Role of cognitive parameters in dengue hemorrhagic fever and dengue shock syndrome

Jih-Jin Tsai1,2,3*, Kulkanya Chokephaibulkit4, Po-Chih Chen5,6, Li-Teh Liu7, Hui-Mien Hsiao8, Yu-Chih Lo9,10 and Guey Chuen Perng8,10,11*

Abstract

Dengue is becoming recognized as one of the most important vector-borne human diseases. It is predominant in tropical and subtropical zones but its geographical distribution is progressively expanding, making it an escalating global health problem of today. Dengue presents with spectrum of clinical manifestations, ranging from asymptomatic, undifferentiated mild fever, dengue fever (DF), to dengue hemorrhagic fever (DHF) with or without shock (DSS), a life-threatening illness characterized by plasma leakage due to increased vascular permeability. Currently, there are no antiviral modalities or vaccines available to treat and prevent dengue. Supportive care with close monitoring is the standard clinical practice. The mechanisms leading to DHF/DSS remains poorly understood. Multiple factors have been attributed to the pathological mechanism, but only a couple of these hypotheses are popular in scientific circles. The current discussion focuses on underappreciated factors, temperature, natural IgM, and endotoxin, which may be critical components playing roles in dengue pathogenesis.

Keywords: Flavivirus, Dengue, DHF, DSS, Hemorrhagic, Endotoxin, Fever

Introduction

Dengue, a vector-borne human disease, has been recognized recently as one of the most significant public health threats, causing high morbidity and mortality worldwide. The disease is caused by the infection of dengue virus that is transmitted to human beings by the bite of a mosquito—domestic Aedes aegypti being the principal vector—although some other species, such as Aedes albopictus, are of importance. There are four serotypes (DENV1, DENV2, DENV3, and DENV4), each being capable of inducing typical dengue manifestations. The spectrum of illness is wide, ranging from inapparent or asymptomatic, mild febrile with varying degrees of thrombocytopenia, hemorrhaging and increased vascular permeability typical of dengue hemorrhagic fever (DHF), to plasma leakage and severe shock syndrome. The resurgence of dengue endemicity has resulted from numerous oscillating environmental, social and economical factors. It is estimated that about 40% of the world’s population is at risk of dengue virus infection, with approximately 25 million of these requiring hospitalization and about 25,000 resulting in death [1]. Currently, there are no antiviral modalities or preventive vaccines available to alter disease outcomes. The mortality rate is varying, ranging from 1 to 5%, dependent upon the country and region. The exact mechanism by which dengue virus induces plasma leakage or disease severity remains poorly understood.

A large majority of the dengue infections occur in humans without any noticeable illness. However there are many incidences of symptomatic disease; they can be partitioned into two syndromes: dengue fever (DF) and DHF/dengue shock syndrome (DSS). While DF is a simple, self-limited febrile illness, DHF is a severe and potentially life-threatening condition. DHF/DSS is characterized by thrombocytopenia and hemorrhagic manifestations; additionally, there is increased vascular permeability that leads to depleted intravascular volume and shock. Severe, profound shock, as well as multiorgan failure, is known to occur in extreme cases and is associated with high mortality.

There are many excellent reviews on dengue pathogenesis, including the topics of dengue viral biology, the
immune-mediated hypothesis, intervention strategies, and dengue diagnostic issues [2-7]. These aspects will not be included in the focus of the current article; readers who are interested in these details are encouraged to refer to the literature. The current article highlights other recent knowledge and developments in the field, and proposes a new mechanism for biological enhancement to dengue pathogenesis.

**Epidemiology**

Initially, dengue disease predominantly affected the people living in tropical and subtropical zones. However the regions of the world that are endemic has spread and the incidence in dengue disease has climbed due to a number of contributing factors. Increased human migration is one culprit; individuals often travel between rural areas and city dwellings and even to other countries via air travel for the purpose of making money or personal enjoyment. A person carrying dengue virus acquired in one location can be bitten again by a mosquito and introduce it into new areas [8]. Another factor is the weather; global warming and climate change has lead to the augmentation of zones hospitable for mosquito survival. Issues with unplanned urban development (including inadequate vector control and poor waste management) have resulted in the presence of many vessels for the accumulation of water, which are exploited by *Aedes aegypti* for breeding and larvae/pupae production [9,10]. All these factors have contributed to the spread of dengue virus in endemic regions.

Recently, dengue has even been spotted in the US territories [11]. In order to avoid a significant impact on the world's economy and avert potentially extensive burdens to society and the public health sector, a greater amount of research has focused on dengue virus surveillance [12,13]. Consequently, as of today, dengue has been documented in over 100 countries, increasing the number of people at risk for an infection to 2.5 billion people. It is estimated that 50–100 million cases of dengue occur annually, resulting in 250,000–500,000 cases of dengue hemorrhagic fever (DHF) and 25,000 deaths, depending on epidemic activity. However, these figures are reliant on a number of assumptions and the true incidence is unknown [14-16].

**Diagnosis and clinical presentation**

Accurate diagnosis of dengue requires serological testing and identification of viral material in the blood, which is dominantly performed in the clinic. Symptomatology cannot be relied upon because the early symptoms experienced by dengue patients are very similar to most other tropical pathogens and common febrile illnesses. Thus, it is very difficult for attending physicians to attribute the correct pathogen to each clinical presentation when they are often highly variable. Once the physicians determine the differential diagnosis, the second layer of difficulty is to distinguish whether the patient has dengue fever or dengue hemorrhagic fever. The former is likely a self-limited illness and patients normally recover without having noticeable sequelae; in contrast, the latter, if treatment is not instituted immediately, the progression of the condition can quickly escalate and result in life-threatening situations, including death. According to the old WHO guidelines [17], the initial phase of clinical manifestations for DF and DHF were quite similar. In general, the onset of DF and DHF are both very abrupt, beginning with fever. The common initial symptoms at the febrile stage are headache, malaise, weakness, chills, aches and pains, and gastrointestinal symptoms. Physical examination often reveals flushing of the face, lethargy, irritability (in young children), abdominal pain, hepatomegaly, and the presence of petechial hemorrhages or other bleeding manifestations. Initial complete blood counts reveal leucopenia, and after 2–5 days of fever, thrombocytopenia and depletion of coagulation factors often develop.

