7-9 days after onset of illness, after which they decrease to normal levels within 2 weeks (Kuo *et al,* 1992; Kalayanarooj *et al,* 1997; Trung *et al,* 2010; Tantawichien, 2012; Tantawichien, 2015). Potential mechanisms of liver injury could involve a range of potential insults, including direct effects of infected virus serotypes, an adverse consequence of abnormal host immune responses to liver cells, compromised circulation and/or hypoxia due to hypotension or localized vascular leakage with the liver capsule, drug-related hepatotoxicity such as hepatotoxicity to acetaminophen or traditional herbal remedies, co-infection with other hepatitis-causing viruses such as hepatitis A, B, and C, as well as pre-existing underlying diseases (*eg*, hemoglobinopathies and alcoholic liver diseases) (Parkash *et al,* 2010).

Clinicians should consider carefully using hepatotoxic drugs such as acetaminophen, antibiotics, and antiemetic drugs, all of which have the potential to aggravate liver damage in patients with dengue. Acetaminophen overdose may play an important role in causing acute liver failure in dengue patients (Ling *et al,* 2007; Kye Mon *et al,* 2016), and adult dengue patients probably have relatively more liver impairment than child dengue patients. Pre-existing liver diseases such as chronic infection with virus hepatitis B or C, alcoholic liver disease, and cirrhosis may aggravate the severity of liver impairment during a dengue infection.

Dengue patients with vascular leakage and abnormal bleeding who have abnormal liver enzyme levels have been associated with disease severity and poor outcomes (Kittitrakul *et al,* 2015; Treeprasertsuk and Kittitrakul, 2015). Increased levels of bilirubin and alkaline phosphatase have been reported in a few patients. Severe liver impairment such as acute hepatic failure contributing directly to severe hemorrhaging as well as potentiating the severity of DIC may occur in the late stage of dengue disease, complicating the outcome of the patient (Ling *et al,* 2007; Kye Mon *et al,* 2016). Severe jaundice and high mortality are observed in dengue patients with fulminant hepatic failure. The management of fulminant hepatic failure in dengue is primarily intensive supportive care; however, therapies with N-acetylcysteine or artificial liver support have been described in the literature (Treeprasertsuk and Kittitrakul, 2015).

The unusual manifestations of dengue infection that have been recognized, include severe internal hemorrhage, fulminant hepatic failure, encephalopathy, cardiomyopathy, cardiac arrhythmia, adult respiratory distress syndrome, rhabdomyolysis, pancreatitis, appendicitis, co-infection with other viruses or tropical infectious diseases, and neurological complications (*eg*, altered consciousness, seizures, paresis, and coma resulting from encephalitis and encephalopathy)

(Thakane *et al,* 1996; Solomon *et al,* 2000; Garcia-Rivera and Rigue-Perez, 2002; Davis and Bourke, 2004; Promphan *et al,* 2004; Misra *et al,* 2006; Premaratna *et al,* 2007; Sam *et al,* 2013).

The neurological manifestations secondary to dengue infection, including encephalopathy, encephalitis, myelitis, neuro-ophthalmic complications, polyradiculopathy, neuropathy, and neuromuscular complications were ascribed in 0.5-21 % of hospitalized patients (Solomon *et al,* 2000; Garcia-Rivera and Rigue-Perez, 2002; Misra *et al,* 2006; Carod-Artal *et al,* 2013). Possible causes of encephalopathy in patients with dengue, include hypotension, cerebral edema, focal hemorrhage, hypernatremia, fulminant hepatic failure, and the direct invasion of dengue virus in the central nervous system (Lum *et al,* 1996; Chokephaibulkit *et al,* 2001).

Acute renal failure is an accompanying presentation in DSS or dengue-associated fulminant hepatic failure. Previous studies have revealed that 5.5% of the patients with DHF/DSS also had dual infection (*eg*, urinary tract infection, diarrhea, or bacteremia) (Pancharoen and Thisyakorn, 1998; Tantawichien, 2012). Dual infection should be suspected in patients with atypical manifestations; for example, fever for more than 10 days, mucus diarrhea, jaundice, persistent abdominal pain, recurrent fever, WBC >10,000/mm^3 (>10x10^9/l) with neutrophilia, or presence of the band form of neutrophil and acute renal failure (Lee *et al,* 2005).

The patient with severe dengue infection may have secondary bacterial sepsis (*eg*, bacteremia or UTI after hospitalization). Failure in making a diagnosis of concurrent infection in patients with dengue may lead to otherwise preventable mortality (Trunfio *et al,* 2016).

DIAGNOSIS

Most efforts to clinically differentiate dengue infection from other acute febrile illnesses are likely to be unsuccessful. The diagnostic attempt may be assisted by laboratory examination, indicating leukopenia, neutropenia, thrombocytopenia, or mildly elevated AST levels.

Early definite diagnosis of dengue infection allows the clinician to initiate supportive care and adequate fluid management early on and also identifies patients with severe dengue for close monitoring for signs of plasma leakage, bleeding, and end-organ damage. A definite diagnosis might also prevent the use of potentially harmful drugs, ensure the adequate use of treatment guidelines, and promote effective control of dengue outbreaks.

