EVALUATION OF DENGUE INFECTION: DETERMINATION OF RISK FACTORS AND OUTCOMES OF ACUTE KIDNEY INJURY IN HOSPITAL UNIVERSITI SAINS MALAYSIA

by

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ACEIs	Angiotensin-converting enzyme inhibitors
ACS	Abdominal Compartment Syndrome
ADQI	Acute Dialysis Quality Initiative
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
aPTT	Activated partial-thromboplastin time
ARBs	angiotensin receptor blockers
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute renal failure
AST	Aspartate Aminotransferase
ATN	acute tubular necrosis
C3	Complement C3 component
CD	Conventional definition
CDC	Centers of Disease Control and Prevention
CHF	Congestive Heart Failure
CKD	Chronic Kidney Disease
CPG	Clinical Practice Guidelines
DAKI	Dengue induced AKI / Dengue associated AKI
DENV	Dengue Virus
DHF	Dengue Hemorrhagic Fever
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
DSS	Dengue Shock Syndrome
DVI	Dengue Viral Infection
EDS	Expanded Dengue Syndrome
EIP	Extrinsic incubation period
GFR	glomerular filtration rate
HCT	Hematocrit
Hg	Hemoglobin

HTN	Hypertension
HUS	Hemolytic uremic syndrome
HUSM	Hospital Universiti Sains Malaysia
ICU	intensive care unit
IgA	Immunoglobulin A
IgG	immunoglobulin G
IgM	Immunoglobulin M
IIP	Intrinsic incubation period
IVF	Intravenous fluid
KDIGO	Kidney disease improving global Outcomes
LFTs	Liver Function Tests
MODs	Multiple organ dysfunctions
NS1	Non-structural glycoprotein 1
NSAIDS	Non-steroidal anti-inflammatory drugs,
PLT	Platelets
PPIs	proton pump inhibitors
PT	Prothrombin time
RBCs	Red Blood Cells
RIFLE	Risk, Injury, Failure, Loss of function, End stage renal disease
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SCr	serum creatinine
SEARO	Regional office for South East Asia
SLE	Systemic Lupus Erythmatosis
WBCs	White Blood Cells
WHO	World Health Organization

PENILAIAN JANGKITAN DENGGI: PENENTUAN FAKTOR-FAKTOR RISIKO DAN TERHASILNYA KECACATAN GINGAL AKUT DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Walaupun pelbagai tindakan agresif telah diambil oleh pihak berkuasa tempatan, Malaysia masih menghadapi krisis denggi yang semakin buruk beberapa tahun kebelakang ini. Ini menunjukkan langkah-langkah segera diperlukan untuk menilai kes denggi bagi memahami spektrum klinikal-makmal untuk menghadapi penyakit ini. Tambahan pula, data terkini menunjukkan laporan semakin meningkat ke atas kecederaan gingal akut (AKI) kalangan pesakit denggi. Walaupun bukti ketara menunjukkan peningkatan sementara dalam kreatinin serum (SCr) yang dikaitkan dengan peningkatan morbiditi dan mortaliti, AKI masih lagi tergolong dalam komplikasi yang diabaikan dalam denggi. Terdapat kekurangan siasatan epidemiologi denggi-aruhan AKI (DAKI). Malangnya, tidak ada laporan mengenai DAKI di Malaysia. Di samping itu, data pasca AKI pemulihan renal juga kurang. Oleh itu, dua kajian fasa retrospektif dan prospektif dijalankan untuk menilai ciri-ciri klinikal-makmal dan peramal bagi demam denggi berdarah (DHF) dan untuk menilai epidemiologi AKI semasa dan selepas discaj daripada hospital. Semasa Fasa-I (retrospektif), sejumlah 667 pesakit denggi telah dikaji semula dan DHF diperhatikan dalam 79 kes (11.8%). Analisis regresi multivariat menunjukkan kehadiran umur melebihi 40 tahun (OR: 4,1), jangkitan sekunder (OR: 2.7), kencing manis (OR: 2,8), lesu (OR: 3.1), pundi hempedu tebal (OR: 1.7) dan terlewat dimasukkan ke hospital (OR: 2.3) sebagai peramal bebas bagi DHF. Keseluruhan mortaliti adalah 1.2% dan hampir separuh daripada pesakit-pesakit telah menginap lama di hospital

(≥3 hari). Beberapa faktor berkaitan hospitalisasi berpanjangan dan mortaliti juga telah dinilai dalam fasa retrospektif. Prevalens AKI adalah 14.2% mengikut kriteria oleh AKIN, 12.6% oleh RIFLE dan 4.2% dengan takrifan konvensional. Kehadiran DHF (OR 8.0), rabdomiolisis (OR 7.9), disfungsi organ berganda (OR: 17.9), diabetes (OR 4.7), hospitalisasi lewat (OR: 2.1) dan penggunaan ubat-ubatan nefrotoksik (OR: 2.9) adalah berkaitan dengan AKI. Tambahan pula, pesakit dengan AKI disahkan mempunyai morbiditi signifikan, mortaliti, dan lebih lama tinggal di hospital. Semasa (prospektif) kajian Fasa-II, 526 pesakit telah diuji dan AKI diperhatikan dalam 13.7% kes. Pemulihan renal telah dinilai kalangan mangsa AKI (n = 71) pada tempoh 6 minggu dan minggu ke-12 dengan menggunakan beberapa kriteria pemulihan. Dengan menggunakan takrifan ketat bagi pemulihan renal daripada kurang (pemulihan ± 50% kepada garis dasar) kepada lebih (± 5% pemulihan ke garis dasar) menunjukkan kadar pemulihan daripada 88.9% kepada 2.8% bagi SCR dan 94.4% kepada 5.6% bagi eGFR, sebagai penanda bio bagi fungsi renal.. Pada akhir kajian, lapan pesakit mempunyai AKI dengan 7 daripadanya mengikut AKIN-II dan seorang pesakit bagi AKIN-III. Pesakit tua dengan ko-morbiditi, MODS dan penggunaan ubat-ubatan nefrotoksik ketika di hospital mempunyai hasil renal yang lemah bagi kriteria pemulihan renal yang digunakan. Kajian ini menunjukkan bahawa DF dan DHF mempunyai perbezaan profil klinikal-makmal yang ketara. Sebaliknya, AKI hadir dalam sebahagian besar pesakit denggi dan mereka yang mempunyai AKI menandakan morbiditi yang signifikan, mortaliti dan hospitalisasi yang lama. Pengenalpastian awal pesakit-pesakit yang berisiko tinggi akan mempunyai kelebihan yang jelas dari segi keputusan yang sesuai tentang rawatan dan pengurusan dalam unit pergantungan tinggi. Selain itu, jangkitan denggi AKI menunjukkan hasil renal yang tidak memuaskan dan memerlukan penjagaan

dan susulan yang lebih lama, terutama di bawah penjagaan nefrologi. Terdapat keperluan untuk melakukan kajian lanjutan di pelbagai pusat dengan tempoh susulan yang lebih penjang untuk mengesahkan penemuan kajian ini serta menjelaskan pengunaan kriteria bagi pemulihan renal pada pesakit-pesakit denggi.