In DF, the fever abates after 3–7 days and the patients recover. In DHF, signs of progressive intravascular fluid leakage, such as petechiae, ecchymosis, epistaxis, gingival or gastrointestinal bleeding, occur after 3–5 days of fever. Frequently, confluent petechial convalescent rashes with scattered sparing spots develop in patients that undergo plasma leakage, allowing doctors a way to differentiate between DHF and typical DF. Conditions arising from plasma leakage, including pleural effusion, ascites, and hypoproteinaemia, are common in severe dengue. This is the so-called critical stage, when the fever typically begins to dissipate but the patients' condition may worsen. At this point many patients may develop shock from depletion of intravascular volume and bleeding. Some patients deteriorate rapidly from circulatory failure, experiencing a condition called dengue shock syndrome (DSS), presenting with a rapid and weak pulse, narrow pulse pressure or hypotension, cold clammy skin, and altered mental status. Disease severity is classified as either mild (grades I and II) or severe (grades III and IV), the presence of shock being the main difference. This stage lasts no more than 48 hours, after which the patients usually recover [16].

This WHO classification has been mostly adequate and used for many decades; however there have been occasional difficulties in classifying patients who present with unusual manifestations. Atypical or abnormal clinical presentations have been reported such as encephalopathy, severe hepatitis, and myocarditis, in which the patients have severe disease but do not fit the DHF definition. In 2009, WHO published another case classification system for guiding dengue management [16]. This
new classification includes dengue without warning signs, dengue with warning signs, and severe dengue, which improved sensitivity for detection but reduced specificity [18,19]. To improve upon the specificity of the 2009 dengue classification system, in 2011 WHO SEARO published an amendment, which expanded case definitions based on the previous DF/DHF (WHO 1997 [17]) description to include unusual manifestations [20]. Both WHO guidelines, the 2009 and SEARO 2011 versions, are in use in several countries. However, this is dependent upon the country’s public health administrative leaders; some advocate classifying disease according to the new guidelines, while others still triage patients according to the expanded older classification of DF and DHF.

Due to the nonspecificity and complexity of dengue patient clinical manifestations, it is imperative to confirm the clinical diagnosis with biological and/or laboratory assays. Since dengue disease management is time sensitive, onsite rapid screening tests at the point-of-care is a critical component in assisting decision-making. Unfortunately these rapid screening tests perform poorly, having low specificities and sensitivities. Other diagnostic tools, such as virus isolation or dengue virus genome, antigen or specific IgM/IgG detection, are more informative but are also very time consuming and expensive to perform. Even though these results do not directly contribute to decisions for the patients in real time, the confirmatory information can provide a guideline for future decision making on patient care in general, as well as lead to improved precision on diagnostic rapid screening tests under development. In addition, the results obtained with confirmatory assays can serve to advance our understanding of human dengue virus pathogenesis and guide the development of preventive modalities.

Parameters associated with DHF/DSS

Although the majority of dengue-infected individuals are asymptomatic, a small percentage of the subjects will progress to apparent clinical illness including life-threatening DHF/DSS. The available information suggests that multiple factors, including the presence of cross-reactive, sub- or non-neutralizing antibodies, viral virulence, genetic predisposition, age, nutritional status and underlying chronic disease, can all be a risk and/or contributive factor to the pathogenesis of DHF/DSS [21-23]. However none of these factors has been substantiated because there are no reliable in vivo model systems to perform the necessary side-by-side comparisons. Consequently, the causes of dengue disease remain poorly understood in spite of many decades of intensive investigations. Some known but under-appreciated factors are briefly discussed here.

Fever temperature

Temperature has captured the attention of the media, owing to growing concerns about the environment and global warming. In line with this, the change in the global climate has significantly impacted the geographic distribution of the mosquito vector and thus dengue disease. Accordingly, WHO has reported that a temperature rise of only 1–2°C could increase the risk of dengue virus infection to the population by several hundred million, potentially resulting in 20,000–30,000 more fatal cases annually [24]. Additionally, the biological importance of temperature, particularly in the form of fever, and its role in medical science has not received the appropriate attention [25]. Dengue fever, as the name indicates, has fever as one of its most salient clinical features. This is also the case for many other common febrile illnesses. The increase in body temperature during infection is commonly viewed as one way to interfere with pathogen replication directly. Additionally this biological alteration may also promote the production of the appropriate host transcriptional and translational profiles, which may work to eradicate some microorganisms. Despite these known phenomena, the contribution of fever to pathogenesis has not been investigated. Researchers more frequently attribute the symptoms as directly or indirectly caused by the pathogen rather than a direct result of the fever. Refocusing the interpretation of the clinical data in light of the degree of fever may allow for a better understanding of disease presentation.

Viremia is another major finding on the pathophysiology of dengue patients. Interestingly, it has been noticed that viremia is correlated highly with temperature in dengue patients [26]. A cumulative result from a pilot study supports this observation as well (Figure 1). Viremia kinetics are characterized by a downward trend, with a peak in the plasma viral RNA levels corresponding to the first day after onset of fever, which decreases to undetectable levels by the 7th day of fever. The downward trends in viral RNA level and body temperature are very similar to each other and are correlated highly with each other as a function of time (P < 0.000, $R^2 = 0.9535$). The results were in line with reports on the highly correlation of body temperature with viral load in samples collected from acute dengue patients [26]. One alternative interpretation of this data would suggest that the lower body temperature contributes to the clearance of viremia. However the results could also suggest that dengue virus may enter cells or replicate more efficiently at higher temperatures [27,28]. Interestingly, it has been reported that Flaviviruses in Aedes albopictus cell cultures adapted to 34.5°C replicate to a higher viral titer than those adapted to 28°C [29] and that Japanese encephalitis virus yields are increased by 0.2–2.5 log PFU/ml in heat shock-treated BHK-21 cultures at 41°C.
compared to control cultures at 37°C [30]. However, why viral titers are amplified when culturing at higher temperatures remains to be further investigated. Foreseeably, understanding the factors or mechanisms leading to efficient viral replication during fever, would provide a new avenue of strategies to improve the quality of life of affected patients and perhaps a preventive modality to dengue as well.

