DESCRIPTION, CLASSIFICATION, AND PREDICTION OF DENGUE ILLNESSES IN A THAI PEDIATRIC COHORT

A Dissertation Presented

By

JAMES A. POTTS, MPH

Submitted to the Faculty of the

University of Massachusetts Graduate School of Biomedical Sciences, Worcester

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

MAY 12TH, 2010

CLINICAL AND POPULATION HEALTH RESEARCH

DESCRIPTION, CLASSIFICATION, AND PREDICTION OF DENGUE ILLNESSES IN A THAI PEDIATRIC COHORT

A Dissertation Presented

By

James Aaron Potts

The signatures of the Dissertation Defense Committee signifies

completion and approval as to style and content of the Dissertation

Alan L. Rothman, MD, Thesis Advisor

Robert Goldberg, Ph.D., Member of Committee

Sharone Green, MD, Member of Committee

Jared Aldstadt, PhD, Member of Committee

The signature of the Chair of the Committee signifies that the written dissertation meets

the requirements of the Dissertation Committee

George Reed, Ph.D., Chair of Committee

The signature of the Dean of the Graduate School of Biomedical Sciences signifies

that the student has met all graduation requirements of the school.

Anthony Carruthers, Ph.D.,

Dean of the Graduate School of Biomedical Sciences

Clinical and Population Health Research

MAY 12TH, 2010

COPYRIGHT NOTICE

ABSTRACT

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are emerging infectious diseases which are endemic in many regions of the globe, many of which are resource-poor areas. DHF and DF impose a severe economic health burden in tropical and subtropical areas. Dengue virus causes an acute febrile illness that can be a self-limited febrile illness, as seen in most cases of DF, or a life-threatening illness with plasma leakage and shock, as seen in cases of DHF. A systematic review of the literature revealed gaps in the knowledge base of clinical laboratory findings of dengue illness with regards to longitudinal dynamics and classification and predictive modeling of disease severity. The objective of this thesis was to investigate the utility of clinical laboratory variables for classification and prediction of disease outcomes.

The data used in this investigation was derived from a prospective study of Thai children presenting to either of two study hospitals within 72 hours of onset of an acute febrile illness. Systematic data collection, including clinical laboratory parameters, and routine clinical management continued each day until 24 hours after the fever had subsided. A final diagnosis of DHF, DF, or other febrile illness (OFI) was assigned by an expert physician after chart review.

The first research objective of this study was to describe the temporal dynamics of clinical laboratory parameters among subjects with DHF, DF, or OFI. Data were analyzed using lowess curves and population-average models.

Quadratic functions of clinical variables over time were established and demonstrated significantly divergent patterns between the various diagnostic groups.

The second research objective was to establish and validate tools for classification of illness severity using easily obtained clinical laboratory measures. Bivariate logistic regression models were established using data from one hospital in an urban area of Thailand as a training data set and validated with a second data set from a hospital in a rural area of Thailand. The validated models maintained a high sensitivity and specificity in distinguishing severe dengue illnesses without using the hallmark indicators of plasma leakage.

The third research objective used classification and regression tree (CART) analysis to established diagnostic decisions trees using data obtained on the day of study enrollment, within the first 3 days of acute illness. Decision trees with high sensitivity were established for severe dengue defined either as: 1) DHF with evidence of shock (dengue shock syndrome, DSS); or 2) DSS or dengue with significant pleural effusion.

This study expands existing knowledge of the potential utility of clinical laboratory variables during different phases of dengue illness. The application of the results of these studies should lead to promising opportunities in the fields of epidemiological research and disease surveillance to reduce the health burden, and improve the clinical management, of dengue illness. Future directions

involve application of these algorithms to different study populations and age groups. Additionally, other analytical techniques, such as those involving CART analysis, can be explored with these data.

Table of Contents

List of Tables

List of Figures

Chapter I Introduction

IA. Overview of dengue illness

"The pains which accompanied this fever were exquisitely severe in the head, back, and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs. In some people, the pains were so acute in their backs and hips that they could not lie in bed…. A few complained of their flesh being sore to the touch, in every part of the body. From these circumstances, the disease was sometimes believed to be a rheumatism. But its more general name among all classes of people was Break-bone fever". (Benjamin Rush's description of dengue epidemic in Philadelphia in 1780 taken from Nelson and Williams[1]) .

IA. 1 Dengue virus

The etiologic agent of "break-bone fever" was found to be dengue virus (DENV). DENV is a flavivirus of the family Flaviviridae. Other flaviviruses in the same genus include Japanese encephalitis, yellow fever, West Nile, and tick-borne encephalitis viruses. Dengue viruses are single-stranded positive-sense RNA viruses [2]. The DENV genome is 11kb in length and encodes three structural and seven nonstructural proteins [2]. DENV has four different serotypes: DENV1, DENV2, DENV3, and DENV4. Infection with one serotype provides lifelong immunity to the infecting serotype only but has been associated with increased risk of severe dengue illness upon secondary infection with a different serotype [3].

It is debatable if one serotype is more infectious or causes a more severe infection compared to another. Some studies have suggested there are differences in the pathophysiology of the different dengue serotypes, but currently no one serotype is considered more dangerous than another [4, 5, 6].

IA. 2 Dengue illness

Dengue viruses are transmitted through the bite of an infected mosquito, usually *Aedes aegypti* or *Aedes albopictus* [7]*.* Once a susceptible host is infected, symptoms of dengue infection may occur and usually appear after an incubation period typically between 4 and 7 days, with a range from 3 to 14 days [8]. Dengue illness can range from an uncomplicated febrile illness, as seen in most dengue fever (DF) cases, to a more severe illness with bleeding tendency, thrombocytopenia, and plasma leakage as seen in dengue hemorrhagic fever (DHF). DF and DHF are emerging infectious diseases that are endemic in tropical and subtropical areas [9, 10, 11].