A positive tourniquet test has been considered by the WHO to have utility as a clue for probable dengue infection for a long time (WHO, 2009). Unfortunately, recent reports have found that the sensitivity and specificity of tourniquet test is poor (34-56% and 68-94%, respectively), and a negative test does not exclude the disease (Gregory *et al,* 2011; Mayxay *et al,* 2011; Halsey *et al,* 2013).

Laboratory diagnosis of dengue infection is established either directly by isolation or detection of viral components in serum or tissue, or indirectly by detection of virus-specific antibodies in serum (de Oliveira *et al,* 2005). The sensitivity of either molecular or serological testing depends partially on the duration and severity of the illness in a patient.

Only reverse transcriptase polymerase chain reaction (RT-PCR) or dengue virus NS1 antigen assay can reliably confirm the diagnosis of dengue during the 2 to 3 days after the onset of illness. The presence of the dengue virus in serum, tissues, saliva or urine can be definitely detected most satisfactorily by RT-PCR, and this molecular testing modality can detect dengue viruses up to 7 days after the onset of the symptoms, especially in severe cases (Alcon *et al,* 2002; Yamada *et al,* 2002; Lanciotti, 2003).

Various ELISA assays in the plasma and/or sera of dengue patients have shown a high circulating level of dengue virus NS1 in early stage of dengue infection (Vazqueza *et al,* 2010). Detecting antibodies for rapid diagnosis in the early stage of the illness is not practical due to their adequate detection ability starting to occur around 5 days after the onset of the illness. To date, ELISA for

detecting acute phase (IgM) and convalescent phase (IgG) antibodies has been considered the test of greatest utility in diagnosing dengue owing to its high sensitivity and ease of use.

There are several commercial kits of rapid tests for IgM and IgG detection; nevertheless, they vary in sensitivity, specificity, and accuracy (Kittigul and Suankeow, 2002; Blacksell *et al,* 2006). Various combination tests for elevated levels of NS1 and dengue IgM/IgG in serum have reported sensitivities ranging from 75.5% to 92.9% and specificities ranging from 75% to 100%, and these combination tests are a pragmatic diagnostic approach in a patient with a suspected dengue infection.

Even without the availability of laboratory tests that are accurate and facilitate early detection, the clinician must consider dengue infection in the differential diagnosis of an acute undifferentiated febrile illness in every presenting child or adult in a dengue endemic area.

MANAGEMENT

Until now, due to the lack of a specific therapeutic agent for dengue infection, treatment is supportive care, especially careful fluid administration. Reduction in morbidity/mortality rates in patients with dengue infection could be aided by the early recognition of warning signs, plasma leakage, abnormal bleeding, signs of circulatory collapse, and other serious complications. Dengue patient without warning signs may be treated at home with oral hydration and antipyretics with instructions to follow up at outpatient care. The clinician needs to provide appropriate safety netting advice by telling the patient return to the hospital promptly if hemorrhaging or warning signs suggestive of severe dengue develop.

Oral rehydration is indicated to replace fluid lost by vomiting and high fever. Patients and their care givers should be made aware that acetaminophen, salicylates, non-steroidal anti-inflammatory drugs (NSAIDs) and traditional medicines that are commonly prescribed to febrile patients may cause either severe bleeding or hepatic injury if used or improperly used as appropriate. If warning signs develop, the patient requires close observation and hospitalization with appropriate use of intravenous fluids in patients with inadequate oral intake or a rapidly increasing hematocrit (WHO, 2009).

Monitoring all of the patients with warning signs to identify developing warning signs of severe disease, which is recommended by the WHO, may greatly burden healthcare services in limited-resource countries. Nonetheless, the clinician should hospitalize a patient with dengue infection immediately if any of the followings are observed: severe nausea/vomiting, restlessness or lethargy, severe hemorrhage (*eg*, hematemesis or hematochezia), narrowing of pulse pressure (≤20 mmHg) or hypotension, a sudden rise in hematocrit or continuously elevated hematocrit despite the administration of fluid, a platelet count of ≤20,000/mm^3 (≤20x10^9/l), AST or ALT >500 U/ml, oliguria or acute renal failure, liver failure, heart failure, severe hypoxemia, pregnancy, and no opportunity to be followed up in an out-patient setting (Nimmannitaya, 1987; Ngo *et al,* 2001; Royal College of Physicians of Thailand, 2015).

Close clinical monitoring of patients with severe dengue or suspected DHF/DSS and intensive supportive treatment are lifesaving and have reduced fatality rates.

Critical activities for the clinician taking care of hospitalized dengue patients are monitoring of abnormal bleeding, circulation and vascular leakage by serial clinical assessments of hypovolemia/shock and rising of hematocrit to inform the decision to give intravenous fluid or blood component transfusion.

Assessment of the severity of plasma leakage by close monitoring measurements (*eg*, vital signs, urine output, and serial hematocrit levels) and timely appropriate intravenous fluid resuscitation with crystalloid to counteract massive plasma leakage are necessary to reduce morbidity and mortality in the critical stage of DHF. DHF patients require close monitoring for signs of shock until at least 24-48 hours after defervescence. The main therapy for patients with plasma leakage and shock

(DSS) is early and appropriate replacement of lost plasma. The WHO recommends immediate volume replacement with Ringer's lactate or physiologically normal saline solution, followed by a plasma expander such as fresh frozen plasma or colloid solutions (albumin and dextran) if shock persists.