EVALUATION OF DENGUE INFECTION: DETERMINATION OF RISK FACTORS AND OUTCOMES OF ACUTE KIDNEY INJURY IN HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Despite aggressive measures taken by the relevant authorities, Malaysia is still facing worsening dengue crisis over the past few years. It warrants an urgent need to evaluate dengue cases for better understanding of clinico-laboratory spectrum in order to combat this disease. Moreover, recent data indicate the increasing reports on acute kidney injury (AKI) among dengue patients. Despite notable evidence that transient increase in serum creatinine (SCr) is linked to increased morbidity and mortality, AKI is still a neglected complication of dengue. There has been a dearth of investigation on epidemiology of dengue-induced AKI (DAKI). Unfortunately, there is no report on DAKI in Malaysia. In addition, the data on post-AKI renal recovery is currently lacking. Therefore, two phase retrospective and prospective study was conducted to evaluate the clinico-laboratory characteristics and predictors of dengue hemorrhagic fever (DHF) and to assess epidemiology of AKI and post-AKI renal recovery among patients attending Hospital Universiti Sains Malaysia (HUSM). During Phase-I (retrospective), a total 667 dengue patients were reviewed. DHF was observed in 79 (11.8 %) cases. Multivariate regression analysis demonstrated presence of age > 40 years (OR: 4.1), secondary infection (OR: 2.7), diabetes mellitus (OR: 2.8), lethargy (OR: 3.1), thick gallbladder (OR: 1.7) and delayed hospitalization (OR: 2.3) as independent predictors of DHF. Overall mortality was 1.2 % and approximately half of the patients had prolonged hospital stay (≥3 days). Several factors associated with prolonged hospitalization and mortality have also

evaluated in retrospective phase. The prevalence of AKI was 14.2% by AKIN (Acute Kidney Injury Network) criterion, 12.6% by RIFLE (Risk, Injury, Failure, Loss of function, End stage renal disease) and 4.2% with conventional definition. Presence of DHF (OR: 8.0), rhabdomyolysis (OR: 7.9), multiple organ dysfunction (OR: 17.9), diabetes mellitus (OR 4.7), late hospitalization (OR: 2.1) and use of nephrotoxic drugs (OR: 2.9) were associated with AKI. Additionally, patients with AKI had significant morbidity, mortality and longer hospital stay. During Phase-II (prospective) study, 13.7% of 526 patients had AKI. Renal recovery was assessed among AKI survivors (n=71) after twelve weeks by using several criteria of recovery. The use of less (± 50% recovery to baseline) to more (± 5% recovery to baseline) stringent definitions of renal recovery demonstrated recovery rates from 88.9% to 2.8% by SCr and 94.4% to 5.6% by eGFR, as renal function biomarker. At the end of study, eight patients had AKI with AKIN-II in 7 and AKIN-III in one patient. Elderly patients with comorbidities, multiple organ dysfunctions and use of nephrotoxic drugs during hospitalization had poor renal outcomes with either criterion of renal recovery. Current study demonstrated that DF and DHF present significantly different clinico-laboratory profile. On the other hand, AKI is present in substantial proportion of dengue patients and those with AKI portended significant morbidity, mortality and prolonged hospitalization. Early identification of high risk patients will have obvious advantages in terms of appropriate decisions about treatment and management in high dependency units. Moreover, AKI in dengue infection indicates unsatisfactory renal outcomes and deserve a careful and longer follow-up, especially under nephrology care. There is a need for appropriately powered multi-center trials with longer follow-up period to validate the research findings and to clarify the utility of criteria for renal recovery in dengue patients.

CHAPTER 1

INTRODUCTION

1.1 Dengue Viral Infection (DVI)

Dengue viral infection (DVI) is a dangerous, debilitating and among the most important arthropod-borne disease that has rapidly been spread in several regions of the world in recent years (Messina *et al*., 2014). The disease is widespread throughout the tropics, with local variations in risk, influence by rainfall, temperature and unplanned rapid urbanization. The spectrum of disease varies from mild self-limiting illness, dengue fever (DF) to more severe and fulminating forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Deen *et al*., 2006).

1.1.1 Dengue Virus, Vector and Host

Dengue infections are believed to be caused by dengue virus (DENV), a mosquito-borne single positive stranded RNA virus (family: *Flaviviridae*, genus *Flavivirus)*. There are four related but antigenically distinct serotypes of dengue virus designated as DENV-1, DENV-2, DENV-3, and DENV-4 (Khan *et al*., 2008). These four serotypes are genetically similar and share approximately 65% of their genomes (Gubler, 2002). However, fifth serotype (DENV-5) has been detected during the screening of viral samples in Sarawak state of Malaysia and announced in October 2013 (Normile, 2013). Dengue virus is transmitted to non-human primates (sylvatic form) and humans (human form) via a mosquito vector; primarily of the genus *Aedes* (Subgenus: *Stegomyia*). The two most prominent species responsible for DENV transmission are *Aedes aegypti* (Origin: Africa) and *Aedes albopictus* (Origin: Asia) (Mustafa *et al*., 2015). The first infection by one serotype produces life long,

serotype-specific immunity but not lasting protection against infection by another serotype. Humans are the main amplifying host of the virus that is transmitted to them by the bite of an infective mosquito. The virus undergoes an intrinsic incubation period (IIP - time taken by the virus to complete its development in humans/animals) of 3 to 14 days (average, 4 to 7 days), after which the person may experience acute onset of fever accompanied by a variety of nonspecific signs and symptoms. During this acute febrile period (2 – 10 days) dengue viruses may circulate in the peripheral blood. This human febrile viremic phase (2 days before and 4-5 days after onset of fever) is a source of viruses for other mosquitoes. Dengue virus circulating in the blood of viremic humans is ingested by female mosquitoes during feeding. The virus then infects the mosquito mid-gut and subsequently spreads systemically over a period of 8 – 12 days. After this extrinsic incubation period (EIP - time taken by the virus to complete its development in mosquitos), the virus can be transmitted to other humans during subsequent probing or feeding. The EIP is influenced in part by environmental conditions, especially ambient temperature. Thereafter the mosquito remains infective for the rest of its life (WHO/SEARO, 2011).

Aedes aegypti is a small, dark mosquito that can be identified by the white bands on its legs and white lyre shaped markings on its body. It is highly resilient with the ability to rapidly bounce back to initial numbers after disturbances caused by the natural disaster or human interventions. *Aedes albopictus,* also known as Asian tiger mosquito, is also a small, dark mosquito with a white dorsal stripes and banded legs. It is slightly hardier than *Aedes aegypti* and feeds both on human and animals (Jansen & Beebe, 2010). Both vectors differ characteristically from each other as summarized in Table 1.1.