Patients with confirmed dengue are classified as having DF if fever and any two of the following are present: headache, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia [12]. Patients are classified as having DHF according to World Health Organization (WHO) guidelines based on the presence of all four of the following four signs: fever, thrombocytopenia (platelet count <100,000/µL), bleeding tendency (positive tourniquet test or spontaneous bleeding), and evidence of plasma leakage (evidence of pleural effusion, ascites

or ≥20% hemoconcentration) [11]; however, these findings may not appear until patients are already critically ill.

DHF is categorized by severity into four grades [11]. A diagnosis of DHF grades 3 and 4, termed dengue shock syndrome (DSS), includes all DHF criteria with the addition of circulatory failure. There is not a reliable definition of what constitutes a severe dengue illness and much controversy surrounds the WHO definition of DHF. This classification system is often impractical in the clinical setting, which leads to inconsistency of scientific data, such as under- or over-reporting of severe dengue cases. Studies have shown that the WHO classification of DHF doesn't account for all severe dengue illnesses [13, 14, 15, 16, 17]. Setiati et al found that a modified classification system using only hemoconcentration with either thrombocytopenia or hemorrhagic tendency was in better agreement with the treating physician's diagnosis of DHF than the WHO criteria [16]. Harris et al found that strict adherence to the WHO classification of DHF excluded severe dengue patients that had shock, defined as hypotension for age or narrow pulse pressure with clinical signs of shock, but lacked thrombocytopenia or hemoconcentration, so they set up another category in their study "dengue with signs associated with shock", which included 3% of 1,027 patients [16].

The term 'hemorrhagic' in DHF can lead to the false assumption that suspected dengue cases must have hemorrhage before being classified as

severe; however, 1) dengue can be severe even without significant hemorrhage, 2) hemorrhage is not the sole criterion for DHF, and 3) dengue can be severe without meeting all the criteria for DHF. For example, Murgue et al found that when dengue cases were classified according to severity score (developed after close examination of clinical and laboratory data), the 50 most-severe cases were characterized by hemorrhage, decreased platelet count, and associated hepatic disorders, of which 17 were DF cases as classified by WHO criteria [14]. Specifically, the most severe DF cases were characterized by severe hemorrhage, miscellaneous (cardiac, renal, pulmonary) manifestations, and elevated serum transaminase levels [14]. These studies demonstrate the failure of the WHO classification system to account for disease severity in all dengue cases. Recently, Srikiatkhachorn et al studied a cohort of Thai children (the same cohort of patients presented in this study) and used the need for clinical intervention (fluid intervention or blood transfusion) as an indicator of disease severity; 15% of DF cases met the criteria for a severe dengue illness and 42% of physician-diagnosed DHF cases did not meet the criteria for a severe dengue illness [17]

IA. 3 Dengue vector

The female *Aedes aegypti* mosquito, the most important vector for transmission of DENV, is known to be a nervous feeder and will disrupt a feeding at the slightest movement and return later to continue feeding on the same individual or a different individual [8]. Due to this type of feeding, the female *Aedes*

aegypti can infect numerous individuals in a single blood meal spreading the virus to each person it feeds on [8]. Furthermore, *Aedes aegypti* are indoor mosquitoes, in that they prefer to feed inside a residence, making control efforts more cumbersome due to the inability to effectively reach breeding sites with spraying of insecticides.

Despite major efforts from the Pan American Health Organization and the CDC to prevent and control dengue, such strategies have proven to be poorly implemented and mostly ineffective [18]. Community-wide participation and active involvement in prevention is needed to sustain any mosquito control effort. In a survey of knowledge, attitudes, and practices in rural Thailand, a negative association was observed between respondent's knowledge of mosquito development sites and the number of unprotected containers; however, preventative practices were only carried out after already having mosquito infestation [19].

IA. 4 History of dengue and current magnitude

The first known report of symptoms similar to dengue-like illness was in China between AD 265 to 420; however, the first dengue virus was not isolated until the 1940s during World War II [20]. There has been an increased occurrence of dengue illness in the 20th century which has been attributed to poor vector control, rapid urbanization, and increased globalization [20, 21]. DHF first appeared in Asia in the 1950s and the first major epidemic of DHF/DSS in the Americas

was caused by the introduction of DENV2 shortly after a large DENV1 outbreak in Cuba in 1981[22]. The first cases of DHF in the Americas were due to secondary DENV2 infections that followed a DENV1 outbreak in 1977 [22, 23]. Dengue infection continues into the 21st century; recent estimates are that 3.6 billion people (55% of the global population) are at risk for dengue infection and that 70-500 million dengue virus (DENV) infections occur annually, 2.1 million of which are severe dengue illnesses with ~21,000 deaths [24]. Moreover, industrialized nations, such as the United States and European countries, are not unsusceptible to dengue outbreaks, as it is the most common systemic febrile illness among American and European travelers returning from Southeast Asia, the Caribbean, and South America [25]. Additionally, recent outbreaks of dengue fever have occurred in south Texas and Hawaii [26, 27].