Biological enhancement
Pathophysiologic responses to dengue virus infection are dynamic. Biological components circulating in the patient's blood stream may modify the body's physiology and alter the presentation of the disease. The biological response to the disease is a double-edged sword; it could be both beneficial and harmful to the host. Shock syndrome often occurs during or at the end of the viral clearance stage and is a dangerous complication of dengue virus infection that is associated with a high mortality rate in some countries [4]. Increased vascular permeability is one of the remarkable clinical manifestations that have been observed in patients with severe dengue. This event may provide a mechanism for the translocation of microbial products from the intestinal lumen into the circulatory system [32,33]. Although sepsis can be observed clinically in patients experiencing DSS and those with bacteremia [34], the mechanism leading to the development of dengue shock is complex and remains largely unknown. Interestingly, gut injury has been correlated significantly with multiorgan failure in hemorrhagic shock [35]. The degree of this mucosal injury in dengue patients has been reported to correlate with the severity of the illness [36]. Recent reports indicate that more than 35% of DF patients have evidence of bleeding in the gut as well [37]. Also, there appears to be an escalating problem with opportunistic pathogen infections in dengue patients (Table 1). This suggests that substances in the gut may translocate and become systemic. One of these such materials is lipopolysaccharide (LPS) or endotoxin [38], a strong immune response inducer. Interestingly, it has been reported that endotoxin is detected in 50% of serum samples collected from DHF/DSS patients [39], and that the levels of LPS in these samples correlate with dengue severity [40]. In a study with cumulative data, we observed that endotoxin levels were significantly higher in dengue patients confirmed with gastrointestinal bleeding (GIB) and that 48.6% of sera from acute DF patients were considered to be endotoxin positive compared to healthy controls (Figure 2A). Kinetic studies demonstrate that the highest levels of endotoxin were seen at the end of disease from days 6 to 9, during defervescence (Figure 2B); this time point also corresponds with the critical stage, during which patients need to be closely monitored for the occurrence of shock. The sera from the GIB group were sampled from the 4th day after the onset of fever. However, although results were derived from limited patients and the actual percentage of GIB remains unknown, literature reports have estimated that the percentage of GIB has been varied from regions to regions, ranging from 1 to 39 percent [37,41,42]. Therefore, clinically, GIB is not only largely unknown but also underestimated because there may be some occult bleeding that were overlooked and asymptomatic presentation as well. The demographic and clinical data of the enrolled patients have been previously described [43,44].

One mechanism known to rid the blood stream of endotoxin is through antibodies or immune complex formation. Serum antibodies are a heterogeneous mixture of immunoglobulins (Ig), all of which share the ability to bind individually to specific antigens. In mammals there are five classes of antibody: IgA, IgD, IgE, IgG and IgM, with 4 IgG and 2 IgA subclasses present in humans. The IgM antibody is the first class of antibodies produced during a primary response. Studies from mice reared germfree and receiving an antigen-free diet have the same serum IgM levels as mice held under conventional housing conditions, while IgG and IgA levels in these mice are greatly reduced, suggesting that a distinct antibody, natural IgM, is induced independently of external stimulation [60]. Later, it was shown that IgM is the major component of natural antibody in humans [61,62]. The levels of natural antibodies vary among
individuals, but increase with age and with good nutrition [63-65]. Characteristics of the natural IgM antibody include low affinities and broad specificities to both foreign and self antigens [66]. Circulating natural IgM antibody provides the first line of defense against invasion by pathogens [67-69]. Importantly, natural IgM has been demonstrated to play an important role in the clearance of endotoxin [70]. Pilot results from sequential samples suggested that the levels of total IgM are dose dependent with disease severity; DHF patients have significantly lower levels of total IgM in sera than DF patients, in spite of similar levels of dengue specific IgM; and the total IgM from the sera of both DF and DHF were significantly lower than that of healthy controls (Figure 3). The results imply that dengue patients typically have lower IgM than that of healthy controls and possible a reduced capability to clear LPS, lending support to the hypothesis that endotoxin may contribute to disease pathology.

Natural IgM is high avidity of polymeric antibody, which may contribute to the initial immune defense and to the control of invading pathogens until immune

| Cases | Pathogens                                                                 | Day of fever | Reference |
|-------|---------------------------------------------------------------------------|--------------|-----------|
| 5     | Staphylococcus aureus                                                    | 8-10         | [45]      |
| 4     | Staphylococcus aureus, Haemophilus influenzae, Coagulase-negative staphylococcus | 7-10         | [46]      |
| 14    | Burkholderia pseudomallei, Varicella zoster, Salmonella, Shigella, Escherichia coli, Herpes simplex, Mycobacterium tuberculosis, Streptococcus pneumoniae, Mycoplasma pneumoniae | 7-10         | [47]      |
| 2     | Salmonella typhi                                                          | 8            | [48]      |
| 1     | Shigella sonnei                                                           | 9            | [49]      |
| 7     | Rosemonas species, Klebsiella pneumoniae, Moraxella lacunata, Klebsiella ozaenae, Enterococcus faecalis | 8-14         | [50]      |
| 3     | Enterococcus faecalis, Klebsiella pneumoniae                              | unspecified  | [51]      |
| 4     | Aspergillus fumigatus                                                    | 8-13         | [52]      |
| 5     | Plasmodium vivax, Plasmodium falciparum                                  | 3-10         | [53-56]   |
| 2     | Leptospira                                                               | 5-7          | [57,58]   |
| 1     | Candida tropicalis                                                        | 14           | [59]      |

Figure 2 Endotoxin observed in sera of dengue patients. (A) The levels of endotoxin in 37 randomly chosen samples were measured by ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit (GenScript USA Inc, Piscataway, NJ). Two distinct patterns were observed in DF patients. A high range of endotoxin levels were observed in a fraction of DF patients and in DF patients with noticeable gut bleeding, while a subset of DF patients had endotoxin levels within the range of healthy controls. (B) The levels of endotoxin in general, increased with time, being higher on days 6 to 9 after onset of fever.
system has time to launch a specific adaptive response [73]. Importantly, natural IgM antibody has been shown to directly neutralize or inhibit pathogens as well as aid the initiation of adaptive immune response from follicular B cells, which together play critical roles in protection against bacterial and viral infection [67,69,74-76]. Consequently, despite with limited number of specimens, we feel confident that the levels of the IgM in acute dengue patients could be lower than that of healthy subjects. Interestingly, recent evidence also suggests that lipopolysaccharide levels are elevated in dengue virus infected patients and correlate with disease severity [40].