Table 1.1: Characteristics of two major species of *Aedes* vector

Aedes aegypti	*Aedes albopictus*
• Origin: Africa • Highly domesticated • Strong antrhopophilic[1] • Nervous feeder[2] • Discordant species[3] • High vectorial competency • Strong vectorial capacity in urban areas while poor in rural regions	• Origin: Asia • Maintain feral moorings • Feeds on both human and animals • Aggressive feeder[4] • Concordant species[5] • High vectorial competency • Poor vectorial capacity in urban areas while strong in rural regions

[1]prefer human beings over animals
[2]bite more than one host to complete one blood meal
[3]need more than one meal for completion of gonotropic cycle
[4]complete blood meal in one person in one go
[5]doest not require second blood meal for completion of gonotropic cycle
Reference: (WHO/SEARO, 2011)

1.1.2 Transmission of dengue virus

The transmission of DENV occurs in three cycles including enzootic (a primitive sylvatic cycle by monkeys-*Aedes*-monkeys), epizootic (crosses over to non-human primates from adjoining human epidemic cycles) and epidemic cycle (human-*Aedes*-human). After ingestion of blood from human, virus replicates in epithelial cell lining of mid-gut and infects the salivary glands. Afterwards, infectious saliva transmitted to human primates during probing. DENV also infect genital tract and may enter to fully developed eggs (De-Silva *et al*., 1999). Dengue transmission usually occurs during rainy season when temperature and humidity are more suitable for vector population breeding, while in regions where rainfall is scanty, vector breeds in man-made storage containers. However, during dry season, the life cycle of *Aedes aegypti* hastens that result in small-size mosquitos and shorter EIP. Small size females are required to take more blood meals for protein needed for egg production, thereby increasing the number of bites and hence infected

individuals. Urbanization and increased global travels are some other factors promoting vector breed, thus resulting in high transmission potential (WHO/SEARO, 2011).

1.2 Clinical course of Dengue Infection

After the incubation period, the illness often begins abruptly with fever and follows three phases i.e. febrile, critical and recovery phase (Figure 1.1) (WHO, 2009; Mustafa & Bee, 2011, CDC, 2014; Clinical Practice Guidelines Malaysia, 2015). The hallmark features of febrile phase include onset of symptoms along with viremia-derived high grade fever. Critical phase, also termed as plasma leak phase, is characterized by sudden onset of varying degrees of plasma leak into the pleural and abdominal cavities. Recovery phase, also refer as convalescence or reabsorption phase, is a sudden arrest of plasma leak with concomitant reabsorption of extravasated plasma and fluids (CDC, 2014). The characteristics, clinical manifestations, laboratory findings and complications of each phase have been described below.

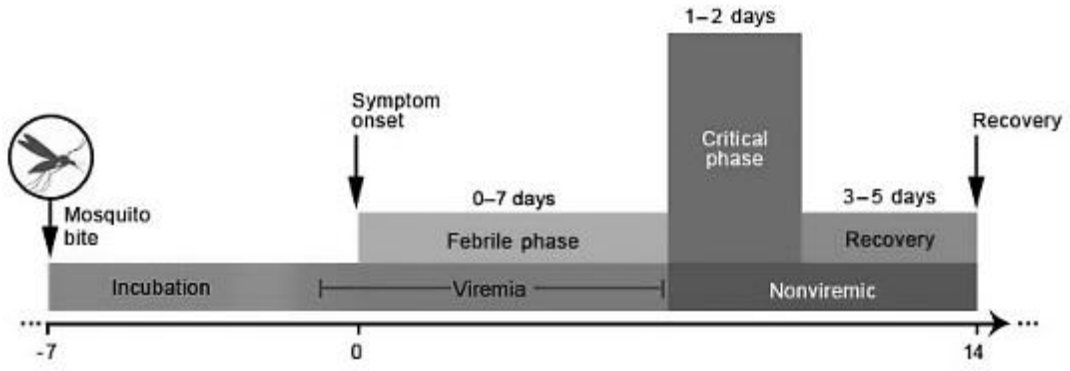

Figure 1.1: Three Phases during clinical course of dengue infection

Descriptive note: Figure is reprinted from (CDC, 2014) – Centers for Disease Control and Prevention, Dengue Clinical Case management. Version 1.1, March, 2014

1.2.1 Febrile phase

1.2.1(a) Characteristics

Typical duration for this phase ranges from 0 to 7 days and biphasic fever can occur. Monitoring of defervescence (may occur between 3-8 days of illness) and warning signs is crucial to identify progression to critical phase (CDC, 2014).

1.2.1(b) Clinical Manifestations during Febrile Phase

This phase is often accompanied by onset of high temperature plus severe headache, retro-orbital pain, myalgia, arthralgia, transient macular or maculopapular rash, facial flushing or erythema, injected oro-pharynx, nausea, vomiting, anorexia, and minor hemorrhagic manifestations such as petechia, ecchymosis, purpura, epistaxis, gum bleeding, hematuria, vaginal and gastrointestinal bleeding or positive tourniquet test. Liver may be large or tender after few days of fever. Onset of fever may also cause limited daily activity (CDC, 2014; WHO/SEARO, 2011).

1.2.1(c) Laboratory Findings during Febrile Phase

Leucopenia, mild to moderate thrombocytopenia, hyponatremia and elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can occur during this phase (CDC, 2014; WHO/SEARO, 2011).

1.2.1(d) Complications during Febrile Phase

Febrile phase may cause several complications such as dehydration, hyponatremia; seizures in young children (due to fever) and neurological manifestations (encephalitis and aseptic meningitis) (CDC, 2014; WHO/SEARO, 2011).

1.2.2 Critical Phase

1.2.2(a) Characteristics

This phase typically starts around the time of defervescence; however, it may begin early among patients who are febrile on third day of onset of fever and lasts about 24 – 48 hours. During the febrile to afebrile transition, patients without increased capillary permeability improve and do not go through the critical phase. On the other hand, patients with increased capillary permeability may manifest with the warning signs, primarily due to plasma leakage. Onset of critical phase can be identified by rapid decline in platelet (PLT) count with a rise in hematocrit (HCT) and presence of warning signs for severe disease. Moreover, patients may develop leucopenia up to 24 hours before platelet drop is recognized (CDC, 2014; WHO/SEARO, 2011). The presence of warning signs indicates the beginning of critical phase. Therefore, the following warning signs should be observed before or at the time of defervescence during dengue infection (CDC, 2014).

- Clinical fluid accumulation (ascites, pleural effusion)
- Liver enlargement > 2 cm
- Severe abdominal pain or tenderness
- Persistent vomiting (at least 3 episodes/24 hours)
- Mucosal bleed
- Lethargy or restlessness
- Sometimes rapid decline in PLT count with concurrent increase hematocrit in (HCT) is also considered warning sign

1.2.2(b) Clinical Manifestations during Critical Phase

Progressive leukopenia followed by a rapid reduction in PLT count and increased HCT above baseline usually precedes plasma leakage. The degree of plasma leakage varies and usually reflected with the degree of hemoconcentration (as determined by elevated HCT). However, degree of hemocentration is affected by intravenous fluid (IVF) therapy. An early IV therapy can reduce hemoconcentration; therefore, frequent monitoring of HCT levels is essential for the adjustment of IVF therapy. Pleural effusion and ascites are usually only clinically detectable after IVF therapy, unless plasma leakage is significant. In addition to the plasma leakage, hemorrhagic manifestations such as easy bruising and bleeding at venipuncture sites occur frequently (CDC, 2014; WHO/SEARO, 2011).