IA. 5 Economic impact of dengue

The economic impact of dengue in developing countries is substantial and has been associated with higher costs and longer disease duration when compared to non-dengue febrile illnesses [28, 29, 30, 31]. As with most severe illnesses, individuals and families are impacted by lost wages from missed work, costs of seeking care, costs of treatment, missed school, and extended effects of recovery. For families who are already in the low socioeconomic bracket, a severe infectious illness could be detrimental both physically and economically. Clark et al estimated that an amount equal to approximately 82% of the average Thai family's monthly household income is lost for each household member with

dengue infection [29]. Although healthcare treatment for children in Thailand is paid for by the government under the "30-baht to cure every disease project" (Dr. Pra-on Supradish, personal communication), Clark et al also found that most families first sought care at a private clinic that was not government funded [29]. Transportation costs for seeking care have also been shown to substantially impact the economic burden of disease in Thailand [28]. Meltzer et al found that the average disability-adjusted-life years lost due to dengue illness in Puerto Rico is 658 per year per million population [32]. These studies may under-estimate the actual impact of dengue given the difficulties in diagnosis of dengue and under-reporting of cases (See section IB).

IA. 6 Treatment of dengue illness

Currently, there is no vaccine to prevent dengue infection. For a dengue vaccine to be effective, it must provide long-lasting protection against all four dengue serotypes. The standard treatment for patients with suspected dengue is supportive care consisting of oral rehydration therapy, bed rest, paracetamol (to reduce fever), and avoidance of aspirin [11]. In practice, patients suspected to have DF or DHF are usually treated the same up to defervescence (initial febrile phase is subsiding); they are sometimes hospitalized and their condition is closely monitored with routine laboratory tests and maintenance of fluid intake. Around the time of defervescence, usually within 24 hours after defervescence, patients with DHF will develop severe symptoms and may become severely ill often with decreases in platelet count, hemorrhage, and signs of plasma leakage.

However, many DF cases are considered mild and may not require hospitalization but are often hospitalized until 24 hours after defervescence to ensure that the characteristics of DHF do not manifest. Currently, there are no early diagnostic/prognostic tools available to distinguish dengue from OFI or DF from DHF or severe dengue from non-severe dengue. If such tools were developed, they could potentially impact clinical practice in many ways, including: 1) decreasing the number of un-necessary hospitalizations, 2) improving utilization of limited hospital resources to treat more severely ill patients, 3) improving outcomes of severely ill patients by getting them the care they need earlier, and 4) improving the capability of physicians in developing or rural areas to make a more accurate early diagnosis with limited resources.

IB. Importance of early dengue diagnosis

Most developing countries have epidemics of febrile illnesses that can be confused with DF, including measles, typhoid fever, and leptospirosis [33, 34, 35, 36, 37]. At initial presentation, DF and other febrile illnesses may have similar clinical features, including fever, headache, myalgia, and rash. The distinguishing clinical features of DHF, such as bleeding and signs of plasma leakage, are seen around the time of defervescence, typically the third or fourth day after the onset of fever. Suspected dengue patients, who include patients with other febrile illnesses, are sometimes hospitalized unnecessarily for observation until at least 24 hours after defervescence to ensure that the characteristics of DHF do not occur.

Hospitalization of patients with suspected dengue has been shown to be a significant financial burden in developing countries [29, 30]. Ideally, only severe DF and DHF cases should be hospitalized.

Confirming a dengue diagnosis by serologic tests may take days due to the time required for development of an antibody response, and plasma leakage may be difficult to detect and measure [38]. Polymerase chain reaction (PCR) testing for DENV RNA is rapid but is presently only available as a research tool. Furthermore, expensive laboratory tests may not be available in many developing countries and remote areas. Areas that do not have access to sophisticated laboratory tools need early clinical and/or simple laboratory indicators that can provide an accurate and reliable diagnosis of dengue prior to the burden of an unnecessary hospitalization.

The most common clinical measurements of plasma leakage include a chest x-ray or ultrasound (to measure the amount of pleural effusion) or serial hematocrits for the detection of hemoconcentration. These measurements add additional economic burden and may be unavailable in resource-poor areas. Furthermore, detection of hemoconcentration requires baseline and convalescent blood samples which are often not available. Classification tools that do not require these expensive and burdensome clinical measures are needed by researchers and epidemiologists to maintain active and reliable dengue disease surveillance in endemic, resource-poor areas.

IC. Distinguishing dengue from other febrile illnesses

Differences in clinical and laboratory features between dengue and other febrile illnesses have been reported; however, published studies differ in terms of duration of symptoms, age of patients, and quality of the study, which could impact the clinical applicability of their findings [39].

IC. 1. Systematic review

I conducted a systematic review to evaluate studies that analyzed differences in clinical and laboratory variables between patients with dengue and patients with OFI to determine which factors, or combination of factors, best distinguished between the two.

IC. 1.1 Search strategy

An electronic search of Pubmed and Global Health databases using combinations of Medical Subject Headings (MeSH) and text words was conducted. Search terms were grouped as follows: (indicators OR "Dengue/diagnosis" OR clinical aspects OR clinical features OR clinical manifestations OR clinical characteristics OR clinical presentations OR physical signs OR physical symptoms) AND (dengue OR dengue fever OR dengue hemorrhagic fever OR dengue haemorrhagic fever). Articles were obtained electronically or in paper form.

IC. 1.2 Selection criteria

Studies were included if they met the following criteria: published between 1990 and October 2007, in English, and included comparisons between patients with DF and/or DHF and OFI patients in the abstract. The exclusion of studies prior to 1990 was to improve the reliability and global distribution of clinical experience with and the laboratory diagnosis of dengue. Studies were excluded if they used “travel” or “travelers” as MeSH terms in order to assess only populations in dengue-endemic areas. An assessment of titles and abstracts was done to exclude non-human studies, studies that assessed only molecular detection methods, and studies that did not compare patients with dengue and those with OFI.