One of the alternative contributing factors is the amount of platelets. Dysfunctional platelets and thrombocytopenia are a salient clinical finding in dengue patients and are correlated with the severity of disease [77]. A platelet-endotoxin interaction is a necessary step for the final removal of LPS by the reticuloendothelial system [78]. The evidence also suggests that the levels of detectable endotoxin in patients may be inversely correlated with the platelet counts. Some percentage of dengue shock cases may result from increased gut mucosa permeability, which could lead to abnormally high endotoxin levels in the peripheral blood. This phenomenon in combination with reduced platelet counts and reduced IgM specific to LPS could lead to inefficient clearance of endotoxin and consequently another mechanism will need to be induced to promote its removal from the bloodstream.

Scientifically, it has been known that phagocytic cells such as primary monocytes and macrophages are very difficult to get infected by dengue virus [79]. But, if these cells are pretreated with endotoxin (LPS) [80], the infectivity rate increases significantly, likely as a result of enhanced phagocytic activity [72]. Monocytes potentially acquire the virus when they engulf dengue-containing platelets, a frequent occurrence in dengue patients on days 6–8 after the onset of fever [81,82]. In addition, LPS is known to bind to the CD14 receptor of macrophages and B cells and promote the secretion of pro-inflammatory cytokines [83,84]. Interestingly, it has been suggested that activated macrophages from secondary DENV infected patients display enhanced phagocytic behavior of opsonized platelets, through a mechanism involving milk fat globule-epidermal growth factor 8 [85]. Taken together, a hypothetical scenario can be drawn; endotoxin, usually kept at a low frequency in the circulation by functioning platelets, may leak into the periphery through a damaged gut-endothelial barrier in dengue patients, whom likely have dysfunctional platelets, thrombocytopenia, or low natural IgM and are unable to clear off the endotoxin in a timely manner. This combination of events may result in the induction of activated macrophages or monocytes, enhancing their engulfment activities and triggering a tsunami of inflammatory cytokine production and inciting septic shock. However this alternative hypothesis requires further investigation.

**Pre-existing immunity**

The pathophysiology of severe dengue is very complex and may involve multiple factors. Epidemiological data tabulated from dengue endemic locales suggest that...
serologically defined primary dengue virus infection and/or subsequent homologous serotype infection is known to be associated with less severe disease as compared with secondary subsequent heterologous serotype infection, a term has been coined as antibody dependent enhancement [86]. However, our understanding of these interacting components that contribute to the development of dengue disease is obstructed by the lack of suitable animal models that can recapitulate the cardinal features of human dengue. As a result, the exact mechanism(s) leading to the development of DHF/DSS remains poorly understood, in spite of several decades of intensive investigations. One of the factors believed to play a role in pathogenesis is pre-exposure. Results available from dengue epidemic countries have indicated that severe disease more frequently occurs not with the primary but during subsequent viral infections [87,88]. Without experimentation with the appropriate comparison groups and controls, it became assumed that pre-existing immunity following a challenge with a heterogeneous serotype is a risk for DHF/DSS. Consequently, the hypothesis suggests that DHF/DSS results from an abnormal or exaggerated host immune response -- particularly due to the cross-reactive antibodies, which bind similar epitopes on other dengue viral strains -- that augments the rate of virus uptake [4,71,89]. However, recent results accumulated from non-dengue endemic regions [90] and from travelers suggest that the frequency of DHF in primary infections in naïve individuals is similar to that of secondary infection [91]. Also, Libraty et al's cohort study reveals no association between maternal antibodies and development of severe dengue in infants [92]. Collectively, multiple causes may play a critical role in dengue pathogenesis. The cause of pathology in naïve individuals and in infants infected by dengue virus may be distinctively distinguishable from that of primary and secondary infection, respectively, in dengue epidemic zones.

According to the WHO guidelines, it is required that several specimens within a certain time interval be processed to clearly define the infection as primary or secondary. But, very often, in the clinical setting, multiple sample collection is inconvenient or dangerous to collect. Thus the term primary and secondary in dengue epidemic zones are often defined with a single collection sample by the ratio of IgM/IgG; if the value is \(>1.2\), then it is a primary infection, but if the value is \(\leq 1.2\), it is a secondary. However, a very high dengue antibody prevalence rate of 85-95% is seen in school-aged children in epidemic countries [88,93,94]. Also, IgG is characteristically unusually low at the onset of disease in secondary dengue patients [95]. Consequently, the definition cannot distinguish between current infection and previous infection. Frequently it is furthermore complicated by samples with similar (at 1) or slightly below 1.2 ratios for IgM and IgG. This case is very often arbitrarily assigned to be secondary infection, and thus the definition has been called into question [96]. To test whether this practice can accurately distinguish between primary and secondary sequential samples were obtained from a cohort study. Sera were collected daily for 7 days from 30 confirmed dengue patients. The IgM/IgG ratio was measured after antibody titers were determined. This study indicated that if the early time point samples were used to define the primary and secondary, then the wrong category was often assigned since the IgM/IgG ratios at the later time samples clearly suggested that the allocation should be to the opposite category (Figure 4).

Although the actual percentage of the erroneous allocation of the category is unknown, primary and Secondary dengue assignments based on the \(>1.2\) IgM/IgG ratio may intrigue the pathogenic cause of dengue in endemic countries. However, we observed about 26.7% (8/30) abnormal antibody response in current investigation, the percentage therefore seemed to be underestimated in dengue epidemic zones. Thus, a better test that can differentiate primary from secondary dengue virus infection is urgently needed.

As a whole, evidence for the role of pre-existing immunity in human disease is still by and large circumstantial [23,97,98]. Thus, in order to further advance the understanding of the causes of DHF/DSS, reported disease should be divided into three major categories (naïve primary infection, defined primary infection in endemic zones, and secondary infection) and considered separately [99]. With a clearer definition of the virus pre-exposure history, the search for the identity of the pathogenic cause for DHF/DSS may be much simpler to assess and faster to acquire and likely make much more sense.