In most of the cases with plasma leakage, circulatory changes are minimal or transient and many of these patients recover spontaneously or after fluid/electrolyte therapy. However, patients with severe plasma leakage may develop shock due to loss of critical plasma volume. With profound and/or prolonged shock, hypoperfusion results in metabolic acidosis, progressive organ impairment, and disseminated intravascular coagulation (DIVC) which lead to severe hemorrhage causing reduced HCT in severe shock. Instead of the leukopenia usually seen during this phase of dengue, the total white cell count (WBCs) may increase as a stress response in patients with severe bleeding. In addition, severe organ involvement may develop. Some patients progress to the critical phase of plasma leakage and shock before defervescence (WHO/SEARO, 2011).

1.2.2(c) Laboratory findings during Critical Phase

Critical phase causes marked disturbances in laboratory parameters that include increased HCT (hemocentration), moderate to severe thrombocytopenia, leukopenia and transient increase in activated partial-thromboplastin time (aPTT) with decrease in fibrinogen (WHO/SEARO, 2011).

1.2.2(d) Complications during Critical Phase

Hypovolemic shock from plasma leakage, end organ impairment due to prolonged shock, severe hemorrhage and encephalopathy are some major complications during critical phase (WHO/SEARO, 2011).

1.2.3 Recovery Phase

1.2.3(a) Characteristics

If patient survives critical phase, a gradual reabsorption of extravascular fluid in next 48 to 72 hours. However, recovery depends on the severity of illness and treatments provided during febrile and critical phase (CDC, 2014).

1.2.3(b) Clinical Clues (Manifestations) of Recovery Phase

Clinical clues of recovery phase include patient`s improvement, hemodynamic stability, fatigue and increased diuresis. Moreover, second rash that might be macular or erythematous with small circular islands of normal, unaffected skin. This convalescent rash can be very pruritic and desquamate. Bradycardia and electrocardiographic changes are common during this stage. Moreover, patients may experience severe fatigue during this phase (CDC, 2014).

1.2.3(c) Laboratory Findings during Recovery Phase

Stable HCT or slightly elevated due to dilutional effect of reabsorbed plasma (hemodilution), rise in WBCs soon after defervescence, increase PLT after WBC recovery

1.2.3(d) Complications during Recovery Phase

Respiratory distress may occur from massive pleural effusion and ascites. Hypervolemia, congestive heart failure (CHF) and acute pulmonary edema can occur if IVF therapy has been excessive or extended too long. Organ impairment can result due to prolonged or refractory shock. This might include ischemic hepatitis and hepatic encephalopathy. Nosocomial infections can occur, especially in infants and elderly patients. All the three phase of clinical course of dengue infection are characterized by specific clinico-laboratory features and serology and virology pattern. Summary of the clinical course of DVI is presented in Figure 1.2.

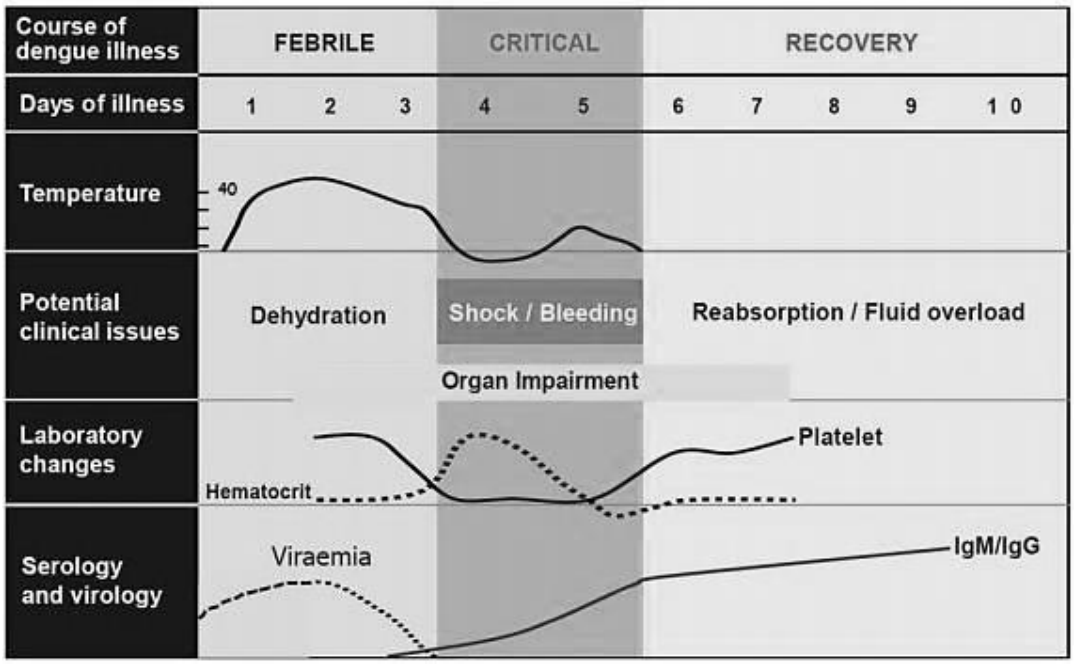

Figure 1.2: Summary of Clinical Course of Dengue Infection

Descriptive note: Figure is reprinted from (CPG, 2015) – Clinical Practice Guidelines of Malaysia on Dengue management

1.3 Diagnosis of Dengue Infection

Dengue can be diagnosed either by isolation of virus or by serological and molecular methods. Early and accurate diagnosis of DVI is of paramount importance for surveillance, management, trials, research and detection of circulating serotypes. Diagnostic tests used to confirm DVI are described in Table 1.2 (Clinical Practice Guidelines, 2015). The use of diagnostic tests varies among hospitals depending upon the availability and nature of dengue infection. However, diagnostic tests include point of care testing such as dengue NS1 antigen test and rapid combo tests (NS1 antigen and dengue IgM/IgG antibodies) (Blacksell *et al*., 2011).

Dengue rapid combo test (NS1 and IgM) or NS1 antigen should be carried out among suspected cases. However, type of diagnostic test should be based on patient`s clinical history (Clinical Practice Guidelines, 2015). Table 1.3 demonstrates the recommendations of diagnostic tests according to the clinical history of patients.

