# PREDICTORS FOR EARLY DETECTION OF SEVERE DENGUE WITH SHOCK

**QUAH CHAI HOONG**

**FACULTY OF MEDICINE**
**UNIVERSITY OF MALAYA**
**KUALA LUMPUR**

**2019**

# PREDICTORS FOR EARLY DETECTION OF SEVERE DENGUE WITH SHOCK

**QUAH CHAI HOONG**

**DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ANAESTHESIOLOGY**

**DEPARTMENT OF ANAESTHESIOLOGY**
**UNIVERSITY OF MALAYA**
**KUALA LUMPUR**

**2019**

**UNIVERSITY OF MALAYA**

**ORIGINAL LITERARY WORK DECLARATION**

Name of Candidate: Quah Chai Hoong

Matric No: MGE 150010

Name of Degree: Masters of Anaesthesiology

Title of Project Predictors for Early Detection of Severe Dengue with Shock

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

## ABSTRACT

### Background

It is vital to detect severe dengue with severe plasma leakage in the early stage to avoid worsening shock. Traditional methods to detect shock in severe dengue are based on the clinical assessment of the physicians in charge. They depend on the presence of symptoms such as cold and sweaty skin, blood pressure, heart rate , and presence of bleeding to aid in disease identification for treatment, epidemiologic surveillance, and studies of dengue pathogenesis. This has delayed the management of severe dengue which substantially increases the likelihood of morbidity and mortality. This study is conducted to identify features that are more informative in relation with severe dengue with shock.

### Methods

We studied the admissions to the General Intensive Care Unit at the University Malaya Medical Centre (UMMC), Kuala Lumpur, Malaysia in the past 1 year after obtaining approval from the Medical Ethical Committee UMMC. We included all patients who were diagnosed with dengue infection and aged 18 years old and above.

Physiological data of patients who were diagnosed with dengue are collected from University Malaysia Medical Centre electronic medical records. We merged various clinical and biochemical parameters to build the prediction model for early detection of shock in dengue patients because any single clinical parameter does not have strong discriminative power. Therefore, major data mining methods are adopted to train the

multi-parameters model. Type of data collected ranges from primary measures and secondary measures of vital signs to laboratory data that require longer time to process and obtain. Data were collected for this study are:

A) Physiological parameters/Vital signs:

Primary measures: Heart rate, respiratory rate (RR), oxygen saturation (SpO2), blood pressure (systolic, diastolic and mean blood pressure)

B) Laboratory data:

Full blood count (FBC): hemoglobin (Hb), hematocrit, platelet count, white blood cell count

Liver function test (LFT): total protein, total bilirubin, aspartate aminotransferease (AST), Alanine aminotransferase (ALT), albumin, alkaline phosphatase,

Arterial blood gas (ABG): oxygen saturation, pO2, pCO2, FiO2, bicarbonate, pH, base excess, Lactate

Coagulation test: partial thromboplastin time (ATT), INR

Renal function test (RFT): Urea, creatinine, sodium, potassium

We classified the cases in this study based on the definition of disease severity in 2009 WHO guidelines. Cases will be characterized as non-severe dengue (non-SD) and severe dengue (SD). Shock is defined as systolic blood pressure < 90 mmHg or narrow pulse pressure < 20 mmHg.

**Results:**

Of the 155 patients, 7 patients had dengue shock syndrome compared to 148 patients did not experienced any shock symptoms.

This study identifies platelet count is the only significant factor that was associated with subsequent development of severe dengue with shock.

For the baseline results, there were no differences in most of the variables being analysed. Only the platelet levels showed a significant difference between the shocked and non-shocked subjects (81.00 vs 16.00, p=0.015). There were no significant differences between the groups for age (p=0.408), BMI (p=0.183), haemoglobin (p=0.408), haematocrit (p=0.390), total white cells (p=0.461), INR (p=0.384) and APTT time (p=0.388), ALT (p=0.301), AST (p=0.274) and lactate levels (p=0.198).

For the 2nd day analysis, it was only the platelet parameter again which showed a significant difference between the shocked and non-shocked subjects (61.00 vs 21.50, p=0.011). There were no significant differences between the groups for haemoglobin (p=0.389), haematocrit (p=0.454), total white cells (p=0.875), INR (p=0.559) and APTT time (p=0.470), ALT (p=0.715), AST (p=0.667) and lactate levels (p=0.314).

**Conclusions:**

Thrombocytopenia during acute febrile phase is an early predictor of severe dengue with shock. Patients with low platelets in acute febrile phase of dengue fever need more medical attention as there is a higher chance of progression to severe dengue with shock. But age, BMI, haematocrit, haemoglobin, platelet, WBC, INR, APTT, ALT, AST and lactate in acute febrile phase are unlikely to predict DHF.

## ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to my supervisor, Dr. Chaw Sook Hui for her thorough guidance and wisdom in helping me with this study.

I would like to thank Associate Professor Dr. Sharifah Faridah Binti Syed Omar, from Department of Medicine, for giving me permission to access the data for dengue patients.

I would also like to thank Encik Awaludin Bin Bakar and Puan Nor Azean Bt. Modan Johari from the Medical Record Department for approving my request to access patients' Electrical Medical Record (EMR).

I also appreciate all my lecturers and colleagues from the Department of Anaesthesiology, UMMC for the memorable journey through this Master's Programme.

Last but not least, special thanks to my lovely family and husband whose encouragement and support made all the difference.

Thank you very much!

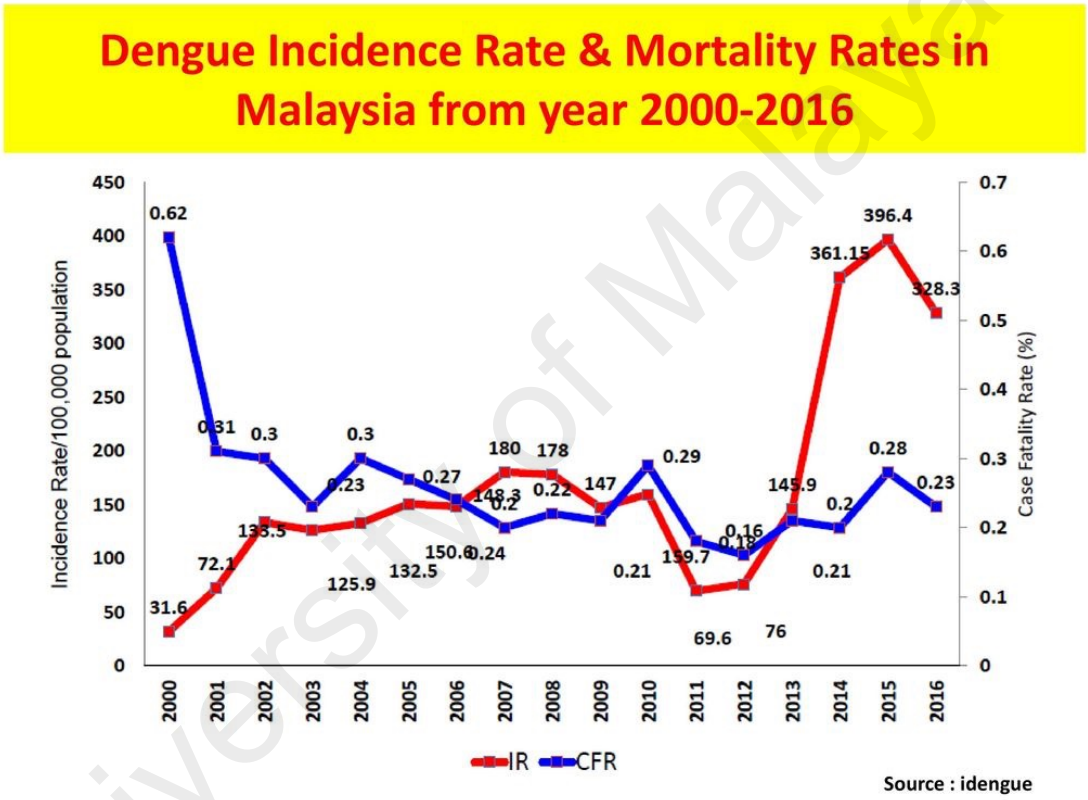
## TABLE OF CONTENTS

## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| BMI | Body Mass Index |
| WBC | White Cell Count |
| INR | International Normalised Ratio |
| APTT | Activated Partial Thromboplastin Time |
| ALT | Alanine Transaminase |
| AST | Aspartate Transaminase |
| IQR | Inter-Quartile Range |
| DHF | Dengue Haemorrhagic Fever |
| DSS | Dengue Shock Syndrome |
| EMR | Electronic Medical Record |

# CHAPTER 1: INTRODUCTION

Dengue is a common viral disease in tropical and subtropical countries and has now become an important national public health problem in Malaysia. It is an ongoing transmission with increased risk in urban and rural areas. Peak transmission occurs in the late monsoon season (October through February in east peninsular Malaysia, Sabah,




and Sarawak; July through August in west peninsular Malaysia).

Dengue is caused by one of the four serotypes of dengue viruses (DENV1-DENV4). An increase in infection has been seen in recent years due to many factors including urbanization and air travel. Over 2.5 billion people of the world's population are now at risk for dengue.