**Viral strains**

The occurrence of DHF/DSS in primary naïve individuals and the high frequency of asymptomatic secondary infections implicates that the immune-enhancement hypothesis alone is inadequate to explain dengue pathogenesis. An alternative explanation for the pathogenesis of DHF/DSS is the virulence of different viral strains [100]. Although the in vivo scientific data on the topic is quite sparse, it can be interpreted that some dengue viral strains are more virulent for man than others. Reports based upon the epidemiological data advocate that particular serotypes appear to be more virulent than others with certain ethnic groups [101-106]. In addition, experimental results also suggest that certain genotypes within a serotype encode determinants for virulence, attenuation, and tissue tropism [107-111]. However, substantiation of the virulent strain hypothesis of dengue
pathogenesis still awaits the availability of an adequate disease model for validation [112,113].

Other factors
As aforementioned, the factors that place patients at higher risk of developing DHF/DSS are not clearly identified yet. Multiple factors have been correlated with DHF/DSS: age, sex, underlying disease, nutritional status, ordering of serotype pre-exposure, individual genetic background including HLA type and ethnic variation [114-119]. These factors have yet to be further evaluated.

Treatment and prevention
Currently, there is not a specific antiviral treatment for dengue. Even if there were a drug available that could reduce viral replication or entry it would have limited usage. Treatment of dengue disease is time-sensitive; in other words, as time progresses, the presence of the virus and the ability to accurately detect it decreases, while the risk of severe immune-mediated disease increases. The best treatment currently available is immediate supportive or palliative care with vigilant monitoring by the professional healthcare staff. Patients usually recover after fluid and electrolyte supportive therapy. Early recognition of DHF and immediate treatment are of utmost importance to reduce the case fatality rate.

Since there is no antiviral therapeutic modality or vaccine against dengue available, the only possible preventive method that can be instituted is mosquito control. However, the effectiveness of current insecticides is diminishing and the successfulness of this strategy is compromised by its high cost. Thus, a dengue vaccine is urgently needed to prevent the virus from further spreading.

Review; Conclusion
Dengue has been associated with human beings for more than two centuries and yet its pathogenic cause(s) remain poorly defined. Lack of a suitable animal model recapitulating the cardinal features of human dengue further hinders the progress of our understanding. Numerous factors and hypotheses have been associated with or attributed to the pathogenesis of dengue. There are only limited results suggestive that some of these theories may be the primal instigator of severe disease; however they remain to be circumstantial and require further verification. Complexity of severe dengue suggests that other factors, such as fever and endotoxin, are important as well. These factors are often underappreciated and may not only provide the critical link in understanding the cause(s) of dengue pathogenesis, but offer a
new strategy for the amelioration and/or prevention of dengue.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JJT designed and drafted the IRB protocol and enrolled the patients, KC drafted the IRB protocol and enrolled the patients and edited the manuscript, PCC enrolled the patients and collected the samples for the studies, LTL assisted in performing the laboratory assays and analyzing results, HVH performed the laboratory assays, YCL assisted in data analysis and edited the manuscript, and GCP wrote and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgements
We would like to thank the clinical staffs at the Tropical Medicine Center and Division of Infectious Diseases of Kaohsiung Medical University Hospital, and at the Division of Infectious Diseases in the Department of Pediatrics at the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. This study was partially supported by a startup grant from the National Health Research Institutes (NHIR-EK102-101295C) and National Science Council (NSC 101-2318-B-008-MY3) (YCL), and Grants from Taiwan National Science Council (NSC 99-2745-B-037-002) (JJT).

Author details
1Tropical Medicine Center, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan. 2Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan. 3Department of Internal Medicine, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan. 4Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. 5Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan. 6Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan. 7Department of Medical Laboratory Science and Biotechnology, College of Medicine and Life Science, Chung-Hwa University of Medical Technology, Tainan, Taiwan. 8Department of Pathology and Laboratory Medicine, Emory Virus Center, Emory University School of Medicine, Atlanta, GA, USA. 9Institute of Bioinformatics and Biosignal Transduction, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan. 10Center of Infectious Disease and Signaling Research, National Cheng Kung University Hospital, Tainan, Taiwan. 11Department of Microbiology and Immunology, Medical College, National Cheng Kung University, Tainan, Taiwan.

Received: 24 September 2013 Accepted: 26 November 2013
Published: 5 December 2013