Table 1.2: Types of Diagnostic tests for Dengue Infection

Technique	Recommended time, Specificity and Sensitivity
Antibody detection	
IgM detection	4 days after onset of symptoms and up to 3 months in primary dengue. 3 days after onset of symptoms and sometimes hindered by large scale IgG production in secondary dengue (Sensitivity: 61.5-100%, Specificity: 52-100%)
IgG detection	10 days after onset of symptom in primary dengue and 3 days after onset of symptoms in secondary dengue (Sensitivity: 46.4-99%, Specificity: 80-100%)
Rapid IgM detection (Strips)	5 days after onset of symptoms and up to 2 months (Sensitivity: 20.5-97.7%, Specificity: 76.6-96.6%)
Antigen/ Antibody Combined Detection	
NS1 and IgM Combo Kit	As this is a combo test, useful in early stage of infection (day 3 onwards) and up to sero-conversion period (up to two weeks onwards) (Sensitivity: 89.9-92.9%, Specificity: 75-100%)
NS1 and IgM/IgG Combo kit	As this is a combo test, useful in early stage of infection (day 3 onwards) and up to sero-conversion period (up to two weeks onwards). In the event of both NS1 and IgM are non-reactive and IgG is reactive, case can be interpreted as secondary dengue. (Sensitivity: 93%, Specificity: 100%)
Viral Detection	
Virus Isolation (cell culture)	1-5 days of onset of symptoms in Primary Dengue and 1-4 days after onset of symptoms in secondary dengue (Sensitivity: 40.5%, Specificity: 100%)
Virus Isolation (mosquitoes)	Same as above, (Sensitivity: 71.5-84.2%, Specificity: 100%)
Viral RNA RT-PCR (Conventional)	Same as above, (Sensitivity: 48.4-100%, Specificity: 100%)
Viral RNA RT-PCR (Real Time)	Same as above, (Sensitivity: 58.9-100%, Specificity: 100%)
Viral Antigen (NS1)	1-7 days of onset of symptoms in primary dengue and 1-5 days after onset of symptoms in secondary dengue (Sensitivity: 54.2-93.4%, Specificity: 92.5-100%)

Abbreviations: IgM: immunoglobulin M, IgG: immunoglobulin G, NS1: non-structural protein 1, RNA: ribonucleic acid, RT-PCR: reverse transcriptase polymerase chain reaction

Reference: Malaysian Clinical Practice Guidelines on Dengue Infection (2015)

Table 1.3: Recommendation of type of dengue test based on Clinical History of Patients and interpretation of their results

Clinical History (Test)	Results	Interpretations
Fever < 5 days (Dengue NS1 or RCT)	Positive	Acute dengue infection
	Negative	DVI still cannot rule out. Repeat for Dengue IgM after day 5 of fever
Fever >5 days (Dengue IgM)	Positive	Suggestive of recent dengue infection
	Intermediate	Repeat
	Negative	The result does not rule out dengue infection. Repeat sample for dengue IgM after day 7 of fever or dengue IgG test.
Fever > 5 days and Dengue IgM and/or NS1 was negative (Dengue IgG)	Positive	Elevated IgG levels are seen in acute or past infections. A titre of ≥ 1:2560 is consistent with acute secondary infection
	Intermediate	Repeat if clinically indicated
	Negative	The absence of elevated IgG is presumptive evidence that the patient does not have secondary dengue infection.

Abbreviations: NS1: non-structural protein 1, RCT: rapid combo test, DVI: dengue viral infection, IgM: immunoglobulin M, IgG: immunoglobulin G

Reference: Malaysian Clinical Practice Guidelines on Dengue Infection (2015)

1.4 Dengue Case Classification

The World Health Organization (WHO) in 1997 classified DVI as undifferentiated fever, dengue fever (DF) and dengue hemorrhagic fever (DHF), where DHF was further divided into four grades (Grade I-IV). Grades III and IV are referred to as dengue shock syndrome (DSS) (WHO, 1997). In 2009, WHO proposed new classification of DVI due to complexity and applicability of previous 1997 criteria. According to new definition, dengue severity is divided into non-severe (dengue with and without warning signs) and severe dengue (WHO, 2009).

However, older classification is still widely used, as WHO`s regional office for South-East Asia (SEARO) has included 1997 definition in its revised and expanded guidelines for DVI (WHO/SEARO, 2011). Although, there is move towards using the 2009 classification due to its ease of use but WHO still favors both case definitions. Nevertheless, there has been a considerable debate regarding the value of both 1997 and 2009 classifications (Hadinegoro, 2012). More recently, centers of disease control and prevention (CDC) classified DVI into three types i.e. dengue, dengue like illness and severe dengue. Dengue like illness is defined by fever and included in the list of notifiable infectious conditions (CDC, 2015). The use of criteria for dengue classification depends on discretion of treating physician and hospital or national dengue guidelines. The details of WHO and WHO/SEARO criteria is given below.

1.4.1 Dengue Case Classification (WHO, 2009)

This classification provides information to identify probable and confirm dengue cases that can further be classified into severe and non-severe dengue infection as demonstrated by Figure 1.3 (WHO, 2009).

1.4.2 Dengue Case Classification (WHO/SEARO, 2011)

The regional office of WHO for South-East Asia (SEARO) has revised dengue guidelines in 2011 and classified DVI into asymptomatic infection, viral syndrome, dengue fever (DF) and dengue hemorrhagic fever (DHF) including dengue shock syndrome (DSS) (Figure 1.4). However, the classification used in these contemporary guidelines corresponds to WHO 1999 criteria (WHO/SEARO, 2011).

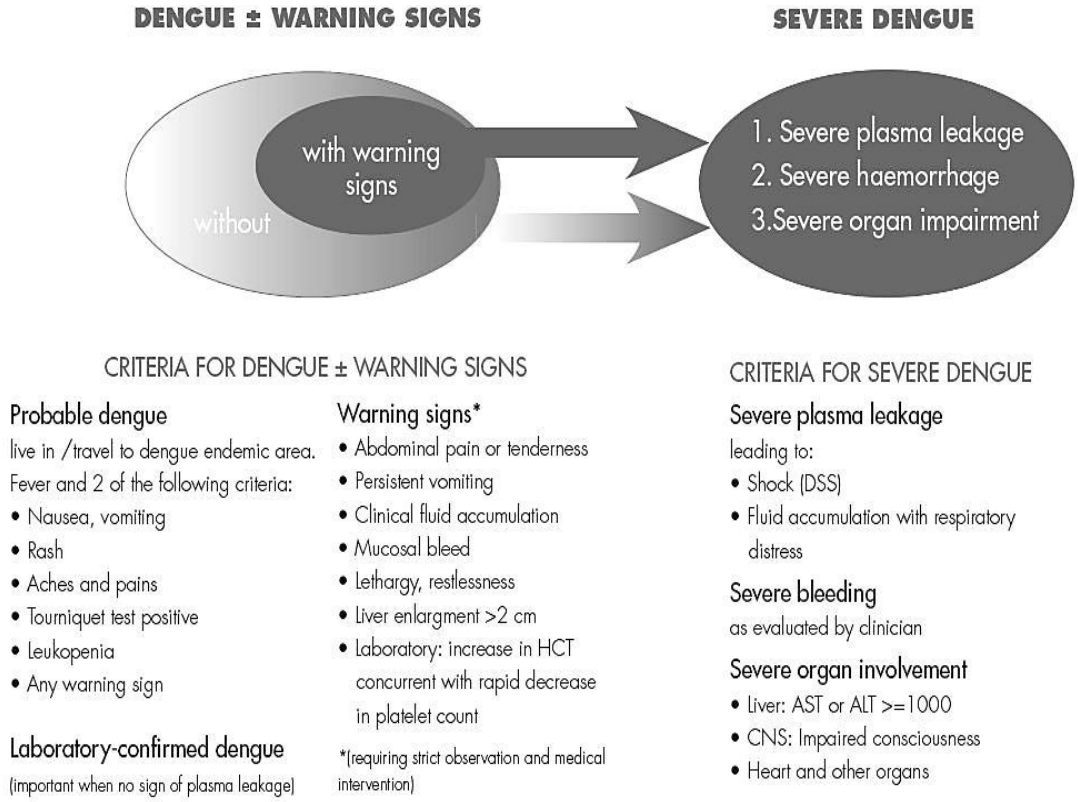

Figure 1.3: WHO 2009 Criteria for dengue Classification

Descriptive note: Figure is reprinted from (WHO, 2009) – World health Organization. Dengue Guidelines for Diagnosis, Treatment, Prevention and Control