Dengue virus infection presents a wide spectrum of manifestations including asymptomatic condition, dengue fever (DF), or severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) in affected individuals.

The clinical manifestation of dengue has a wide spectrum from asymptomatic to vague complaints such as lethargy to classical symptoms such as fever, vomiting, abdominal pain which can mimic many other illness. Severe dengue is defined as a suspected dengue patients with one of the following features: severe plasma leakage that leads to shock, severe bleeding, and severe organ impairment, which refers to brain, heart and liver. Despite the advances in medical care, severe dengue remains as the major cause of morbidity and mortality in Southeast Asia.

Due to the dynamic nature of symptomatic dengue infection, early detection of warning signs and clinical deterioration are critical in determining the clinical outcome of dengue. Clinical manifestations offer the earliest markers in predicting severe dengue disease.

A recent meta-analysis of signs and symptoms of severe dengue shows that bleeding, nausea and vomiting, abdominal pain, skin rashes, and hepatosplenomegaly are associated with severe dengue disease. Patients with dengue fever are clustered into two groups; one with warning signs including abdominal pain, mucosal bleeding and liver enlargement that warrant ICU admission and the other without those signs

Mortality of patients with severe dengue is largely due to the progressively worsening shock and multiorgan failure. The mechanism of worsening shock is not fully understood, but it is postulated that increased vascular permeability due to malfunction of vascular

endothelial cells occurs in severe dengue. Therefore, it is vital to detect severe dengue with severe plasma leakage in the early stage to avoid refractory shock. Traditional method to detect shock in severe dengue is based on the clinical assessment of the physicians. They depend on the presence of symptoms such as cold and sweaty skin, blood pressure, heart rate, and presence of bleeding to aid in disease identification for treatment, epidemiologic surveillance, and studies of dengue pathogenesis. This has delayed the management of severe dengue which substantially increases the likelihood of morbidity and mortality.

The possibility to examine the progression of shock with integration of patients' physiological information and biochemical parameters would help in understanding of progression of the disease and early detection of shock. The individualized model that is learned based on the measurement of patients' physiological data, could be the basis for effective treatment and prevention of development of shock in critically ill patients.

Since severe dengue has emerged as the most important arthropod-borne viral diseases of humans since mid-1950s, there is a lot of cases documented that can be used to learn a prediction model for development of shock in severe dengue. The early prediction of severe dengue in patients without any warning signs who may later develop severe DHF is very important to choose appropriate intensive supportive therapy since available vaccines for immunization are yet to be approved. An ideal biomarker should be able to identify individuals who are at risk of developing severe dengue

Severe dengue responses include T and B cell activation and apoptosis, cytokine storm, hematologic disorders and complement activation. Cytokines, complement and other

unidentified factors may transiently act on the endothelium and alter normal fluid barrier function of the endothelial cells and cause plasma leakage.

To date, there is a dearth of study that evaluate a model that can predict the development of shock in severe dengue patients by using various clinical and biochemical parameters. In addition, the large volume of data brings new challenges to build an accurate prediction model.

# CHAPTER 2: LITERATURE REVIEW

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following a self-limiting non-severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without haemorrhage.

Peripheral blood parameters change during the course of the illness. Dengue fever is characterized by leucopenia (WBC) < 5000 cells/mm3), thrombocytopenia (< 150,000 cells/mm3), rising haematocrit (5–10%) and there should be no evidence of plasma leakage.

In Dengue Hemorrhagic Fever (DHF) there is evidence of plasma leakage which is usually evidenced by ascites or pleural effusion. Peripheral blood parameters characterized by thrombocytopenia (< 100,000 cells/mm3) and haematocrit rise > 20%. A drop in platelet count below 100,000 cells/mm3, may be occasionally observed in dengue fever but is almost a feature in DHF. There is also a tendency towards haemorrhage associated with severe thrombocytopenia. Thrombocytopenia and hypofibrinogenemia are the two most common hemostatic defects. Increase intravascular clotting seemed to be a responsible factor, though not an outstanding one. It is evidenced by mildly and variably low factors II, V, VII, VIII, IX, X, and XII, and by mild to moderate increase of fibrin degradation products as well as low platelet count and fibrinogen.

Presently, changes in platelet count by dengue virus–platelet interaction have been clearly demonstrated. Luckily, most patients have compensated consumptive coagulopathy that seldom requires treatment. Bleeding is likely caused by activated platelets, resulting from damaged capillary endothelium, and can be safely treated with

normal saline. Petechial skin rash, epistaxis, and gum bleedings are common in mild and moderately severe cases.