References
1. WHO: Dengue Virus Development: The role of the WHO South-East Asia Regional Office. Geneva: World Health Organization; 2010.
2. Clark KB, Onilaman O, Nisao HM, Perrin GC, Villinger F: Can non-human primates serve as models for investigating dengue disease pathogenesis? Front in microbial 2013, 4:305.
3. Gusman MG: Dengue vaccines: new developments. Drugs Future 2011, 36:45–62.
4. Halstead SB: Dengue. Lancet 2007, 370(9599):1644–1652.
5. Murphy BR, Whitehead SS: Immune response to dengue virus and prospects for a vaccine. Annu Rev Immunol 2011, 29:587–619.
6. Peeling RW, Artsob H, Pelegrino JL, Bucy P, Cardosa MJ, Devi S, Enria DA, Farrar J, Gubler DJ, Gusman MG, Halstead SB, Hunsperger E, Kikis S, Margolis HS, Nathanon CM, Nguyen VC, Razo N, Vazquez S, Yoksan S: Evaluation of diagnostic tests: dengue. Nat Rev Microbiol 2010, 8(12):Suppl:S30–S38.
7. Rothman AL: Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol 2011, 11(8):532–543.
8. Chastel C: Eventual role of asymptomatic cases of dengue for the introduction and spread of dengue viruses in non-endemic regions. Front in phsiol 2012, 3:10.
9. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, Hay SJ: The global distribution and burden of dengue. Nature 2013, 496(7446):504–507.
10. Immanuel R, Suresh M: Global climate change and its potential impact on disease transmission by salinity-tolerant mosquito vectors in coastal zones. Front in physiol 2013, 3:198.
11. CDC: Locally acquired dengue–Key West, Florida, 2009–2010. MMWR 2010, 59(19):577–581.
12. Laughlin CA, Morens DM, Cassetti MC, Costero-Saillent Denis A, San Martin JL, Whitehead SS, Fauci AS: Dengue research opportunities in the Americas. J Infect Dis 2012, 206(7):1121–1127.
13. Morens DM, Fauci AS: Dengue and hemorrhagic fever: a potential threat to public health in the United States. Jama 2008, 299(2):214–216.
14. Pinheiro FP, Corber SJ: Global situation of dengue and dengue haemorrhagic fever, and its emergence in the Americas. World Health Stat Q 1997, 50(4):161–169.
15. Rsu-M-Aziz KS, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Verdam AV: Dengue and dengue haemorrhagic fever. Lancet 1998, 352(9123):971–977.
16. WHO: Dengue guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009.
17. WHO: Dengue Haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization; 1997.
18. Barniol J, Gaczkowski R, Barbato EV, da Cunha RV, Salgado D, Martinez E, Segarra CS, Piteiro Sandolov EB, Mitha A, Laksoko I, Lem LC, Martinez GS, Nunez A, Balsameda A, Allende I, Ramirez G, Dimano E, Thomacek K, Akbar NA, Ooi EE, Villegas E, Hien TT, Farrar J, Horstick O, Kroeber A, Jaenisch T: Usefulness and applicability of the revised dengue case classification by disease: multi-centre study in 18 countries. BMC Infect Dis 2011, 11:106.
19. Basuki PS, Budyanto, Pusupitaras D, Husada D, Darmowandowo W, Ismoerdijanto, Soegijanto S, Yamanaka A: Application of revised dengue classification criteria as a severity marker of dengue viral infection in Indonesia. Southeast Asian J Trop Med Public Health 2010, 41(5):1088–1094.
20. WHO: Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever. Revised and expanded edition, Volume 60. Geneva: SEARO Technical Publication Series, 2011.
21. Gusman MG, Halstead SB, Artsob H, Bucy P, Farrar J, Gubler DJ, Hunsperger E, Kroeber A, Margolis HS, Martinez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S, Peeling RW: Dengue: a continuing global threat. Nat Rev Microbiol 2010, 8(12):Suppl:S57–S56.
22. Halstead SB: Epidemiology of dengue and dengue haemorrhagic fever. In Dengue and Dengue Haemorrhagic Fever. Edited by Gubler DJ, A. G. Wallingford, England: CAB International, 1997:23–44.
23. Rothman AL: Dengue: defining protective versus pathologic immunity. J Clin Invest 2004, 113(7):946–951.
24. WHO: The World Health Report, A Vision for all. Geneva: Life in the 21st century, 1998.
25. Chokephaibulkit K, Perleg GC: The importance of temperature for medical science. Can Tap in Viral 2011, 9:43–49.
26. Vaughn DW, Green S, Kalayanaroj S, Innis BL, Nimmanitritya S, Suntayakorn S, Rothman AL, Ennis FA, Niskal A: Dengue in the early febrile phase: Viremia and antibody responses. J Infect Dis 1997, 176(2):322–330.
27. Pratter MK, Lloyd JB: Effects of temperature, metabolic inhibitors and other factors on fluid-phase and adsorptive pinocytosis by rat peritoneal macrophages. Biochem J 1979, 190(3):567–571.
28. Wiegel PH, Oka JA: Temperature dependence of endocytosis mediated by the asialoglycoprotein receptor in isolated rat hepatocytes. Evidence for two potentially rate-limiting steps. J Biol Chem 1981, 256(5):2615–2617.
29. Kuno G, Oliver A: Maintaining mosquito cell lines at high temperatures: effects on the replication of flaviviruses. In Vit Cell Dev Biol 1989, 25(2):193–196.
30. Pananjape SP, Kadam OD, Deolankar RP: Increased yields of Japanese encephalitis virus in heat shocked cell cultures. Acta Virol 1994, 38(6):333–337.
31. Noisakran S, Onilaman N, Hisao HM, Clark KB, Villinger F, Ansari AA, Perleg GC: Infection of bone marrow cells by dengue virus in vivo. Exp Hematol 2012, 40(3):250–259. e254.
32. Chiu YC, Wu KL, Kuo CH, Hu TH, Chou YP, Chush SK, Kuo CM, Kuee KM, Changchien CS, Liu JW, Chiu KW: Endoscopic findings and management...
of dengue patients with upper gastrointestinal bleeding. Am J Trop Med Hyg 2005, 73(2):441–444.

33. Sandler NG, Douek DC: Microbial translocation in HIV infection: causes, consequences and treatment opportunities. Nat Rev Microbiol 2012, 10(9):655–666.

34. Tan PS: Clinical correlates with immunopathogenesis in dengue haemorrhagic fever/dengue shock syndrome. Malays J Pathol 1993, 15(1):41–47.

35. Swank GM, Deitch EA: Role of the gut in multiple organ failure: bacterial translocation and permeability changes. World J surg 1996, 20(4):411–417.

36. Vejchapipat P, Theamboonlers A, Chongsrisawat V, Poovorawan Y: An evidence of intestinal mucosal injury in dengue infection. Southeast Asian J Trop Med Public Health 2006, 37(1):79–82.

37. Melzner E, Heymann Z, Bin H, Schwartz E: Capillary leakage in travelers with dengue infection: implications for pathogenesis. Am J Trop Med Hyg 2012, 86(3):536–539.

38. Ruiz N, Kahne D, Silhavy TJ: Transport of lipopolysaccharide across the cell envelope: the long road of discovery. Nat Rev Microbiol 2009, 7(9):677–683.

39. Usawattanakul W, Nimmannitya S, Sarabenjawong K, Tharavanij S: Role of the gut in multiple organ failure: bacterial translocation and permeability changes. Current Opinion in Gastroenterology 2003, 19(2):271–276.

40. van de Weg K, Koraan P, van Gorp EC, Mairuhu AT, Supriatna M, Soermitro A, van de Vijver DA, Osterhaus AD, Martin SE: Lipopolysaccharide levels are elevated in dengue virus infected patients and correlate with disease severity. J Clin Virol 2012, 53(1):38–42.