Therapeutic responses to colloid and crystalloid solutions from two randomized controlled trials have found that Ringer's lactate is inferior to other options and that the more severely ill patients identified by a narrow pulse pressure have a greater benefit from initial resuscitation with colloid compared with crystalloid solution (Dung *et al,* 1999; Wills *et al,* 2005; Akech *et al,* 2011). Adequate volume replacement should assessed by adjusting the rate of intravenous fluid during the period of plasma leakage with frequent assessments of vital signs, hematocrit, and urine output. Volume replacement should be kept to the minimum needed to maintain cardiovascular stability until vascular permeability returns to a normal level. Internal or concealed bleeding should be suspected in the patient with persistent shock in spite of a declining hematocrit after fluid resuscitation with crystalloid or colloid solutions.

To correct the bleeding tendency, anemia, coagulopathy, and hypovolemia in a dengue patient with severe hemorrhaging, blood transfusion therapy with packed red cell, concentrated platelets, and fresh frozen plasma is still the treatment of choice. Blood or platelet transfusions for prophylaxis against severe thrombocytopenia may cause harm and should not be performed in uncomplicated cases (Lye *et al,* 2009). Invasive procedures should be minimized to avoid hemorrhagic complications.

Because metabolic acidosis and hyponatremia occur more commonly in DSS, sodium bicarbonate infusion should be considered along with early adequate fluid replacement.

Co-morbidities in adult and elderly patients such as coronary artery disease, peptic ulcer, hypertension, diabetes mellitus, cirrhosis, or chronic kidney disease should be considered for proper management, and these co-morbidities may contribute to the severity of dengue infection (Rigau-Perez and Laufer, 2006; Kuo *et al,* 2007; Lee *et al,* 2008; Lye *et al,* 2010; Sam *et al,* 2013).

No evidence supporting the use of chloroquine, corticosteroid, interferon, immune globulin, desmopressin, or carbazochrom sodium sulfonate (AC-17) for severe dengue infection exists (Tassniyom *et al,* 1993; Tassniyom *et al,* 1997; Kularatne *et al,* 2009; Tricou *et al,* 2010).

Vasculopathy and circulatory failure are usually self-limiting, and spontaneous resolution of these can be expected to occur within 2-to-3 days, followed by complete recovery. In the recovery period, the patient usually has improved appetite, bradycardia, and a convalescent rash and may have fatigue or mood disturbance for several weeks.

PREVENTION

To reduce burden of dengue, the WHO has set out specific objectives in global dengue control strategy: estimate the true burden of dengue by 2015 and a reduction of dengue morbidity and mortality by 2020 by at least 25% and 50%, respectively (using 2010 as the baseline reference measurement) (WHO, 2012).

It seems clear that implementations of effectively sustainable vector control and effective dengue vaccines are keys to success for this disease control.

The WHO has recommended the concept of integrated vector management (IVM). This is an evidence-based approach based on evidence specific to a country to promote the optimal use of its resources. Development and deployment of vector-control strategies that effectively minimize dengue replication and transmission are still challenging.

At present, public health and community-based *Ae. aegypti* control programs that use chemical or biological methods to remove and destroy mosquito-breeding sites are the mainstay of dengue prevention (WHO SEARO, 2011). However, the IVM approach has been unsuccessful in most of the Asian countries, especially dengue endemic regions. Thus,

new tools and strategies are needed to prevent and control dengue, including the development of a safe and efficacious dengue vaccine.

The potential dengue vaccine is one consisting of a tetravalent combination of attenuated dengue strains, which simultaneously induce protective and durable immune responses against all four dengue serotypes. Recent studies in Asia and Latin America show that recombinant live-attenuated tetravalent dengue vaccine (CYD-TDV) was safe and moderately efficacious when given three injections at months 0, 6, and 12 to children and adolescents (Capeding *et al,* 2014; Hadinegoro *et al,* 2015). Overall vaccine efficacy of CYD-TDV was estimated to be 60% against virologically confirmed dengue infection (VCD) with high levels of protection offered against hospitalization (80%) in subjects aged 2-16 years. However, variations of vaccine efficacy against VCD were observed in endemic settings, dengue serotype, and the pre-existing dengue antibody type in terms of serotype in the individual (Capeding *et al,* 2014; Hadinegoro *et al,* 2015). Recent pooled analyses of the first 2-3 years of long-term follow-up provided further supportive evidence of efficacy against hospitalized dengue in children 9 years of age or older (Guya *et al,* 2015). This vaccine has recently obtained licensure for use in children 9 years of age or older (9-45 year old) in Mexico, the Philippines, Brazil, Thailand, Singapore, and several other endemic countries. Due to the high disease burden in endemic countries, this vaccine could have a substantial effect on public health despite its moderate overall efficacy (Endo *et al,* 2016; Lopez-Gatell *et al,* 2016; Pang, 2016). The implementation of an efficacious dengue vaccine will shift the burden of disease, the age-related differences in clinical manifestations and prognoses described here, indicating the importance of comparing a wide range of ages in future clinical studies of dengue.