IC. 1.3 Study assessment and data extraction

The quality of selected studies was assessed using a modified version of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [40]. The STROBE is a quality assessment checklist for observational studies that consists of 22 items. The STROBE was modified by adding questions about the serologic method used to confirm dengue diagnosis, use of viral isolation, and whether the study was based on a single dengue outbreak or transmission season. Use of viral isolation increased the score whereas single outbreak studies received no additional points. The quality score was the number of items from the STROBE checklist addressed as a percentage of the total number of items applicable (minimum of 23 and maximum of 25).

Studies with a quality assessment below 50% were excluded. Each selected article was characterized for study design, study location, type of patients (outpatients or inpatients), age of patients, type of dengue illness (primary or secondary; DF or DHF), method to confirm dengue (viral isolation, ELISA), duration of illness, and clinical and laboratory features.

IC. 1.4 Results

IC. 1.4.1 Search

The initial search retrieved 1575 articles/abstracts (Figure 1-1). We excluded 182 studies because they included "travel" or "travelers", 293 published prior to 1990, 112 non-English studies, 147 duplicates (in both databases), and 790 based on title/abstract assessment. A total of 51 articles were selected for data abstraction. Among these, two were unavailable for review [41, 42]. Forty-nine articles were reviewed and an additional 34 (Suppl. 1.1) were excluded for the following reasons: 18 lacked statistical comparison between dengue and OFI, 9 lacked an OFI comparison group, 4 had a quality assessment <50%, one had a limited number of dengue cases (nine), one compared environmental factors only, and one was a short report lacking necessary abstraction data (Table 1-1). A total of 15 published articles were included in this review.

IC. 1.4.2 Characteristics of included studies

The characteristics of the included studies are listed in Table 1-2. There was substantial heterogeneity in study design and inclusion criteria. Among these, 10 were prospective cohort studies and five were case-control studies; 9/15 (60%) were single outbreak studies or concluded within one year (study was concluded within one rainy season). The majority of studies were carried out in dengue-endemic regions of Southeast Asia/Pacific except for three studies from the Americas (Brazil, Nicaragua, and Puerto Rico) and one study from Australia. The included studies had quality assessment ratings ranging from 63% to 88%. One study assessed outpatients only and one study failed to give information on the type of patients included. Four studies assessed adults only (defined as >14 years old), four studies assessed children only (defined as >11 months and <14 years old), and seven studies assessed all age groups (including infants).

The sample sizes of laboratory-confirmed dengue patients ranged from 13 to 2108 and the sample sizes of OFI patients ranged from 37 to 1065. All studies used hemagglutination inhibition and/or enzyme-linked immunosorbent antibody (ELISA) assays for serological confirmation of DENV infection; seven studies also used viral isolation for laboratory confirmation of infection. Six studies relied on a single blood sample for serology. For studies with convalescent serum samples, the shortest time between acute and convalescent samples was three days; however, this study obtained an additional sample at 3-4 weeks [43]. Five studies used duration of fever prior to enrollment as part of their selection criteria

[15, 43, 44, 45, 46], four studies mentioned duration of fever prior to enrollment but did not use it as an enrollment criteria [37, 47, 48, 49] and six studies failed to mention the duration of fever prior to enrollment [35, 50, 51, 52, 53, 54]. The mean duration of illness during the study period, noted in nine of the 15 studies, ranged from 3.3 to 10.5 days. Only two studies analyzed clinical and laboratory symptoms according to day of illness [44, 48]. The percentage of DHF cases was determined in eight studies and ranged from 0% [54] to 47% [44]. The percentage of secondary infections was determined in seven studies and ranged from 43% [43] to 93% [44]. No study statistically compared clinical or laboratory variables of patients with DHF and patients with OFI; however, Kalayanarooj et al listed the frequencies of symptoms separately for DHF and OFI [44]. Only two studies separated primary and secondary infections in the analysis; neither found any significant differences in signs or symptoms between patients with primary and secondary infections [43, 45]. Two studies used a serologically identified comparison group- either SARS or leptospirosis [37, 50]; three additional studies provided information about the specific diagnoses in the OFI group [15, 35, 47]. Seven of the 15 studies used data collected at presentation to make comparisons [15, 37, 43, 44, 45, 47, 50]; the other eight studies did not clearly define which data were used for statistical comparisons.

Table 1-2 indicates the direction of association (increasing ↑ or decreasing ↓ likelihood/frequency of dengue compared to OFI) for clinical and laboratory features that were reported in at least two studies where one (or more) found a significant difference between dengue and OFI patients. Bruce et al (2005),

Deparis et al (1998), Hammond et al (2005), Karande et al (2005), Low et al (2006), Nunes-Araujo et al (2003), and Phuong et al (2006) showed significant increases/decreases in mean likelihood of dengue versus OFI as relative risks or odds ratios. All other studies reported independent associations as differences in proportions (for categorical variables) or means (for continuous variables) between patients with dengue and OFI. For clinical and laboratory features reported in at least two prospective and two retrospective studies, the directions of association were similar except for gender and headache/retro-orbital pain. The consistency score is an evaluation of the direction of association for each variable across all the studies that measured that variable, weighted by the quality assessment percentage of each study.

IC. 1.4.2.1 Demographic indicators

No consistent associations were observed between age and occurrence of dengue across all studies or within age-grouped studies. Two retrospective studies showed a significantly higher frequency of dengue among males [37, 50].