41. Halstead SB, Udomsakdi S, Singraraj P, Nisalak A: Alterations in key innate immune cell components in the peripheral blood of dengue patients detected by FACS analysis. J Innate Immun 2011, 3(5):530–540.

42. Chai LY, Lim PL, Lee CC, Hsu LY, Teoh YL, Lye DC, Krishnan P, Leo YS: Endotoxin and dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health 1986, 17(1):8–12.

43. Tsai JJ, Jen YH, Chang JS, Hsiao HM, Noisakran S, Perng GC: Concurrent dengue and malaria. Emerg Infect Dis 2006, 12(2):370–372.

44. Tsai CJ, Kuo CH, Chen PC, Changcheg CS: Upper gastrointestinal bleeding in dengue fever. Am J Gastroenterol 1991, 86(3):33–35.

45. Nisalak A, Jarman RG, Malasit P, Chokephaibulkit K, Perng GC: The importance of natural IgM: scavenger, protector and regulator. Histol Histopathol 2006, 21(12):1355–1366.

46. Vollmers HP, Brandlein S: Natural IgM antibodies: from parias to parvenus. Histol Histopathol 2006, 21(12):1355–1366.

47. Baumgarth N, Herrmann OC, Jager GC, Brown LE, Herrnberg LA, Chen J: B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J Exp Med 2000, 192(2):271–280.

48. Ehrenstein MR, Notley CA: The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol 2010, 10(11):778–786.

49. Ochsenbein AF, Fehr T, Lutz C, Suter M, Blombacher F, Hengartner H, Zinkernagel RM: Control of early viral and bacterial distribution and disease by natural antibodies. Science 1999, 286(5447):2156–2159.

50. Reid RR, Prodeus AP, Khan W, Hsu T, Rosen FS, Carroll MC: Endotoxin shock in antibody-deficient mice: unraveling the role of natural antibody and complement in the clearance of lipopolysaccharide. J Immunol 1997, 159(2):970–975.

51. Kurane I, Ennis FA: Immunopathogenesis of dengue virus infections. In Dengue and Dengue Hemorrhagic Fever. Edited by Gubler DJ, Kuno G. Wallingford, UK: CAB International; 1997:273–300.

52. Hotta S: Newer problems of dengue research. The Eighth Tropical Medicine Seminar: Kanazawa Medical University; 1984.

53. Baumgarth N, Tung JW, Herrnberg LA: Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. Springer Semin Immunopathol 2005, 26(4):347–362.

54. Boes M, Prodeus AP, Schmidt T, Carroll MC, Chen J: A critical role of natural immunoglobulin M in immediate defense against systemic bacterial infection. J Exp Med 1998, 188(12):2381–2386.

55. Weerkamp F, de Haas EF, Naber BA, Comans-Bitter WM, Bogers AJ, van Dongen JJ, Staal FJ: Age-related changes in the cellular composition of the thymus in children. J Allergy Clin Immunol 2005, 115(4):834–840.

56. Xu G, Dong H, Shi N, Liu S, Zhou A, Cheng Z, Chen G, Liu J, Fang T, Zhang H, Gu C, Tan X, Ye J, Xie S, Cao G: An outbreak of dengue virus serotype 1 infection in Cixi, Ningbo, People’s Republic of China, 2004, associated with a traveler from Thailand and high density of Aedes albopictus. Am J Trop Med Hyg 2007, 76(6):1182–1188.

57. WHO: Pathogenetic mechanisms in dengue haemorrhagic fever: report of an international collaborative study. Bull WHO 1973, 48:117–133.

58. Das J, Schwartz AA, Folkman J: Clearance of endotoxin by platelets: role in increasing the accuracy of the Limulus gelation test and in combating experimental endotoxemia. Surgery 1973, 74(2):335–340.

59. Kuo Z, Lim JY, Bertramello M, Quinn M, Chen H, Liu S, Martinez-Sobrido L, Diamond MS, Schlesinger JJ, de Silva A, Sallusto F, Jin X: Human antibodies against dengue enhance dengue viral infectivity without suppressing
type I interferon secretion in primary human monocytes. Virology 2011, 410(1):240–247.

80. Hotta H, Hotta S: Dengue virus multiplication in cultures of mouse peritoneal macrophages: effects of macrophage activators. Microbiol Immunol 1982, 26(8):665–676.

81. Naisakran S, Onlamoon N, Pattanapanyasat K, Hsiao HM, Songprakhon P, Angkasuwan N, Cheokeyhalkibut K, Villinger F, Ansari AA, Peng GC: Role of CD61(+) cells in thrombocytopenia of dengue patients. Int J Hematol 2012, 96(6):590–610.

82. Tsai J-H, Liu L-T, Chang K, Wang S-H, Hsiao H-M, Clark KB, Peng GC: The importance of hematopoietic progenitor cells in dengue. Ther Adv Hematol 2012, 3(1):59–71.

83. Rietz CR, Whieldon C: Lipopolysaccharide endotoxins. Annu Rev Biochem 2002, 71:635–700.

84. Rittig MG, Kaufmann A, Robins A, Shaw B, Sprenger H, Gemsa D, Foulongne V, Rouot B, Dornand J: Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes. J Leukoc Bio 2003, 74(4):1045–1055.

85. Alonzo MT, Lucarla TL, Dimao EM, Kurosu T, Suarez LA, Mapua CA, Akiya Y, Matas RR, Kuter DJ, Naqsta S, Natividad FF, Oishi K: Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus infections. J Infect Dis 2012, 205(S):1321–1329.

86. Halstead SB: Pathogenesis of dengue: challenges to molecular biology. Science 1988, 239(4839):476–481.

87. Halstead SB, Nimmannitya S, Cohen SN: Observations related to pathogenesis of dengue hemorrhagic fever, IV. Relation of disease severity to antibody response and virus recovered. Yale J Biol Med 1970, 42(5):311–328.

88. Sangkawibha N, Rojanasuphat S, Ahandrik S, Viriyapongse S, Jatanasen S, Salitl V, Phanthumachinda B, Halstead SB: Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. Am J Epidemiol 1984, 120(3):563–669.