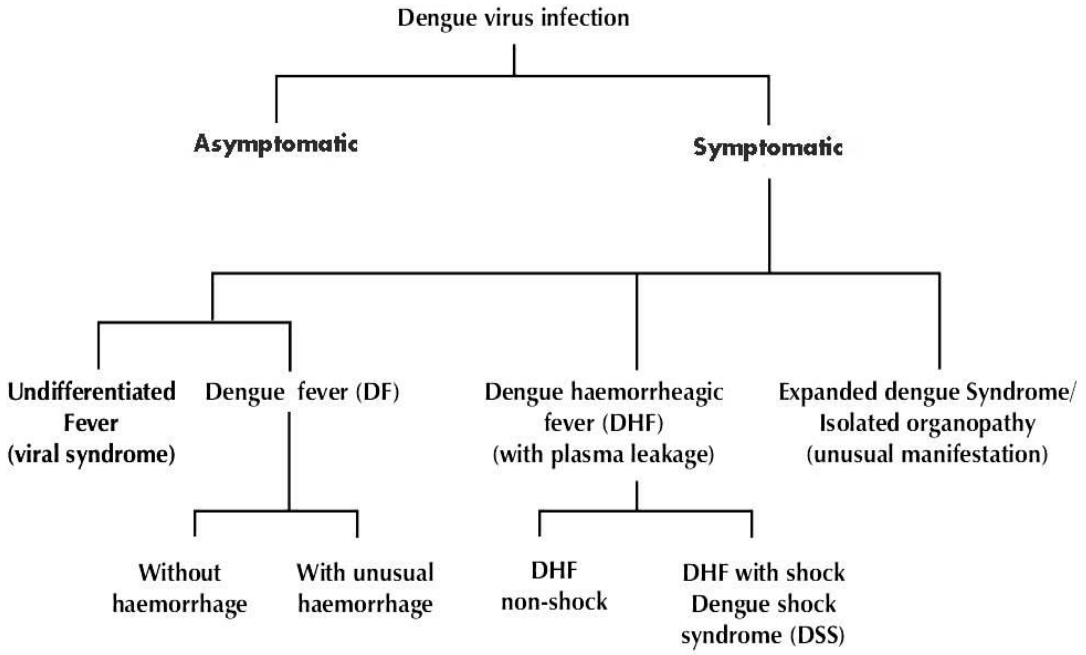

Figure 1.4: WHO/SEARO 2011 or WHO 1999 criteria for dengue classification

Descriptive note: Figure is reprinted from (WHO/SERAO, 2011) – Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever

The severity of DHF is classified into four grades (Table 1.4) where presence of thrombocytopenia with concurrent hemoconcentration differentiates Grade I and Grade II from dengue fever. Grading the severity of the disease has been found clinically and epidemiologically useful in both children and adults.

Table 1.4: Classification of dengue viral infection according to WHO/SEARO (2011)

DVI classification	Sign and Symptoms	Laboratory findings
DF	Fever with two of the following: • Headache • Retro-orbital pain • Myalgia • Arthralgia/bone pain • Rash • Hemorrhagic manifestations • No evidence of plasma leakage	Leucopenia (WBC ≤5000 cells/mm3) Thrombocytopenia (Platelet Count <150 000 cells/mm3) Rising hematocrit (5% – 10%) No evidence of plasma loss
DHF (Grade I)	Fever and hemorrhagic manifestation (positive tourniquet test) and evidence of plasma leakage	Thrombocytopenia <100 000 cells/mm3; HCT rise ≥20%
DHF (Grade II)	As in Grade I plus spontaneous bleeding	Thrombocytopenia <100 000 cells/mm3; HCT rise ≥20%
DHF (Grade III)	As in Grade I or II plus circulatory failure [weak pulse, narrow pulse pressure (≤20 mmHg), hypotension, restlessness]	Thrombocytopenia <100 000 cells/mm3; HCT rise ≥20%
DHF (Grade IV)	As in Grade III plus profound shock with undetectable BP and pulse	Thrombocytopenia <100 000 cells/mm3; HCT rise ≥20%

Abbreviations: DF: dengue fever, DHF: dengue hemorrhagic fever, WBC: white blood cells, HCT: hematocrit
DHF grade III and IV is dengue shock syndrome (DSS)

Reference: (WHO/SEARO, 2015)

1.5 Prevalence and Burden of Dengue Viral Infection

The global burden of dengue is formidable and represents a growing challenge to health authorities. Knowledge of geographical distribution and burden of dengue is essential for understanding its contribution to global morbidity and mortality burdens in order to determine resource allocation needed for dengue control and in evaluating international impact of these control activities (Bhatt *et al*., 2013).

1.5.1 Global burden of DVI

The incidence of dengue has grown dramatically around the world in recent decades. However, the actual numbers of dengue cases are underreported or misclassified. One recent estimate indicates 390 million dengue infections per year, of which 96 million manifests clinically with any severity of disease (Bhatt *et al*., 2013). Although the full global burden of the disease is uncertain but number of cases reported to WHO has increased from 2.2 million in 2010 to 3.2 million in 2015. Before 1970, only nine countries had dengue epidemics. Currently, dengue is endemic in more than 100 countries in five out of the six WHO regions. Today about 40% of world`s population live in dengue risk areas. Every year approximately 5 lac people with severe dengue require hospitalization and 2.5% of those affected die (WHO Fact Sheet 117, 2016).

1.5.1(a) Dengue in Asia

The surge in dengue has been most marked in Asia and accounts 75% of global dengue burden, costing South-East Asia (SEA) US$1billion annually. It is estimated that among 2.5 billion people at risk globally, about 1.8 billion are in Asia. Southeast Asia records approximately 2.9 million dengue episodes and 5906 deaths annually, with a yearly monetary burden of $950 million (Shepard *et al*., 2013). Recurrent

dengue epidemics in Asia have established hyperendemic areas, often in large and heavily populated cities. In South-East Asia, demands of a large health and economic burden for dengue control is more challenging than ever. The year 2015 was characterized by large dengue outbreaks in Asia, with Philippines and Malaysia representing 59.5% and 16% increase in case numbers to the previous year, respectively. Alarmingly, spread of *Aedes albopictus* (a secondary dengue vector) from Asia to North America and Europe may result in high disease burden in these cooler temperate regions (WHO Fact Sheet – 117, 2017).

1.5.1(a)(i) Dengue in Malaysia

The global increase of dengue incidence is also experienced by Malaysia. Since the first outbreak in 1902, dengue has become a notifiable infection by 1973 (Mohd-Zaki *et al*., 2014). Dengue is among top five notifiable diseases in Malaysia and continues to be a formidable public health concern. Since the year 2000, its incidence in Malaysia continues to increase from 32 cases per 100,000 populations to 361 cases per 100,000 populations in 2014. The incidence of dengue is higher in the age group of 15 to 49 years. Most of the cases reported were from urban areas (70–80%) where factors such as high density population and rapid development favor dengue transmission (Clinical Practice Guidelines - Malaysia, 2015).