CONCLUSION

The increasing number of dengue cases is a major public health problem in many countries in South and Southeast Asia where *Ae. aegypti* and *Ae. albopictus* are widespread in both urban and rural areas. Several countries in Asia have reported an epidemic shift of dengue from mainly affecting children to affecting adolescents and young adults with increased severity. The clinical spectrum of dengue ranges from undifferentiated fever or DF to the life-threatening infection associated with plasma leakage (DHF/DSS), severe bleeding or multi-organ failure, which may be fatal.

The early recognition of warning signs, plasma leakage, abnormal bleeding, circulatory collapse, and other serious complications would reduce mortality rates in patients with dengue infection. The implementations of effectively integrated vector management and efficacious dengue vaccines are the keys to success for disease control in hyperendemic/endemic areas.

REFERENCES

Adam I, Jumaa AM, Elbashir HM, Karsany MS. Maternal and perinatal outcomes of dengue in Port Sudan, Eastern Sudan. *Virol J* 2010; 7: 153.

Agarwal R, Kapoor S, Nagar R, *et al.* A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Public Health* 1999; 30: 735-40.

Akech S, Ledermann H, Maitland K. Choice of fluids for resuscitation in children with severe infection and shock: systematic review. *BMJ* 2011; 341: c4416.

Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol* 2002; 40: 376-81.

Anderson KB, Gibbons RV, Cummings DAR, *et al*. A shorter time interval between first and second dengue infections is associated with protection from clinical illness in a school based cohort in Thailand. *J Infect Dis* 2014; 209: 360-8.

Anuradha S, Singh NP, Rizvi SNA, Agarwal SK, Gur R, Mathur MD. The 1996 outbreak of dengue hemorrhagic fever in Delhi, India. *Southeast Asian J Trop Med Public Health* 1998, 29: 503-6.

Anyamba A, Chretien JP, Small J, Tucker CJ, Linthicum KJ. Developing global climate anomalies suggest potential disease risks for 2006-2007. *Int J Health Geogr* 2006; 5: 60.

Barbazan P, Yoksan S, Gonzalez JP. Dengue hemorrhagic fever epidemiology in Thailand: description and forecasting of epidemics. *Microb Infect* 2002; 4: 699-705.

Basurko C, Carles G, Youssef M, Guindi WE. Maternal and fetal consequences of dengue fever during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2009; 147: 29-32.

Bhatt S, Gething PW, Brady OJ, *et al*. The global distribution and burden of dengue. *Nature* 2013: 496: 504-7.

Blacksell SD, Newton PN, Bell D, *et al*. The comparative accuracy of 8 commercial rapid immunochromatographic assays for the diagnosis of acute dengue virus Infection. *Clin Infect Dis* 2006; 42: 1127-34.

Brien D, Tobin S, Brown GV, Torresi J. Fever in returned travelers. *Clin Infect Dis* 2001; 33: 603-9.

Bunyavejchevin S, Tanawattenacharoen S, Taechakraichana N, Thisyakorn U, Tannirandorn Y, Limpaphayom K. Dengue hemorrhagic fever during pregnancy. *J Obstet Gynaecol Res* 1997; 23: 445-8.

Burdino E, Milia MG, Sergi G, *et al*. Diagnosis of dengue fever in North West Italy in travelers from endemic areas: a retrospective study. *J Clin Virol* 2011; 51: 259-63.

Capeding MR, Tran NH, Hadinegoro SRS, *et al*. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* 2014; 384: 1358-65.

Carod-Artal FJ, Wichmann O, Farrar J, Gasc J. Neurological complications of dengue virus infection. *Lancet Neurol* 2013; 16: 906-19.

Carroll D, Toovey S, Gompel AV. Dengue fever and pregnancy. *Travel Med Infect Dis* 2007; 5: 183-8.

Chamnanchanunt S, Kanagaraj D, Thanachartwet V, Desakorn V, Rojnuckarin P. Early predictors of clinically significant bleeding in adults with dengue infection. *Southeast Asian J Trop Med Public Health* 2012; 12: 890-8.

Charoensook O, Foy HM, Teeraratkul A, Silarug N. Changing epidemiology of dengue hemorrhagic fever in Thailand. *Epidemiol Infect* 1999; 122: 161-6.

Chen LH, Wilson ME. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. *Clin Infect Dis* 2004; 39: e56-60.

Chitra TV, Panicker S. Maternal and fetal outcome of dengue fever in pregnancy. *J Vector Borne Dis* 2011; 48: 210-3.

Chiu YC, Wu KL, Kuo CH, *et al*. Endoscopic findings and management of dengue patients with upper gastrointestinal bleeding. *Am J Trop Med Hyg* 2005; 73: 441-4.

Chokephaiblukit K, Kankirawatna P, Apintanapong S, *et al*. Viral etiologies of encephalitis in Thai children. *Pediatr Infect Dis J* 2001; 20: 216-8.