89. Green S, Rothman A: Immunopathological mechanisms in dengue and dengue hemorrhagic fever. Curr Opin Infect Dis 2006, 19(5):429–436.

90. Chao DY, Lin TH, Hwang KP, Huang JH, Liu CC, King CC: Dengue hemorrhagic fever epidemic in Taiwan. Emerg Infect Dis 2004, 10(3):552–554.

91. Melitzer E, Schwartz E: A travel medicine view of dengue and dengue hemorrhagic fever. Travel Med Infect Dis 2009, 7(1):278–283.

92. Librarty DH, Acosta LP, Tallo V, Segure-Mercado E, Bautista A, Potts JA, Jarman RG, Yoon IK, Gibbons RV, Birion JD, Capeding RZ: A prospective nested case-control study of Dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. PLoS Med 2009, 6(10):e1000171.

93. Balmaseda A, Hammond SN, Perez L, Teillez Y, Saborio S, Mercado JC, Cuadra R, Rocha J, Perez MA, Silva SA, Rocha C, Harris E: Serotype-specific differences in clinical manifestations of dengue. Am J Trop Med Hyg 2006, 74(3):449–456.

94. Thai KT, Binh TQ, Gao PT, Phuong HL, Le Hung Q, Van Nam N, Ngia TT, Groen J, Nagelkerke N, de Vries PJ: Seroprevalence of dengue antibodies, annual incidence and risk factors among children in southern Vietnam. Trop Med Int Health 2005, 10(4):379–386.

95. Peng GC: Dengue vaccines: challenge and confrontation. World J of Vaccines 2011, 01(04):109–130.

96. Prince HE, Yeh C, Lape-Nixon M: Utility of IgM/IgG ratio and IgG avidity for distinguishing primary and secondary dengue virus infections using sera collected more than 30 days after disease onset. Clin Vaccine Immunol 2011, 18(11):1951–1956.

97. Kurane I: Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comp Immunol Microbial Infect Dis 2007, 30(5–6):229–340.

98. Stephenson JR: Understanding dengue pathogenesis: implications for vaccine design. Bull World Health Organ 2005, 83(4):308–314.

99. Peng GC, Cheokeyhalkibut K: Immunologic hypo- or non-responder in natural dengue virus infection. J Biomed Sci 2011, 20(1):34.

100. Barnes WJ, Rosen L: Fatal hemorrhagic disease and shock associated with primary dengue infection on a Pacific Island. Am J Trop Med Hyg 1974, 23(3):495–506.

101. Gubler DJ, Reed D, Rosen L, Hitchcock JR: Epidemiologic, clinical, and virologic observations on dengue in the Kingdom of Tonga. Am J Trop Med Hyg 1978, 27(3):581–589.

102. Dengue And Dengue Hemorrhagic Fever. 1st edition. Edited by Gubler DJGK. Wallingford, UK: CABI; 1997.

103. Mata J, Rico-Hesse R: Dengue virus tropism in humanized mice recapitulates human dengue fever. PLoS One 2011, 6(6):e20762.

104. Rico-Hesse R: Dengue virus markers of virulence and pathogenicity. Future Virol 2009, 4(6):581–590.

105. Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C, Boshell J, de Mesa MT, Nogueira RM, da Rosa AT: Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology 1997, 230(2):244–251.

106. Watts DM, Porter KR, Putvutana P, Vasquez B, Calampa C, Hayes CG, Halstead SB: Failure of secondary infection with American genotype dengue 2 to cause dengue hemorrhagic fever. Lancet 1999, 354(9184):1341–1344.

107. Messer WB, Gubler DJ, Harris E, Sivananthan K, de Silva AM: Emergence and global spread of a dengue serotype 3, subtype IV virus. Emerg Infect Dis 2003, 9(7):800–809.

108. Puri B, Nelson WM, Henchall EA, Hoke CH, Eckels KH, Dubois DR, Porter KR, Hayes CG: Molecular analysis of dengue virus attenuation after serial passage in primary dog kidney cells. J Gen Virol 1997, 78(Pt 9):2287–2291.

109. Rico-Hesse R: Microevolution and virulence of dengue viruses. Adv Virus Res 2003, 59:315–341.

110. Rico-Hesse R: Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology 1990, 174(2):479–493.

111. Thant KZ, Morita K, Igarashi A: Detection of the disease severity-related molecular differences among new Thai dengue-2 isolates in 1993, based on their structural proteins and major non-structural protein NS1 sequences. Microbiol Immunol 1996, 40(3):205–216.

112. Shen W, Kawanoh H, Men R, Clark D, Lai CJ: Construction of intertypic chimeric dengue viruses exhibiting type 3 antigenicity and neuroviroviral for mice. J Virol 1995, 69(8):5186–5190.

113. Cologna R, Rico-Hesse R: American genotype structures decrease dengue virus output from human monocytes and dendritic cells. J Virol 2003, 77(7):3929–3938.

114. Guzman MG, Kouri G: Dengue: an update. Lancet Infect Dis 2002, 2(1):33–42.

115. 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(2):118–124.

116. Halstead SB, Streit TG, LaFontant JG, Putvutana R, Russell K, Sun W, Kanesa-Thasan N, Hayes CG, Watts DM: Haiti: absence of dengue hemorrhagic fever despite hyperendemic dengue virus transmission. Am J Trop Med Hyg 2001, 65(1):180–183.

117. Loke H, Bethell DB, Phuong CX, Dung M, Schneider J, White NJ, Day NP, Farrar J, Hill AV: Strong HLA class I-restricted T cell responses in dengue hemorrhagic fever: a double-edged sword? J Infect Dis 2001, 184(11):1369–1373.

118. Malaviye GN, Velathanthri VG, Wijewickrama ES, Fernandez S, Jayatane SD, Aaskov J, Seneviratne SL: Patterns of disease among adults hospitalized with dengue infections. QJM 2006, 99(5):299–305.

119. Thiyakorn U, Nimmannitya S: Nutritional status of children with dengue hemorrhagic fever. Clin Infect Dis 1993, 16(2):295–297.

doi:10.1186/1423-0127-20-88

Cite this article as: Tsai et al.: Role of cognitive parameters in dengue hemorrhagic fever and dengue shock syndrome. Journal of Biomedical Science 2013 20:88.