Currently Malaysia is facing worst dengue crisis while some countries in South-East Asia (e.g. Philippines & Thailand) have seen decreases in DVI activity in 2014. Although, number of dengue cases in 2016 (100,028 cases), is less than that reported in 2015 (118,325 cases) but still dengue is imposing substantial disease burden on health authorities in Malaysia (Figure 1.5) (WPRO-507, 2017).

With regards to case fatality rate (CFR), the national target is less than 0.2%. The case fatality rate has been reduced from 0.6% in year 2000 to 0.2% in year 2014 (CPG, 2015). The year 2015 paints a scary picture as highest number of deaths (n=322) ever recorded in the country (WPRO-507, 2017). However, 231 dengue deaths were reported in 2016 (WPRO-507, 2017) that is comparable to 215 deaths in 2014 (WPRO-456, 2015). Most of the dengue deaths have been observed among individuals with age ≥15 years (Clinical Practice Guidelines - Malaysia, 2015).

Malaysia is "hyperendemic" with all four dengue virus (*DENV*) serotypes co-circulating over the past two decades, predominantly *DENV1* and *DENV2* responsible for the escalating number of cases over the recent years (Mudin, 2015). However, a particular dengue virus serotype can predominate for at least two years before it is replaced by another serotype. In year 2013-2014, the predominant serotype had switched twice from DENV-2 to DENV-1 in February and June 2014 (Clinical Practice Guidelines - Malaysia, 2015). Of the estimated, the annual cost for dengue illness (standard errors in parenthesis) in Malaysia is US$42.4 (±4.3) including per capita cost US$4.73 for one thousand population size (n=1000) with disability adjusted life years (DALYs) equivalent to 8,324 (Suaya *et al*., 2009).

Year	Cases	Mortality
1998	27381	82
1999	10146	37
2000	7103	45
2001	16386	50
2002	15493	99
2003	31545	72
2004	33895	102
2005	39654	107
2006	34386	92
2007	48846	98
2008	49355	112
2009	41486	88
2010	46171	134
2011	13743	36
2012	21444	35
2013	41226	92
2014	108698	215
2015	118325	336
2016	100028	233

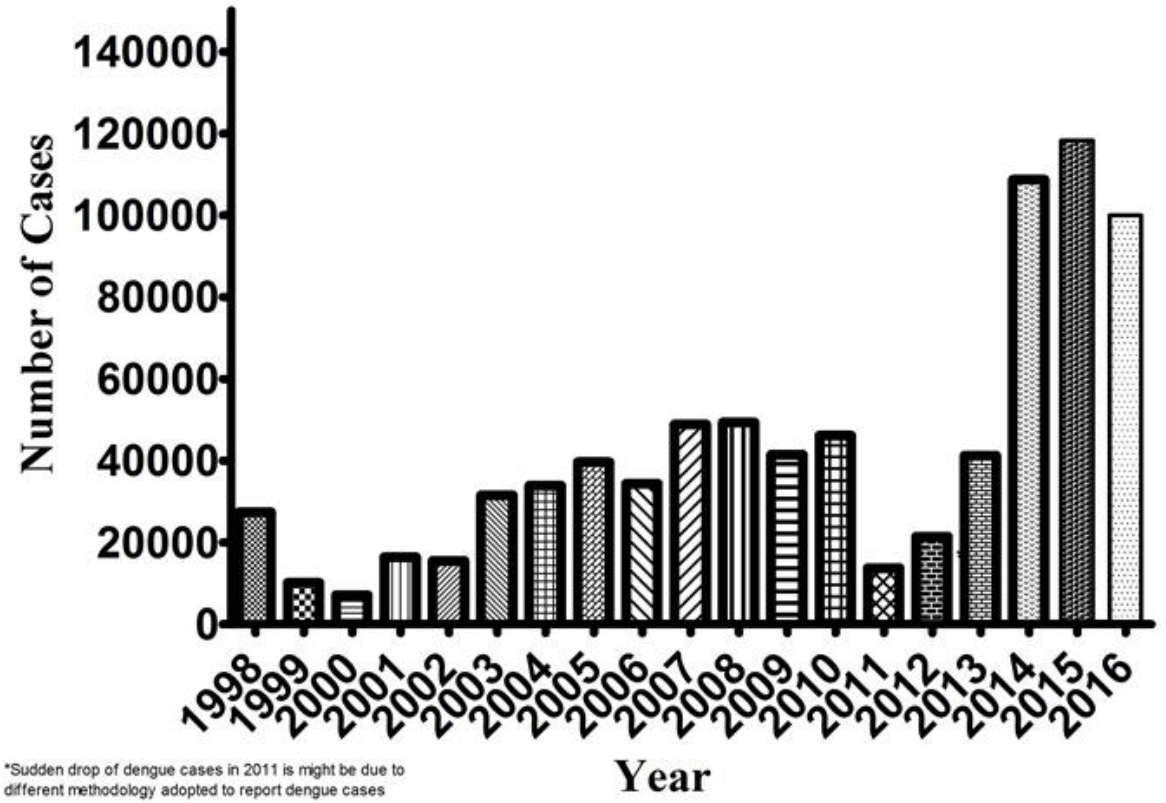

Figure 1.5: Prevalence of Dengue Infection in Malaysia from 1998 to 2016

Descriptive note: Data from statistics of Ministary of health Malaysia and World Health Organization

1.6 Expanded Dengue Syndrome

In recent years with the geographical spread of dengue illness and with more involvement of adults, there have been increasing reports of DVI with unusual manifestations, termed as "Expanded Dengue Syndrome" (EDS). These isolated organopathies include hepatic, renal, cardiac, respiratory and neurological involvements and could be explained as complication of severe profound shock or associated with underlying host conditions or coinfections. The involvements of various organs are increasingly being reported in DHF, while EDS can also occur during DF without any evidence of plasma leakage (WHO/SEARO, 2011). These atypical manifestations were previously termed as "unusual complications" by WHO (WHO, 1997; WHO, 2009). Currently, EDS is new entity incorporated to WHO guidelines. The immunopathological mechanisms during dengue infection primarily target endothelium, resulting in vascular permeability and coagulation disorders that can explain these varied systemic involvements (Gulati *et al*., 2007).

Atypical and unusual manifestations of dengue infection are shown in Figure 1.6. Of these, hepatic complications are more frequent in dengue infection and have been well described in various studies (Wahid *et al*., 2000; Huerre *et al*., 2001; Pancharoen *et al*., 2002; Seneviratne *et al*., 2006; Hien *et al*., 2010). All other complications are less frequent and have been reported in few case reports and small data series (Solomon *et al*., 2000; Gulati *et al*., 2007; Kulratne *et al*., 2007; Wang *et al*., 2007; Acharya *et al*., 2010; Raju *et al*., 2014). However, recently mild to severe renal complications have been frequently observed among dengue patients (Lee *et al*., 2009; Khalil *et al*., 2012; Mehra *et al*., 2012; Lizarraga & Nayer, 2014).