Chuansumrit A, Chaiyaratana W. Hemostatic derangement in dengue hemorrhagic fever. *Thromb Res* 2014; 133: 10-6.

Corles G, Peiffer H, Talarmin A. Effects of dengue fever during pregnancy in French Guiana. *Clin Infect Dis* 1999; 28: 637-40.

Costa SD, da Silva GB, Jacinto CN, *et al*. Dengue fever among renal transplant recipients: a series of 10 cases in a tropical country. *Am J Trop Med Hyg* 2015; 93: 394-6.

Cummings DA, Iamsirithaworn S, Lessler JT, *et al.* The impact of the demographic transition on

dengue in Thailand: insights from a statistical analysis and mathematical modeling. *PLOS Med* 2009; 6: e1000139.

Davis JS, Bourke P. Rhabdomyolysis associated with dengue virus infection. *Clin Infect Dis* 2004; 38: e109-11.

de Oliveira PC, Pavoni DP, Queiroz MH*, et al.* Dengue virus infections: comparison of methods for diagnosing the acute disease. *J Clin Virol* 2005; 32: 272-7.

Dung NM, Day NP, Tam DT*, et al.* Fluid replacement in dengue shock syndrome: a randomized, double-blind comparison of four intravenous-fluid regimens. *Clin Infect Dis* 1999; 29: 787-94.

Endo IC, Ziegelmann PK, Patel A. The economic promise of developing and implementing dengue vaccines: evidence from a systematic review. *Vaccine* 2016; 34: 6133-47.

Freedman DO, Weld LH, Kozarsky PE*, et al.* Spectrum of disease and relation to place of exposure among ill returned travelers. *N Engl J Med* 2006; 354: 119-30.

Gamble J, Bethell D, Day NP*, et al.* Age-related changes in microvascular permeability: a significant factor in the susceptibility of children to shock? *Clin Sci (Lond)* 2000; 98: 211-6.

Garcia-Rivera EJ, Rigue-Perez JG. Encephalitis and dengue. *Lancet* 2002; 360: 261.

Gautret P, Gaudart J, Leder K*, et al.* Travel-associated illness in older adults (>60 y). *J Travel Med* 2012; 19: 169-77.

Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. *Curr Opinon Infect Dis* 2006; 19: 429-36.

Gregory CJ, Lorenzi OD, Colon L, *et al.* Utility of the tourniquet test and the white blood cell count to differentiate dengue among acute febrile illnesses in the emergency room. *PLOS Negl Trop Dis* 2011; 5: e1400.

Guilarde AO, Turchi MD, Siqueira JB*, et al.* Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. *J Infect Dis* 2008; 197: 817-24.

Guya B, Briand O, Lang J, Saville M, Jackson N. Development of the Sanofi Pasteur tetravalent dengue vaccine: one more step forward. *Vaccine* 2015; 33: 7100-11.

Guzman Mg, Harris E. Dengue. *Lancet* 2015; 385: 453-65.

Guzman MG, Kouri G, Bravo J, Valdes L, Vazquez S, Halstead SB. Effect of age on outcome of secondary dengue 2 infections. *Int J Infect Dis* 2002; 6:118-24.

Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas. *J Clin Virol* 2003; 27: 1-13.

Hadinegoro R, Taurel AF, Capeding MR*, et al.* Symptomatic dengue disease in five Southeast Asian countries: epidemiological evidence from a dengue vaccine trial. *PLOS Negl Trop Dis* 2016; 10: e0004198.

Hadinegoro SR, Arredondo-Garcia JL, Capeding MR*, et al*. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med* 2015; 373: 1195-206.

Hadinegoro SR. The revised WHO dengue case classification: does the system need to be modified? *Paediatr Int Child Health* 2012; 32(suppl): 33-8.

Halsey ES, Vilcarromero S, Forshey BM*, et al.* Performance of the tourniquet test for diagnosing dengue in Peru. *Am J Trop Med Hyg* 2013; 89: 99-104.

Hammond SN, Balmaseda A, Perez L*, et al.* Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. *Am J Trop Med Hyg* 2005; 73:1063-70.

Hanafusa S, Chanyasanha C, Sujirarat D, Khuankhunsathid I, Yaguchi A, Suzuki T. Clinical features and differences between child and

adult dengue infections in Rayong Province, Southeast Thailand. *Southeast Asian J Trop Med Public Health* 2008; 39: 252-9.

Horstick O, Farrar J, Lum L*, et al.* Reviewing the development, evidence base, and application of the revised dengue case classification. *Pathog Glob Health* 2012; 106: 94-101.

Imbert P, Sordet D, Hovette P, Touze JE. Splenic rupture in a patient with dengue fever. *Trop Med Parasitol* 1993; 44: 327-8.

Jelinek T. Dengue fever in international travelers. *Clin Infect Dis* 2000; 31: 144-7.

Kalayanarooj S, Vaughn DW, Nimmannitya S*, et al.* Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 1997; 176: 313-21.

Kittigul L, Pitakarnjanakul P, Sujirarat D, Siripanichgon K. The differences of clinical manifestations and laboratory findings in children and adults with dengue virus infection. *J Clin Virol* 2007; 39:76-81.