Recently, Putintseva and colleagues studied bone marrow specimens obtained from patients with dengue infection and found megakaryocytic hyperplasia in 60%. They proposed that dengue viruses provoke a transitory alteration in the humoral regulation of thrombopoiesis, which possibly may be the consequence of the lymphoid tissue damage and extends the thrombocytopenic state and contributes to the appearance of hemorrhagic complication.

Azin FR1 et. al found that thrombocytopenia and elevated transaminases were observed in patients with classic dengue fever. The main laboratory abnormalities found in dengue hemorrhagic fever were thrombocytopenia, hemoconcentration and elevated transaminases, similar to severe dengue with the exception of hemoconcentration.

There are some recent interesting studies on the platelet count and its clinical correlation in dengue infection. Chang and colleagues studied 15 dengue cases and reported that all patients had varying degrees of hepatomegaly and pleural effusion on their chest radiographs accompanied by a rapid increase in the hematocrit of more than 20% and a decrease in the platelet count to less than 100,000/μL.

George and colleagues reported a wide range of hemorrhagic manifestations in the patients with dengue infection. They noted that those manifestations were common in severe cases .

Tripathi and colleagues reported in their experience in an outbreak in Delhi that the mortality of dengue infection is low if the patients came early to the hospital.

Concerning hematologic diagnostic markers, Narayanan and colleagues noted that there was no correlation between platelet count and bleeding in dengue infection.

Garcia and colleagues noted that most dengue patients had their maximal thrombocytopenia within the fifth day from the onset of constitutional symptoms. In addition, they identified three phases which are proteinuria and hypoalbuminemia; maximal cytopenia as well as bradycardia and liver enzyme elevation, in the sequence of events seen in the majority of patients with dengue fever accompanied by thrombocytopenia.

Udaya Ralapanawa et. al found that there was a significant difference in mean values of platelets and haemoglobin observed during acute phase in non-leakers compared to the patients who progressed to DHF, while no significant difference was observed for white blood cells, neutrophils, lymphocytes and haematocrit values. A significantly higher mean value was observed in white blood cells and hemoglobin in leakers compared to non-leakers during day 5. Mean day 5 platelet value was significantly lower among leakers compared to non-leakers but no significant difference between haematocrit, neutrophil and lymphocyte values were observed [(10)].

From a literature reiew from Pubmed 2004, a summative report on the platelet count and its clinical correlation to duration of fever in 35 Thai children is presented. Most of the subjects visited to the physician with a complaint for fever. Most patients went to see the physician between the 3rd and the 5th day from the onset of fever. There is no significant correlation between platelet count and duration of fever (ANOVA test, $p = 0.28$). However, there is a trend of increase platelet count in the later days.

Saito et. al concluded that severe thrombocytopenia and increased vascular permeability are two major characteristics of dengue haemorrhagic fever (DHF). To develop a better understanding of the roles of platelet-associated IgG (PAIgG) and IgM (PAIgM) in inducing thrombocytopenia and its severity of disease in patients with secondary dengue virus infection, the relationship between the PAIgG or PAIgM levels and disease severity as well as thrombocytopenia was examined in 78 patients with acute phase secondary infection in a prospective hospital-based study. The decrease in platelet count during the acute phase recovered significantly during the convalescent phase. In contrast, the increased levels of PAIgG or PAIgM that occurred during the acute phase of these patients decreased significantly during the convalescent phase. An inverse correlation between platelet count and PAIgG or PAIgM levels was found in these patients. Anti-dengue virus IgG and IgM activity was found in platelet eluates from 10 patients in an acute phase of secondary infection. Increased levels of PAIgG or PAIgM were significantly higher in DHF than those in dengue fever (DF). An increased level of PAIgM was associated independently with the development of DHF, representing a possible predictor of DHF with a high specificity. Their present data suggest that platelet-associated immunoglobulins involving antidengue virus activity play a pivotal role in the induction of thrombocytopenia and the severity of the disease in secondary dengue virus infections.

Liang Cui et. al developed quantitative and high-throughput tools and discovered circulating serotonin, conceivably platelet-derived, that showed a nearly two-fold decrease in DHF patients compared to mild dengue fever. Because immune mediators may increase the predictive ability, we measured them in blood and identified interferon-gamma as an important cytokine in DHF. When serotonin is used in combination with IFN-γ, this dual-panel predictive panel provides accurate prognosis of

DHF within 96 h from fever onset. These findings may have important clinical implications not just in early dengue prognostication but also in the design of therapeutic strategies against dengue infections.

# CHAPTER 3: METHODS

After approval from the University of Malaya Medical Ethics Committee ( UMREC Number 201848-6208), a total of 155 patients', whose ages were 18 years and above, previous records were retrieved from the Electronic Medical Record ( EMR) and were analysed retrospectively. Datas were divided into two groups, namely dengue fever with warning signs and severe dengue with shock (systolic blood pressure of less than 90mmHg).