IC. 1.4.2.2 Clinical indicators

Studies that assessed adults only reported consistently higher frequencies of rash and hemorrhagic signs in patients with dengue when compared to patients with OFI [37, 47, 53]; however, the frequency of hemorrhagic signs showed no differences between dengue and OFI in the four studies that assessed children only [45, 48, 52, 54]. Hammond et al reported hemorrhagic signs in specific

categories; the frequencies of melena and hematemesis were higher in children with dengue but not in adults [52]. In four of seven studies assessing all age groups, the frequency of rash was also higher in patients with dengue [48, 52, 54]; however, two studies assessing children only found no significant association with rash [35, 49]. Three studies that assessed children only found a higher frequency of petechiae among patients with dengue and one study that measured petechiae in adults only also found a positive association among those with dengue compared to OFI [15, 44, 47, 49]. A greater percentage of patients with dengue reported lethargy/prostration and arthralgia/joint pain in two studies assessing adults only [53]; however, lethargy/prostration was not reported in studies assessing children only and the patterns of arthralgia/joint pain were inconsistent in all other studies (children or all ages). In two studies that only included children, the frequency of anorexia was higher among patients with dengue [15, 44]. Taste alteration and skin sensitivity were more frequently reported in patients with dengue in two studies assessing adults only. Nonspecific symptoms, such as headache/retro-orbital pain, abdominal pain, diarrhea, vomiting, itching/pruritis, and nausea showed inconsistent or non-significant associations when comparing patients with dengue and patients with OFI. Duration of fever prior to or during the study period showed inconsistent or non-significant associations with the occurrence of dengue versus OFI.

IC. 1.4.2.3 Laboratory indicators

Neutrophil and lymphocyte counts were significantly lower in patients with dengue in comparison to patients with OFI among studies that measured these variables [15, 35, 37, 43, 44, 47, 48, 52]. All studies measuring WBC found a lower WBC count among patients with dengue, except for one retrospective study by Sawasdivorn et al [49], which showed no association. Nine of 11 studies found lower platelet counts among patients with dengue compared to OFI patients. Two of three studies that measured prothrombin time found significantly lower values among patients with dengue [37, 47]. Bruce et al and Chadwick et al found lower creatinine levels and a lower percentage of jaundice among patients with dengue [47, 50]. Higher levels of hepatic transaminases (AST/ALT) were found in patients with dengue in three of four studies [37, 44, 47]. Increased hematocrit and hemoglobin levels were observed among patients with dengue in two adult-only studies [37, 47]; however, hematocrit showed inconsistent associations in three children-only studies. Other laboratory measures, such as total protein, APTT, and urea, also showed inconsistent patterns with the occurrence of dengue [37, 47]. Kalayanarooj et al and Hammond et al were the only studies to measure pleural effusion or ascites [44, 52]. Kalayanarooj et al reported a higher frequency of pleural effusion in patients with DHF compared to DF or OFI [44]. Hammond et al found an increased odds of having pleural effusion and ascites among children and adults with dengue [52].

IC. 1.4.2.4 Other indicators

Table 1-3 lists additional symptoms that showed associations between dengue and OFI but were reported in only one of the 15 studies reviewed. Table 1-4 lists symptoms that were measured in only one study and showed no association with dengue or OFI. Other common laboratory tests- sodium, potassium, glucose, alkaline phosphotase, and lactate dehydrogenase- measured in Chadwick et al and Wilder-Smith et al showed no differences between dengue and OFI [37, 47].

IC. 1.4.2.5 Combined indicators

Seven studies [15, 35, 37, 47, 48, 49, 53] carried out multivariable regression analysis in an attempt to distinguish patients with dengue from those with OFI (Tables 1-5 and 1-6). Among these seven studies, all studies that measured WBC included this variable in their final model and showed a reduced WBC count in patients with dengue compared to patients with OFI. Three of these seven studies included some measure of liver function in the final model. Wilder-Smith et al found that increased AST resulted in an increased odds of dengue. Phuong et al found that hepatomegaly resulted in an increased adjusted odds of dengue. On the other hand, Chadwick et al found that lower bilirubin values resulted in increased adjusted odds of dengue [47]. Chadwick et al was the only one of these studies that reported platelet count and did not include this variable in the final regression model [47]. Three studies included signs of bleeding such as petechiae, hematocrit, and positive tourniquet test in their final model and

showed that positive signs of bleeding increased the odds of having dengue [15, 49, 53]. Three studies also showed a higher frequency of rash among dengue patients in their final model [47, 48, 53]. The final model in Karande et al had a negative predictive value (NPV) of 45% and was the only study to report a NPV along with the final model's positive predictive value (PPV) [35].

IC. 1.5 Summary of findings

This review of the literature suggests that several clinical and laboratory measures distinguish patients with dengue from those with OFI. Low platelet count/thrombocytopenia and decreases in WBC and neutrophils were independently associated with the presence of dengue, when compared to patients with OFI in both adults and children. These variables, as well as signs of rash and liver damage, were also used in multivariable models to distinguish patients with dengue from those with OFI.

Low platelet count is used as a criterion for the diagnosis of DHF [11]. The cause(s) of thrombocytopenia in dengue are unknown; but decreased production of platelets in DF and increased destruction of platelets in DHF have been described [55]. Kalayanarooj et al attributed the reduction in WBC to bone marrow suppression by the dengue virus; however, it has been suggested that these laboratory measures are not dengue-specific in the early stages of the disease [44, 48, 56].