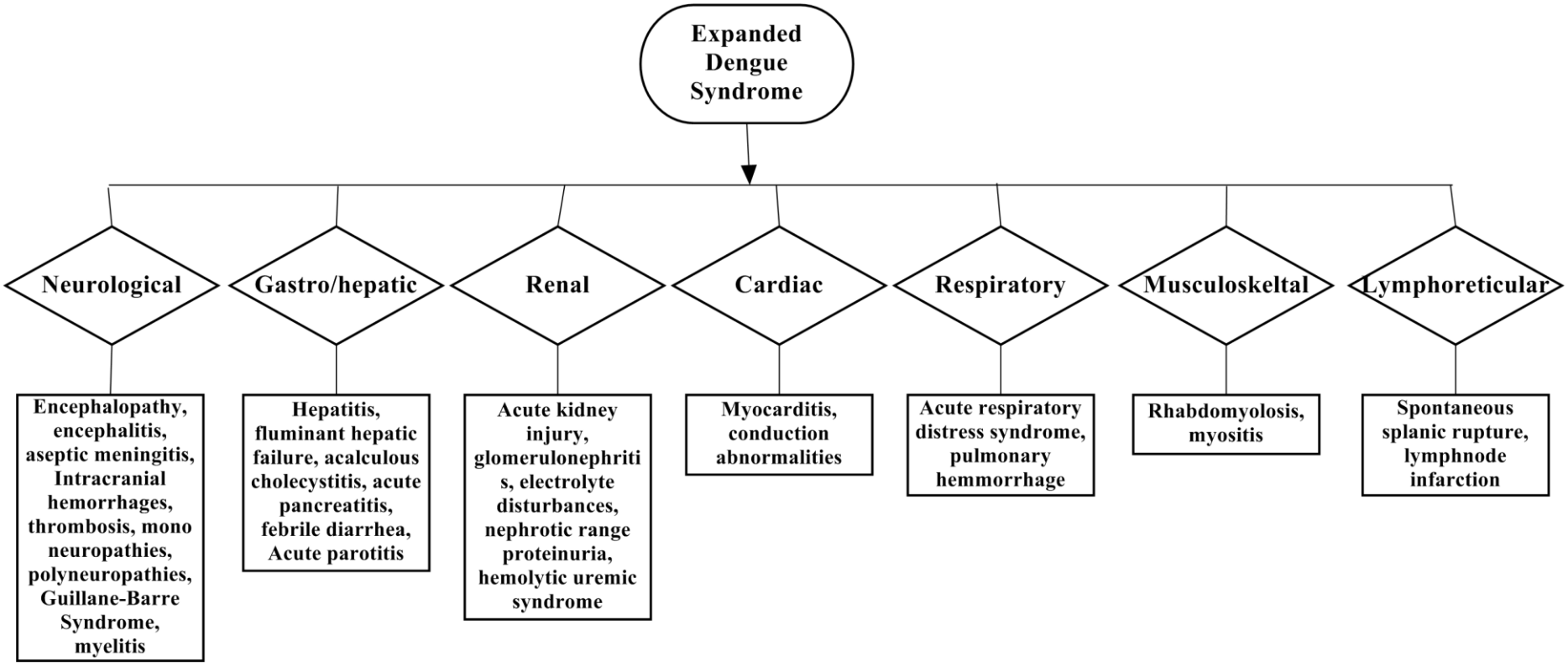

Figure 1.6: Manifestations of Expanded Dengue Syndrome

Reference: World Health Organization Regional Office for South-East Asia. Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic guidelines, 2011.

1.7 Renal Complications in Dengue Infection

The spectrum of renal involvements in dengue infection is described in Figure 1.6, which includes elevated serum creatinine (SCr), dyselectrolytemia, acute tubular necrosis (ATN), hemolytic uremic syndrome, IgA nephropathy, lupus nephritis, glomerulonephritis, nephrotic syndrome, proteinuria, and acute kidney injury (AKI). Renal involvement is more commonly seen in DHF and DSS, and is an independent predictor of mortality (Oliveira & Burdmann, 2015). Several types of dengue induced nephropathies are described in this section below.

1.7.1 Abnormal Urinary Sedimentations

Urinary sediment is a centrifuged deposit suitable for microscopic examination for the presence of cells, casts, bacteria and crystals. Abnormal urinalysis is a general feature of kidney involvement in dengue viral infection. Proteinuria, glycosuria, ketonuria, occult blood, micro or macroscopic hematuria and presence of tubular epithelial cells in urine have been documented among patients with DVI. Urinary sedimentations are more prevalent in DHF and DSS (Vachvanichsanong & McNeil, 2015).

1.7.2 Glomerulonephritis

Glomerulonephritis is an inflammation of glomeruli and has been documented during or after DVI in both mice and human models. Several mice models infected with dengue virus have demonstrated glomerular enlargement, immune-complex deposition and proliferative lesions in glomeruli. Immune complex mediated glomerulonephritis consists predominantly of immunoglobulin G (IgG), immunoglobulin M (IgM) and compliment C3 (Barreto *et al*., 2004).

1.7.3 IgA nephropathy

IgA nephropathy or Berger`s disease is most common form of glomerulonephritis and is characterized by predominant IgA deposition in the glomerular mesangium. Mesangial proliferation along with ATN has been documented in 15-year-old boy with dengue infection. However, resolution of nephropathy has been observed on renal biopsy six weeks later (Upadhaya *et al*., 2010).

1.7.4 Lupus nephritis

Lupus nephritis is inflammation of kidney caused systemic lupus erythmatosis (SLE). Dengue infection evolving into SLE and lupus nephritis has been reported in 22-year-old woman (Rajadhyaksha, & Mehra, 2012).

1.7.5 Hemolytic uremic syndrome

Hemolytic uremic syndrome (HUS) is progressive renal failure and is characterized by hemolytic anemia, thrombocytopenia and acute kidney injury. HUS with renal recovery has been previously reported among dengue patients (Oliveira & Burdmann, 2015).

1.7.6 Acute Kidney Injury (AKI)

AKI, previously known as acute renal failure (ARF), is sudden onset (within a few hours or a few days) of renal dysfunction primarily characterized by elevated SCr and reduction in glomerular filtration rate (GFR). It encompasses a wide spectrum of injury to the kidneys, not just kidney failure. AKI has been reported to affect from 13% to 18% of hospitalized patients and 1% to 25% of intensive care unit

(ICU) patients. AKI is associated with adverse outcomes and high mortality rates ranging from 25% to 80% (KDIGO, 2012; Bellomo *et al*., 2012).

1.7.6(a) Clinical Manifestations and Etiology of AKI

Clinical manifestations and causes of AKI are described in Table 1.5. Sign and Symptoms of AKI are associated with the disturbances of normal kidney functions. However, on the basis of etiology, AKI is divided into three categories including pre-renal AKI (due to decreased renal perfusion, often because of volume depletion), intra-renal or intrinsic AKI (due to processes within the kidney) and post-renal AKI (caused by inadequate drainage of urine crystals to the kidneys) (Greenberg & Cheung, 2005).