Age, body mass index (BMI), haematocrit, haemoglobin, platelet, total white cell, INR, APTT, ALT, AST and lactate were collected respectively and classifed accordingly. Univariate analysis was done to compare the results between the two groups. The blood parameters were also analysed after collection on Day 2.

Patient's variables were summarized as means and standard deviations for continuous normally-distributed variables, as medians and interquartile ranges for non-normally distributed variables. The variables of the patients in both the shocked and non-shocked groups were compared using Mann Whitney U test to analyse for univariate significance. The threshold for statistical significance was set at $p<0.05$. All analyses were performed using SPSS version 21.

# CHAPTER 4: RESULTS

| Variables | Non-shock | | Shock | | p-value[a] |
|---|---|---|---|---|---|
| | Median (n) | IQR | Median (n) | IQR | |
| **Age** | 32.50 (148) | 22.00 | 26.00 (7) | 18.00 | 0.408 |
| **BMI** | 24.20 (125) | 6.68 | 26.62 (7) | 8.20 | 0.183 |
| **Haematocrit** | 45.00 (148) | 8.00 | 43.00 (7) | 8.00 | 0.390 |
| **Haemoglobin** | 15.00 (148) | 3.00 | 14.90 (7) | 4.00 | 0.408 |
| **Platelet** | 81.00 (148) | 82.00 | 16.00 (7) | 36.00 | 0.015 |
| **Total white cell** | 3.50 (147) | 1.90 | 4.50 (7) | 2.30 | 0.461 |
| **INR** | 1.10 (122) | 0.20 | 1.10 (6) | 0.50 | 0.384 |
| **APTT** | 39.55 (122) | 10.70 | 46.75 (6) | 27.00 | 0.388 |
| **ALT** | 73.00 (143) | 79.00 | 171.00 (6) | 221.00 | 0.301 |

| | | | | | |
|---|---|---|---|---|---|
| **AST** | 116.00 (102) | 150.00 | 277.00 (6) | 367.00 | 0.274 |
| **Lactate** | 1.55 (76) | 1.00 | 1.90 (5) | 0.60 | 0.198 |

Table 1. Univariate analysis of the different variables between the patients in the shocked and non-shocked group at Day 1 presentation (baseline)

[a] Mann Whitney U test

IQR = inter-quartile range

| Variables | Non-shock | | Shock | | p-value |
|---|---|---|---|---|---|
| | Median (n) | IQR | Median (n) | IQR | |
| **Haematocrit** | 43.00 (145) | 8.00 | 39.00 (6) | 16.00 | 0.454 |
| **Haemoglobin** | 14.50 (145) | 2.90 | 13.20 (6) | 6.18 | 0.389 |
| **Platelet** | 61.00 (145) | 74.00 | 21.50 (6) | 34.50 | 0.011 |
| **Total white cell** | 4.10 (145) | 2.75 | 4.00 (6) | 2.20 | 0.875 |
| **INR** | 1.10 (42) | 0.20 | 1.20 (3) | - | 0.559 |
| **APTT** | 43.40 (41) | 14.80 | 44.70 (3) | - | 0.470 |
| **ALT** | 83.00 (111) | 99.00 | 149.00 (7) | 239.00 | 0.715 |
| **AST** | 128.50 (114) | 176.00 | 193.00 (5) | 439.00 | 0.667 |
| **Lactate** | 1.20 (29) | 1.10 | 1.75 (2) | - | 0.314 |

Table 2. Univariate analysis of the different variables between the patients in the shocked and non-shocked group at Day 2 presentation

[a] Mann Whitney U test

IQR = inter-quartile range

Of the 155 patients, 7 patients had dengue shock syndrome compared to 148 patients did not experienced any shock symptoms.

For the baseline results, there were no differences in most of the variables being analysed. Only the platelet levels showed a significant difference between the shocked and non-shocked subjects (81.00 vs 16.00, p=0.015). There were no significant differences between the groups for age (p=0.408), BMI (p=0.183), haemoglobin (p=0.408), haematocrit (p=0.390), total white cells (p=0.461), INR (p=0.384) and APTT time (p=0.388), ALT (p=0.301), AST (p=0.274) and lactate levels (p=0.198).

For the 2$^{nd}$ day analysis, it was only the platelet parameter again which showed a significant difference between the shocked and non-shocked subjects (61.00 vs 21.50, p=0.011). There were no significant differences between the groups for haemoglobin (p=0.389), haematocrit (p=0.454), total white cells (p=0.875), INR (p=0.559) and APTT time (p=0.470), ALT (p=0.715), AST (p=0.667) and lactate levels (p=0.314).