Kittigul L, Suankeow K. Use of a rapid immunochromatographic test for early diagnosis of dengue virus infection. *Eur J Clin Microbiol Infect Dis* 2002; 21: 224-6.

Kittitrakul C, Silachamroon U, Phumratanaprapin W, Krudsood S, Wilairatana P, Treeprasertsuk S. Liver function tests abnormality and clinical severity of dengue infection in adult patients. *J Med Assoc Thai* 2015; 98(suppl): 1-8.

Kulanatne SAM, Gawarammana IB, Kumarasiri PRV. Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults. *Southeast Asian J Trop med Public Health* 2005; 36: 686-92.

Kularatne SAM, Walathara C, Mahindawansa I, *et al.* Efficacy of low dose dexamethasone in severe thrombocytopenia caused by dengue fever: a placebo controlled study. *Postgrad Med J* 2009 85: 525-9.

Kuo CH, Tai DI, Chang-Chien CS*, et al.* Liver biochemical tests and dengue fever. *Am J Trop Med Hyg* 1992; 47: 265-70.

Kuo MC, Chang JM, Lu PL, Chiu YW, Chen HC, Hwang Hj. Case report: difficulty in diagnosis and treatment of dengue hemorrhagic fever in patients with chronic renal failure. *Am J Trop Med* 2007; 76: 752-6.

Kye Mon K, Nontprasert A, Kittitrakul C, Tangkijvanich P, Leowattana W, Poovorawan K. Incidence and clinical outcome of acute liver failure caused by dengue in a hospital for tropical diseases, Thailand. *Am J Trop Med Hyg* 2016; 95: 1338-44.

Kyle Jl, Harris E. Global spread and persistence of dengue. *Ann Rev Microbiol* 2008; 62: 71-92.

Lanciotti RS. Molecular amplification assays for detection of flaviviruses. *Adv Virus Res* 2003; 61: 67-99.

Leder K, Torresi J, Brownstein JS*, et al*. Travel-associated illness trends and clusters, 2000-2010. *Emerg Infect Dis* 2013; 11: 1049-57.

Lee IK, Liu JW, Yang KD. Clinical characteristics and risk factors for concurrent bacteremia in adults with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2005; 72: 221-6.

Lee IK, Liu JW, Yang KD. Clinical and laboratory characteristics and risk factors for fatality in elderly patients with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2008; 79: 149-53.

Lee IK, Liu JW, Yang KD. Clinical characteristics, risk factors, and outcomes in adults experiencing dengue hemorrhagic fever complicated with acute renal failure. *Am J Trop Med* 2009; 80: 651-5.

Leo YS, Thein TL, Fisher DA*, et al.* Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study. *BMC Infect Dis* 2011; 11: 123.

Ling LM, Wilder-Smith A, Leo YS. Fulminant hepatitis in dengue haemorrhagic fever. *J Clin Virol* 2007; 38: 265-8.

Lopez-Gatell H, Alpuche-Aranda CM, Santos-Preciado JI, Hernandez-Avila M. Dengue vaccine: local decisions, global consequences.

Bull World Health Organ 2016; 94: 850-5.

Lum LCS, Lam SK, Choy YS, *et al*. Dengue encephalitis: a true entity? *Am J Trop Med Hyg* 1996; 54: 256-9.

Lye DC, Lee VJ, Sun Y, Leo YS. Lack of efficacy of prophylactic platelet transfusion for severe thrombocytopenia in adults with acute uncomplicated dengue infection. *Clin Infect Dis* 2009; 48: 1262-5.

Lye DC, Lee VJ, Sun Y, Leo YS. The benign nature of acute dengue infection in hospitalized older adults in Singapore. *Int J Infect Dis* 2010; 14: e410-3.

Massed E, Wilder-Smith A. Risk estimates of dengue in travelers to dengue endemic areas using mathematical models. *J Travel Med* 2009; 16: 191-3.

Mayxay M, Phetsouvanh R, Moore CE, *et al*. Predictive diagnostic value of the tourniquet test for the diagnosis of dengue infection in adults. *Trop Med Int Health* 2011; 16: 127-33.

Miranda CH, Borges Mde C, Matsuno AK, *et al.* Evaluation of cardiac involvement during dengue viral infection. *Clin Infect Dis* 2013; 57: 812-9.

Misra UK, Kalita J, Syam UK, Dhole TN. Neurological manifestations of dengue virus infection. *J Neurol Sci* 2006; 244: 117-22.

Mohammed H, Linnen JM, Munoz-Jordán JL, *et al.* Dengue virus in blood donations, Puerto Rico, 2005. *Transfusion* 2008; 48: 1348-54.

Nakhapakorn K, Tripathi NK. An information value based analysis of physical and climatic factors affecting dengue fever and dengue haemorrhagic fever incidence. *Int J Health Geogr* 2005; 4: 13.

Namvongsa V, Sirivichayakul C, Songsithichok S, Chanthavanich P, Chokejindachai W, Sitcharungsi R. Difference in clinical features between children and adult with dengue hemorrhagic shock syndrome. *Southeast Asian J Trop Med Public Health* 2013; 44: 72-9.