Alterations in the microvascular endothelium in patients with dengue are thought to lead to a higher likelihood of hemorrhage [55, 57]. In this review, an increased frequency of hemorrhage was observed in adults with dengue but was not associated with the occurrence of dengue in studies that only included children; however, Hammond et al demonstrated that some types of hemorrhage (e.g., hematemesis and melena) were associated with dengue in children, suggesting that the types of hemorrhagic manifestations seen in patients with dengue may depend on the age of the patient [52].

It is unlikely that any one indicator will be useful in clinical practice because these signs and symptoms are present in other diseases, such as viral hepatitis and leptospirosis, which are also endemic in areas with a high prevalence of dengue. However, a combination of rash and indicators of liver damage in combination with other variables, such as age, myalgia, WBC count, and platelet counts, may help to establish a diagnostic algorithm that can be used to distinguish dengue from OFI patients. Several studies used multivariable regression models to discriminate patients with dengue from patients with OFI; however, most published models had lingering statistical questions or did not describe some statistical issues such as over-fitting, co-linearity, and how variables were categorized. Wilder-Smith et al presented a model with very large odds ratios; however, the confidence intervals for their model were also extremely large and questions of over-fitting and co-linearity were not discussed [37]. Deparis et al presented a model with an unusually small odds ratio for a

categorical variable (low platelet count), which may not be applicable in a clinical setting [48]. None of the regression models were validated using a training and testing dataset approach. Furthermore, the generalizability of these models is questionable since most were derived from single outbreak studies. For example, Karande et al was a single outbreak study and presented a model with 100% PPV but they only had 13 patients with dengue in the model [35].

Any algorithm to identify patients at risk of dengue would need to be applied early after the onset of illness in order to be useful in preventing unneeded hospitalizations. This review highlights a weakness in the literature as few studies indicated which day of illness clinical and laboratory measures were assessed. Only Kalayanarooj et al and Deparis et al separately analyzed clinical and laboratory measures according to day of illness [44, 48]. Kalayanarooj et al showed that positive and negative predictive values for individual variables differed depending on the stage of illness [44]. Deparis et al showed that the frequency of clinical and laboratory symptoms varied according to day of illness [48].

Five of the included studies were case-control studies that relied on the review of medical records or patient recall, which could potentially bias the findings of these studies. Furthermore, two of the case-control studies did not use a standardized data collection form. Six studies relied on serologic testing of a single blood sample, which could increase the risk of misclassification of

patients with dengue. Only two studies serologically confirmed all patients in the non-dengue comparison group and differences found between patients with dengue and patients with OFI depended on the specific comparison febrile illness. Bruce et al used a leptospirosis comparison group and was the only study that showed no differences in platelet count or AST/ALT [50]. Illnesses with similar characteristics, such as dengue and leptospirosis, will clearly be more difficult to discriminate on the basis of any clinical algorithm.

Duration of illness prior to study enrollment did not distinguish patients with dengue from those with OFI in four out of five studies. This finding indicates that patients with dengue do not have an initial febrile period that is different in length from patients with OFI. Duration of illness prior to presentation may be more applicable in distinguishing patients with DHF from patients with DF. On average, patients with DHF have a more severe form of illness and may require hospitalization for a more extended period of time after defervescence in comparison to patients with DF. However, no study in this review statistically compared clinical signs and symptoms in patients with DHF to patients with DF or OFI. We are, therefore, unable to make any conclusions from this review regarding which readily available signs and symptoms, if any, are able to prospectively distinguish patients with DHF from patients with DF or OFI.

This review has several limitations. There was no assessment of inter-rater or intra-rater reliability of quality assessment ratings and the STROBE is

mainly a score of reporting and may reflect the ability to extract information rather than quality of the study itself. There is a lack of established quality assessment rating scales for evaluating observational studies. The STROBE contains items that give merit to a study for addressing its limitations, which may explain why retrospective case-control studies had the highest quality assessment rating. Not all studies had robust statistical methods due to poor study quality and retrospective design. Some studies failed to include duration of fever or duration of illness in their analysis which limits the conclusions that can be made about the impact of this variable as well as other variables that may be time-dependent. Finally, many studies did not include what day of illness the clinical and laboratory data presented were measured, which makes it impossible to determine whether this data can be used to distinguish patients with dengue from those with OFI early in the course of illness. Additional prospective studies are needed to establish a diagnostic algorithm that can be validated and generalized to distinguish dengue from OFI and DF from DHF in the early stages of illness. Furthermore, longitudinal studies that routinely document clinical and laboratory signs and symptoms throughout each patients' course of illness would provide much needed data to develop a predictive model that can distinguish patients with dengue who will require hospitalization from patients with OFI. An easily applicable clinical algorithm could have a favorable impact on the healthcare economies of developing countries that have endemic levels of dengue.

ID. Correlates of clinical laboratory measures and physician's diagnosis of DHF or DF

Using the data source from Thailand covering study years 1994-97, we conducted a preliminary analysis to assess the feasibility of the application of our data to establish disease classification models. Using only data from these study years, we used readily available clinical and laboratory variables from 318 subjects with confirmed dengue to determine which variable(s) distinguished an expert physician's final diagnosis of DHF or DF. Data included in the analysis consisted of each patient's maximum aspartate aminotransferase (AST), alanine aminotransferase (ALT), percent change in hemoconcentration, and receiving any intravenous fluid received throughout their hospitalization, and their minimum albumin and platelet count. There were 301 patients with sufficient data to be used in the modeling. Kappa statistics were used to assess the percent agreement between the expert physician's final diagnosis of DHF and WHO criteria for the diagnosis of DHF, using two different indicators of plasma leakage: percent change in hematocrit and pleural effusion index (PEI). Kappa statistics showed 86% agreement between physician's final diagnosis of DHF and WHO criteria when PEI was used as the indicator of plasma leakage (K=.71); 75% agreement was observed between physician's final diagnosis of DHF and WHO criteria when percent change in hematocrit was used (K=.47). Univariable and stepwise multivariable logistic regression analysis was done on clinical and laboratory variables thought to correlate with a final diagnosis of DF or DHF.