# CHAPTER 5: DISCUSSION

Dengue is a viral infection of increasing global significance that evolves rapidly over a short time-course and displays a wide range of clinical manifestations. Although much has been written from an empirical standpoint about the clinical spectrum of disease, few formal descriptions based on prospectively collected data have been published. Given the highly variable disease evolution, this may reflect the need for enrolment of substantial case numbers to allow meaningful interpretation of the relevance of different clinical events.

Its clinical features and laboratory parameters similarities to other diseases make timely diagnosis of dengue fever and its management difficult. Delayed diagnosis might be associated with higher mortality. From Malaysia registry in 2017, there were 177 dengue mortality out of 83, 849 cases with case fatality rate being 0.21%. The most affected states are Selangor, Kuala Lumpur and Johor.

Early detection of severe dengue is believed to be crucial to reduce the mortality related to dengue. Many small studies have been carried out to look for determinants of severe dengue and mortality but so far they yield mixed results.

This retrospective study consists of 155 patients diagnosed with either dengue fever with warning signs or severe dengue with shock. Using this unique dataset we were able to identify only platelet is the significant factor that was associated with subsequent development of severe dengue with shock.

Interestingly, we did not find evidence of a relationship between haematocrit and risk of progression to severe dengue with shock. We found the absolute platelet count on a given day during the febrile phase to be an important risk factor for developing DSS ( p-value 0.015 and 0.011). As the main underlying pathophysiological abnormality in DSS is plasma leakage [11], these findings suggest a potential role for platelets in the

induction of plasma leakage, a phenomenon supported by the recent work by Hottz et al . Haematocrit may be less important in the early phase of disease, as found in this study, because plasma leakage is less pronounced in this stage.

Platelets contribute to increased vascular permeability by inflammation dependent release of IL-1β. A rapid decrease in platelet count, concomitant with a rising haematocrit, is suggestive of progression to plasma leakage. Even though previous studies have shown lower platelet values among DHF patients, only few study findings reflect acute febrile phase platelet values. In our study among severe dengue with shock patients, a significant drop of the platelets value was observed compared to dengue fever with warning signs even during the acute febrile phase, suggesting a rapid decline of platelet counts before the critical phase. Similar to our findings, a previous research done in the paediatric population, acute febrile phase platelet counts in the patients who developed DSS tended to be lower than the patients who never progressed to DHF.

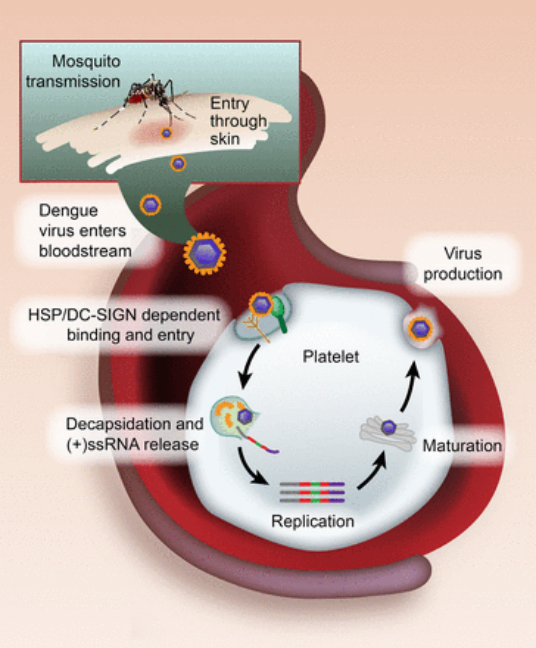



Figure 5.1: Model of DENV binding and replication by platelets

Recent studies have shown that dengue induces platelet activation and apoptosis, which modulate inflammatory responses in target monocytes. Additionally, dengue infection triggers the synthesis and release of interleukin-1β (IL-1β) by human platelets.

Although binding of dengue to the dendritic cell–specific intracellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN) has been implicated in platelet activation, apoptosis, and IL-1β synthesis, it was substantiated that dengue directly binds DC-SIGN on the surface of platelets. Binding of dengue to DC-SIGN, however, also requires heparin sulfate proteoglycan and is significantly enhanced by thrombin. The DENV readily binds platelets at 37°C, but binding is also observed at room temperature (25°C). Interestingly, it was found that trypsinization did not remove all of the surface-bound DENV. It was suggested that DENV may be internalized, initiating a search for replication of the virus in platelets.