Ngo NT, Cao XT, Kneen R, *et al.* Acute management of dengue shock syndrome: a randomized double-blind comparison of 4 intravenous fluid regimens in the first hour. *Clin Infect Dis* 2001; 32: 204-12.

Nimmannitaya S. Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J Trop Med Public Health* 1987; 20: 325-30.

Olivera-Botello G, Coudeville L, Fanouillere K, *et al*. Tetravalent dengue vaccine reduces symptomatic and asymptomatic dengue virus infections in healthy children and adolescents aged 2–16 years in Asia and Latin America. *J Infect Dis* 2016; 214: 994-1000.

Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C. Dengue infection: a global concern. *J Med Assoc Thai* 2002; 85(suppl): 25-33.

Pancharoen C, Thisyalorn U. Coinfection in dengue patients. *Pediatr Infect Dis J* 1998; 17: 81-2.

Pang J, Salim A, Lee VJ, *et al.* Diabetes with hypertension as risk factors for adult dengue hemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. *PLOS Negl Trop Dis* 2012; 6: e1641.

Pang T, Thiam DGY, Tantawichien T, Ismail Z, Yoksan S. Dengue vaccine —time to act now [Letter]. *Lancet* 2015; 385: 1725-6.

Pang T. SAGE committee advice on dengue vaccine. *Lancet* 2016; 16: 880-2.

Parkash O, Almas A, Jafri SM, Hamid S, Akhtar J, Alishah H. Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital, Karachi, Pakistan (South Asia). *BMC Gastroenterol* 2010; 10: 43.

Patumanond J, Tawichasri C, Nopparat S. Dengue hemorrhagic fever, Uttaradit, Thailand. *Emerg Infect Dis* 2003; 9: 1348-50.

Pongsumpun P, Patanarapelert K, Sriprom M, Varamit S, Tang IM. Infection risk to travelers going to dengue fever endemic regions.

Southeast Asian J Trop Med Public Health 2004; 35: 155-9.

Pongsumpun P, Yoksan S, Tang IM. A comparison of the age distributions in the dengue hemorrhagic fever epidemic in Santiago de Cuba (1997) and Thailand (1998). *Southeast Asian J Trop Med Public Health* 2002; 33: 255-8.

Pouliot SH, Xiong X, Harville E*, et al.* Maternal dengue and pregnancy outcomes: a systematic review. *Obste Gyncol Sur* 2010; 65: 107-18.

Prasad D, Kumar C, Jain A, Kumar R. Accuracy and applicability of the revised WHO classification (2009) of dengue in children seen at a tertiary healthcare facility in northern India. *Infection* 2013; 41: 775-82.

Premaratna R, Bailey S, Ratnasena GN, Silva HJ. Dengue fever mimicking acute appendicitis. *Trans R Soc Trop Med Hyg* 2007; 101: 683-5.

Promphan W, Sopontamanarak S, Pruekprasert P, Kajornwattanakul W, Kongpattanayothin A. Dengue myocarditis. *Southeast Asian J Trop Med Public Health* 2004; 35: 611-9.

Pungjitprapai A, Tantawichien T. A fatal case of spontaneous rupture of the spleen due to dengue virus infection: case report and review. *Southeast Asian J Trop Med Public Health.* 2008; 39: 383-6.

Rigau-Perez JG, Laufer MK. Dengue-related deaths in Puerto Rico, 1992–1996: diagnosis and clinical alarm signals. *Clin Infect Dis* 2006; 42: 1241-6.

Royal College of Physicians of Thailand. Practical guideline for management of dengue in adult. *Southeast Asian J Trop Med Public Health* 2015; 46(suppl1): 169-81.

Sam SS, Omar SFS, Teoh BT, Abd-Jamil J, AbuBakar S. Review of dengue hemorrhagic fever fatal cases seen among adults. *PLOS Negl Trop Dis* 2013; 7: e2194.

Schwartz E, Weld LH, Wilder-Smith A, *et al.* Seasonality, annual trends, and characteristics of dengue among ill returned travelers, 1997-2006. *Emerg Infect Dis* 2008; 14: 1081-8.

Setcawan MW, Samsi TK, Pool TN*, et al*. Gallbladder wall thickening in dengue hemorrhagic fever: an ultrasonographic study. *J Clin Ultrasound* 1995; 23: 357-62.

Solomon T, Dung NM, Vaughn DW*, et al*. Neurological manifestations of dengue infection. *Lancet* 2000, 335: 1053-9.

Souza LJ, Alves JG, Nogueira RM*, et al*. Aminotransferase changes and acute hepatitis in patients with dengue fever: *Braz J Infect Dis* 2004; 8: 156-63.

Souza LJ, Pessanha LB, Mansur LC. Comparison of clinical and laboratory characteristics between children and adults with dengue. *Braz J Infect Dis* 2013; 17: 27-31.

Srikiatkhachorn A, Gibbons RV, Green S*, et al.* Dengue hemorrhagic fever: the sensitivity and specificity of the World Health Organization definition for identification of severe cases of dengue in Thailand, 1994-2005. *Clin Infect Dis* 2010; 50:1135-43.

Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W*, et al.* Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. *Pediatr Infect Dis J* 2007; 26: 283-90.

Stephan C, Allwinn R, Brodt HR, Knupp B, Preiser W, Just-Nubling G. Travel-acquired dengue infection: clinical spectrum and diagnostic aspects. *Infection* 2002; 30: 225-8.

Tambyah PA, Koay ESC, Poon MLM, Lin R, Ong BKC. Dengue hemorrhage fever transmitted by blood transfusion. *N Engl J Med* 2008; 359: 1526-7.

Tan F, Loh D, Prabhakaran K. Dengue haemorrhagic fever after living donor renal transplantation. *Nephrol Dial Transplant* 2005; 20: 447-8.

Tangnararatchakit K, Tirapanich W, Tapaneya-Olarn W*, et al.* Severe nonfebrile dengue infection in an adolescent after postoperative kidney transplantation: a case report. *Transplantation*

Proc 2012; 44: 303-6.

Tantawichien T. Dengue fever and dengue haemorrhagic fever in adolescents and adults. *Paediatr Int Child Health* 2012; 32(suppl): 22-7.

Tantawichien T. Dengue fever and dengue hemorrhagic fever in adults. *Southeast Asian J Trop Med Public Health* 2015; 46(suppl1): 79-98.

Tassniyom S, Vasanawathana S, Chirawathul A, Rojanasuphot S. Failure of high-dose methylprednisolone in established dengue shock syndrome. *Pediatrics.* 1993; 92: 111-5.

Tassniyom S, Vasanawathana S, Dhiensiri T, Nisalak A, Chirawatkul A. Failure of carbazocrome sodium sulfonate(AC-17) to prevent dengue vascular permeability or shock. *J Pediatr* 1997; 131: 525-8.

Thaithumyanon P, Thisyakorn U, Deerojnawong J, Innis BL. Dengue infection complicated by severe hemorrhage and vertical transmission in parturient woman. *Clin Infect Dis* 1994; 18: 248-9.

Thakane J, Walhelzar B, Banerjee K. Hemorrhagic manifestations and encephalopathy in case of dengue in India. *Southeast Asian J Trop Med Public Health* 1996; 27: 471-5.

Treeprasertsuk S, Kittitrakul C. Liver complications in adult dengue and current management. *Southeast Asian J Trop Med Public Health* 2015; 46(suppl1): 99-107.

Tricou V, Minh NN, Van TP, *et al*. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLOS Negl Trop Dis* 2010; 4: e785.

Trunfio M, Savoldi A, Viganò O, Monforte AD. Bacterial coinfections in dengue virus disease: what we know and what is still obscure about an emerging concern. *Infection* 2016; 45: 1-10.

Trung DT, Thao le TT, Hien TT*, et al.* Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg* 2010; 83: 774-80.

Tsai CJ, Kuo CH, Chen PC, Changcheng CS. Upper gastrointestinal bleeding in dengue fever. *Am J Gastroenterol* 1991; 86: 33-5.

Vazqueza S, Ruiza D, Barreroa R. Kinetics of dengue virus NS1 protein in dengue 4-confirmed adult patients. *Diagn Microbiol Infect Dis* 2010; 68: 46-9.

Wagner D, With K, Huzly D*, et al.* Nosocomial acquisition of dengue. *Emerg Infect Dis* 2004; 10: 1872-3.

Wang CC, Liu SF, Liao SC*, et al.* Acute respiratory failure in adult patients with dengue virus infection. *Am J Trop Med Hyg* 2007a; 77a: 151-8.

Wang CC, Wu CC, Liu JW*, et al.* Chest radiographic presentation in patients with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2007b; 77a: 291-6.

Wichmann O, Hongsiriwan S, Bowonwatanuwang C, Chotivanich K, Sukthana Y, Pukrittayakamee S. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. *Trop Med Int Health* 2005; 72: 221-6.

Wilder-Smith A, Chen LH, Massad E, Wilson ME. Threat of dengue to blood safety in dengue-endemic countries. *Emerg Infect Dis* 2009; 15: 8-11.

Wilder-Smith A. Dengue infections in travelers. *Pediatr Int Child Health* 2012; 32: 28-32.

Wills BA, Dung NM, Loan HT*, et al.* Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005; 353: 877-89.

World Health Organization (WHO), Regional Office for South-East Asia (WHO SEARO). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. *SEARO Techn Pub Ser* 2011; 60.

World Health Organization (WHO). Dengue haemorrhagic fever: diagnosis, treatment and control. 2nd ed. Geneva: WHO, 1997.

World Health Organization (WHO). Dengue, guidelines for diagnosis, treatment, prevention and control. Geneva: WHO, 2009.

World Health Organization (WHO). Global strategy for dengue prevention and control 2012-2020. Geneva: WHO, 2012.

Wung JY, Tseng CC, Lee SC, Cheng KP. Clinical and upper gastroendoscopic features of patients with dengue virus infection. *J Gastroenterol Hepatol* 1990; 5: 664-8.

Yamada KI, Takasaki T, Nawa M, Kurane I. Virus isolation as one of the diagnostic methods for dengue virus infection. *J Clin Virol* 2002; 24: 203-9.