Two different final models adjusting for age and gender were produced using the different indicators of plasma leakage. Area under the curve (AUC), sensitivity and specificity were generated for the best models using each of the indicators. The multivariable model using PEI as the indicator for plasma leakage that included other indicators such as maximum AST, gender, and minimum platelet count had an AUC of 98%, sensitivity of 91% and specificity of 98%. The multivariable model using percent change in hematocrit as the indicator for plasma leakage had an AUC of 86%, sensitivity of 75% and specificity of 83%, and included other indicators of minimum serum albumin levels, gender, and minimum platelet count. Based on these results, plasma leakage as measured by PEI is the best indicator of a diagnosis of DHF and best mimics real clinical experience. However, PEI may be difficult to measure and resource-poor areas may not have access to equipment to measure it. Percent change in hematocrit also mimicked real clinical experience but to a lesser degree and also required baseline and convalescent hematocrits that are not typically available outside of a research setting.

These results suggest that physicians did not rely on a single measure to distinguish DHF from DF and did not strictly adhere to WHO criteria to make a diagnosis of DHF. However, strong correlations were established with measures of plasma leakage (PEI or hemoconcentration), which may not be measured in resource-poor areas. The multivariable modeling demonstrates that a combination of variables, both WHO criteria and other variables, such as liver

enzymes, albumin, and need for intravenous fluid, may better mimic the clinical experience and better distinguish DHF from DF. Furthermore, this preliminary study suggests that a classification model using a combination of clinical laboratory measurements, but which lacks indicators of plasma leakage, may also have a strong correlation with a physician's diagnosis and may be useful in classifying dengue disease when indicators of plasma leakage are not available.

Additionally, a classification scheme for individuals diagnosed with dengue illness who were not in need of hospitalization was done using the same patients from the Thai 1994-1997 data. Patients who had DHF grade 3 and/or received IVF, and/or had evidence of significant pleural effusion measured by chest x-ray (PEI >5), were classified as patients in need of hospitalization. Using data from 246 patients with dengue who had information available on all classification variables, 142 patients with DF or DHF grade 1 or 2 did not require hospitalization. Of patients with DHF grade 1 or grade 2, one-third did not require hospitalization. In contrast, 16.2% of patients with DF required hospitalization. In conclusion, a diagnosis of DF is not always indicative of mild illness and a diagnosis of DHF isn't always indicative of a severe illness. By using a different categorization that can distinguish between severe and non-severe dengue, physicians will be able to better utilize limited resources by reserving treatment for more severe illnesses.

IE. Summary and significance

Dengue is a significant health burden and consists of a wide range of disease severity from a self-limited febrile illness to severe hemorrhagic fever with shock. Controversy surrounds the practicality of the WHO guidelines regarding DHF classification versus classification of severe dengue disease. After a systematic review of the literature, research gaps exist with regards to prospective longitudinal studies that can describe dengue illnesses in terms of routine clinical laboratory measures and that could potentially establish predictive algorithms that can benefit dengue endemic regions. Preliminary research suggests that more useful classification tools can be developed.

IF. Research objectives

The research objectives of these analyses were to describe the dynamics of dengue diseases over the disease course, establish validated correlation models that can be used to classify dengue illnesses without the need for indicators of plasma leakage (chest x-ray, ultrasound, and hemoconcentration) and investigate the potential utility of clinical laboratory variables as early indicators of dengue illness among children in a hospital setting. Our approach was to apply robust statistical techniques to a well defined clinical dataset. Our dataset was designed to capture early symptomatic dengue illnesses and to systematically record routine clinical data throughout each subject’s illness episode.

Research objective 1

To describe the temporal dynamics of clinical laboratory variables over the course of illness among hospitalized children with DHF, DF, and OFI to determine whether the temporal patterns of these variables differ among these diagnostic groups. Additionally, we identified trends in clinical laboratory variables that could be used to distinguish patients with impending plasma leakage. The analytical techniques used for this analysis were lowess curves and population average models.

Research objective 2

To establish and validate binary logistic regression models using maximum and minimum clinical laboratory variables throughout each patient's hospitalization, without relying on direct measures of plasma leakage (chest x-ray, ultrasound, and hemoconcentration), that are able to distinguish between: DHF vs. DF, DHF vs. DF + OFI, severe dengue vs. non-severe dengue+OFI, and subjects with dengue vs. subjects with OFI.

Research objective 3

To establish and validate predictive diagnostic trees, using clinical laboratory data obtained on the day of presentation, that can distinguish subjects with impending severe dengue from subjects with non-severe dengue and OFI. The analytical technique used for this analysis was classification and regression tree (CART) analysis.