Although there was no prior evidence that platelets replicate DENV, the virus has been detected in platelets isolated from dengue patients. Based on these findings and previous studies demonstrating that anucleate platelets are capable of translating their own

messenger RNA, it was postulated that the DENV could replicate its viral RNA by usurping the translational machinery of platelets. DENV is a positive-sense (+) single-stranded RNA (ssRNA) with a genome of ~11 kb. The dengue genome encodes a polyprotein precursor that is cleaved to three structural proteins (a core protein, membrane-associated protein, and envelope protein) and seven nonstructural (NS) proteins. Platelets synthesize NS1 protein and replicate the dengue viral genome. Intact platelets, but not damaged platelets (ie, freeze-thawed), were capable of replicating all 4 serotypes of dengue and as predicted, the authors' studies revealed that platelets facilitate the generation of infectious DENV progeny. Production of infectious virus also indicates that platelets use functional Golgi components for nucleocapsid assembly and formation of virions.

This story has several implications. First and foremost, it provides compelling evidence that dengue replication occurs in platelets (see figure) and raises the possibility that other (+)ssRNA viruses may be similarly propagated by these anucleate cytoplasts. Although platelets are in a prime position to defend against blood-borne viruses, it appears that dengue takes advantage of this frontline positioning to board platelets and swipe their translational cargo as a mode of survival. Second, de novo synthesis of NS1 may have implications beyond its primary role in dengue replication. NS1 is reported to be released into the plasma, where it is highly immunogenic, and antiplatelet autoantibodies elicited by DENV NS1 induce thrombocytopenia in mice.

Thus, it is conceivable that production of viral antigens may impact platelet clearance by immune complex formation. Finally, the findings by Simon et al provide important new insights relevant to human DENV infection and platelet transfusion practices. Previously, DENV-induced synthesis and release of IL-1β in platelet-derived

microparticles have been linked to injurious systemic inflammatory response syndromes and increased vascular permeability. As DENV is now shown to commandeer human platelets, DENV may also use platelets to ship newly formed virions throughout the circulation and thus extend its infectious reach. Whether the thrombocytopenia that commonly occurs during dengue infection is a host-defense mechanism to limit viral dissemination remains to be determined.

In addition, in the current report, DENV binding and replication in platelets occurred at 25°C, the temperature at which platelet concentrates are commonly stored prior to transfusion into recipient patients. As DENV infection may not always result in clinical symptoms, platelet concentrates collected from asymptomatic DENV-infected donors may serve as a reservoir for DENV transmission during platelet transfusions, especially in tropical or subtropical climates where DENV is endemic.

However, there are few limitations in this study. There is an inadequate sample size as it is too small. Hence, the statistical tests are not able to identify significant relationships within the data set. If this study is of much larger sample size, it could have generated more accurate results. Another limitation is the data collection process. The data collection which I used is by accessing the EMR and look into each patient's data in detail. This method is very time consuming and could be improved if the data were collected via computerised data extraction.Limited study time is the main limitation to this study. Much time is needed to be spend on the manual data collection. Lastly, it is very costly if the data are to be collected via computerized data extraction by the information technologists.

# CHAPTER 6: CONCLUSION

Thrombocytopenia during acute febrile phase is a early predictor of severe dengue with shock. Patients with low platelets in acute febrile phase of dengue fever need more medical attention as there is a higher chance of progression to severe dengue with shock. But age, BMI, haematocrit, haemoglobin, platelet, WBC, INR, APTT, ALT, AST and lactate in acute febrile phase are unlikely to predict DHF. Also, this provides strong support for the WHO recommendation to perform daily full blood counts in dengue patients in febrile phase and more frequent blood taking in critical phase.

## REFERENCES


Azin FR1, Gonçalves RP, Pitombeira MH, Lima DM, Branco IC.APPENDICES
Dengue Update. Epidemiology unit, Ministry of Health, Sri Lanka. Accessed 15 June 2018. Dengue: profile of hematological and biochemical dynamics.
Rev Bras Hematol Hemoter. 2012;34(1):36-41. doi: 10.5581/1516-8484.20120012

Basu A1, Chaturvedi UC.
FEMS Immunol Med Microbiol. 2008 Aug;53(3):287-99. doi: 10.1111/j.1574-695X.2008.00420.x. Epub 2008 Jul 3.
Vascular endothelium: the battlefield of dengue viruses.

Daisy Vanitha John et. al (2015) Biomarkers of severe dengue disease- a review : 1-2

Guidelines on Management of Dengue Fever and Dengue Haemorrhagic Fever in Adults. National Guidelines. Revised and expanded edition 2012. Ministry of Health, Sri lanka.

Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. Blood. 2013;122: 3405–14. 10.1182/blood-2013-05-504449 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
]

Kularatne SAM, Weerakoon KGAD, Munasinghe R, Ralapanawa UK, Pathirage M. Trends of fluid requirement in dengue fever and dengue haemorrhagic fever: a single centre experience in Sri Lanka. BMC Res Notes. 2015;8:130. doi: 10.1186/s13104-015-1085-0. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Lam PK, Van Ngoc T, Thu Thuy TT, Van Hong NT, Nhu Thuy TT, Hoai Tam DT, et al. The value of daily platelet counts for predicting dengue shock syndrome: results from a prospective observational study of 2301 Vietnamese children with dengue. PLoS Negl Trop Dis. 2017;11:1–20. doi: 10.1371/journal.pntd.0005498. [PMC free article] [PubMed] [CrossRef] [Google Scholar

Leonard E. G. Mboera1*, Clement N. Mweya1, Susan F. Rumisha1, Patrick K. Tungu1, Grades Stanley1, Mariam R. Makange2, Gerald Misinzo2, Pasquale De Nardo3, Francesco Vairo3, Ndekya M. Oriyo1
The Risk of Dengue Virus Transmission in Dar es Salaam, Tanzania during an Epidemic Period of 2014
National Institute for Medical Research, Dar es Salaam, Tanzania, 2 Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, Morogoro, Tanzania, 3 National Institute for Infectious Diseases, "L. Spallanzani", Rome, Italy

Liang Cui et. al (2015) Serum Metabolomics Reveals Serotonin as a Predictor of Severe Dengue in the Early Phase of Dengue Fever :2

Malavige GN, Velathanthiri VGNS, Wijewickrama ES, Fernando S, Jayaratne SD, Aaskov J, et al. Patterns of disease among adults hospitalized with dengue infections. J Assoc Physicians. 2006;99:299–305. [PubMed] [Google Scholar]

Med J Armed Forces India. 2013 Jul; 69(3): 254–259.
Published online 2012 Oct 23. doi: 10.1016/j.mjafi.2012.08.021
Autopsy findings in fatal dengue haemorrhagic fever – 06 Cases
K.R. Rathi, Col,a,∗ M.M. Arora, Brig,b K. Sahai, Col,c S. Tripathi, Col,d S.P. Singh, Wg Cdr,e D.K. Raman, Lt Col,f and K.B. Anand, Surg Cdrg

M. SAITO*, K. OISHI*, S. INOUE† E. M. DIMAANO‡, M. T. P. ALERA‡, A. M. P. ROBLES‡, B. D. ESTRELLA JR‡, A. KUMATORI§, K. MOJI††, M. T. ALONZO¶, C. C. BUERANO¶**, R. R. MATIAS¶**, K. MORITA†, F. F. NATIVIDAD¶ & T. NAGATAKE* Departments of *Internal Medicine, †Virology and §Biochemistry, ††Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine Nagasaki University, Japan, ‡Department of Blood Borne Diseases, San Lazaro Hospital, Manila, ¶Research and Biotechnology Division, St Luke's Medical Center, Quezon City, and **Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines

Nivedita Gupta, Sakshi Srivastava,* Amita Jain,* and Umesh C. Chaturvedi
Indian J Med Res. 2012 Sep; 136(3): 373–390. Dengue in India

Putintseva E, et al. Nouv Rev Fr Hematol, 1986, Alterations in thrombopoiesis in patients with thrombocytopenia produced by dengue hemorrhagic fever, 1

Rigau-Pérez JG1, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV.
Lancet. 1998 Sep 19;352(9132):971-7.
Dengue and dengue haemorrhagic fever.

Simmons CP, Farrar JJ, Nguyen van VC, Wills B. Dengue. N Engl J Med. 2012;366: 1423–32. 10.1056/NEJMra1110265 [PubMed] [CrossRef] [Google Scholar]

Tanner L, Schreiber M, Low JGH, Ong A, Tolfvenstam T, Lai YL, et al. Decision tree algorithms predict the diagnosis and outcome of dengue fever in the early phase of illness. PLoS Negl Trop Dis. 2008;2:e196. doi: 10.1371/journal.pntd.0000196. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Tee HP, How SH, Jamalludin AR, Fariz Safhan MN, Mohd Sapian M, Kuan YC, et al. Risk factors associated with development of dengue haemorrhagic fever or dengue shock syndrome in adults in Hospital Tengku Ampuan Afzan Kuantan. Med J Malaysia. 2009;64:316–320. [PubMed] [Google Scholar]

Udaya Ralapanawa,[1] A. T. M. Alawattegama,1 Malinga Gunrathne,1 Sampath Tennakoon,2 S. A. M. Kularatne,1 and Thilak Jayalath1
Value of peripheral blood count for dengue severity prediction
Published online 2018 Jun 20. doi: 10.1186/s13104-018-3505-4

WHO. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Geneva: WHO; 2011. [Google Scholar]