Figure 1-1 Flow-chart of review process

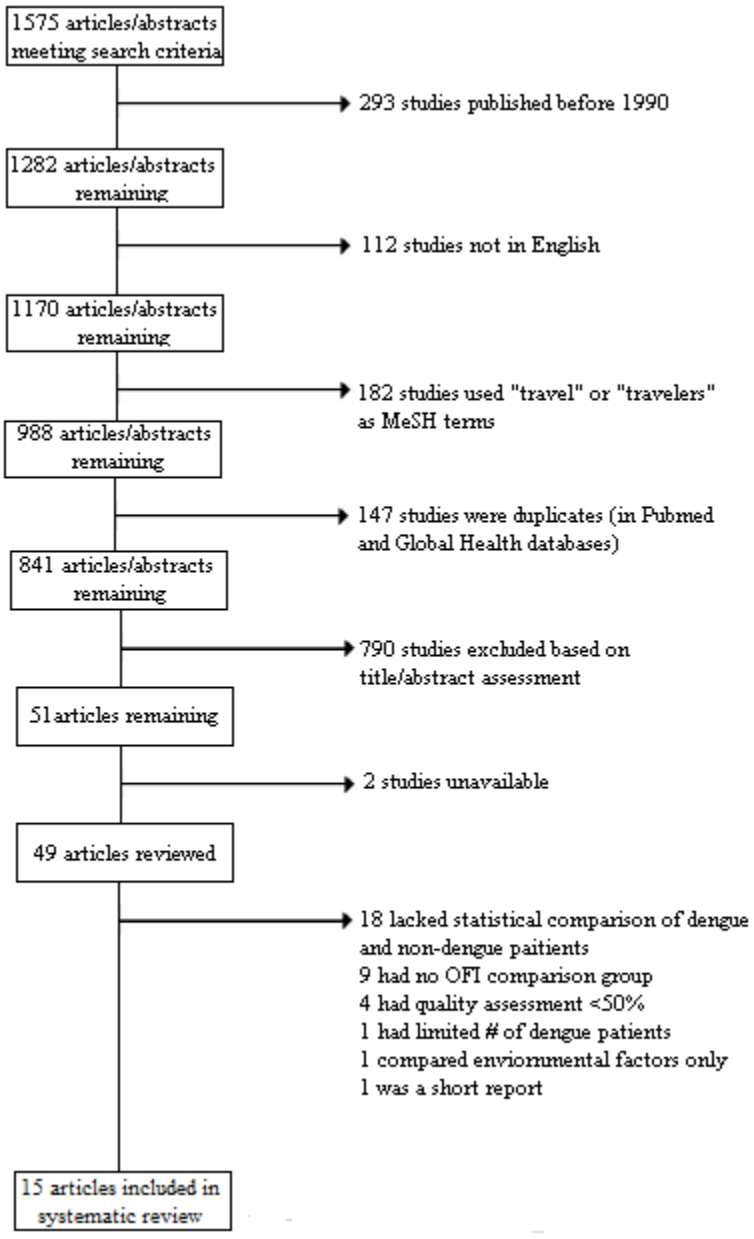


Table 1-1 Characteristics of included studies

First Author	Study design	Study Year(s)	Location	Patients	Age	Sample Size		Viral isolation	Duration of fever prior to enrollment (days)	Duration of illness during study period (days)	Modified STROBE QA (%)
						Dengue	OFI				
Low	Prospective cohort	2005	Singapore	Mixed	Adults	133	321	Yes	<3	Mean 10.5	84%
Phuong (2004)	Prospective cohort	1996-1998	Vietnam	Inpatients	Children	712	85	No	<7	N/A	84%
Chadwick	Prospective cohort	1998-2000	Singapore	Inpatients	Adults	148	233	No	Mean 5.2	N/A	80%
Kalayanarooj	Prospective cohort	1994	Thailand	Inpatients	Children	60	112	Yes	<3	Mean 4.0	80%
Phuong (2006)	Prospective cohort	2001-2002	Vietnam	Mixed	All ages	234	463	No	<14	Median 5	79%
Deparis	Prospective cohort	1996-1997	French Polynesia	Outpatients	All ages	196	102	Yes	Median 2.5	N/A	76%
Hammond	Prospective cohort	1999-2001	Nicaragua	Inpatients	All ages	2108	1065	Yes	N/A	Infants 6.4 Children 6.0 Adults 5.2	76%
Karande	Prospective cohort	2002	India	Mixed	Children	13	37	No	N/A	Mean 5.5	68%
Buchy	Prospective cohort	2001-2002	Vietnam	Inpatients	All ages	108	17	Yes	N/A	Median 3.9	64%
Suwandono	Prospective cohort	2004	Indonesia	Inpatients	All ages	180	92	Yes	<7	N/A	63%

Wilder-Smith	Retrospective case-control	2003; 1997-2000	Singapore	Inpatients	Adults	147	55	No	Median 4	Median 4	88%
Bruce	Retrospective case-control	1996-1997	Puerto Rico	Mixed	All ages	84	42	No	N/A	Median 10	83%
Nunes-Araujo	Retrospective case-control	1993-1998	Brazil	N/A	All ages	495	650	No	N/A	N/A	78%
Sawasdivorn	Retrospective case-control	1998-1999	Thailand	Inpatients	Children	45	38	Yes	Mean 3.71	Mean 3.37	76%
McBride	Retrospective case-control	1995	Australia	Mixed	Adults	399	600	No	N/A	N/A	67%

List of included studies indicating: first author, study design, year the study was performed, location of the study (country), type of patients ("inpatients", "outpatients", or "mixed" which includes both inpatients and outpatients), sample size ("dengue" is the number of confirmed dengue patients, and "OFI" is the number of other febrile illness patients), viral isolation, duration of fever prior to enrollment (among the dengue patients enrolled), duration of fever during study period, and modified STROBE quality assessment rating (shown as a percentage)

Table 1-2 Symptoms and laboratory measures assessed in at least two studies where one study showed an association with dengue

Symptoms	Consistency Score	All ages						
		Phuong[A] (2006)	Deparis[A]	Hammond[A]	Nunes-Araujo[A]	Buchy	Bruce[A]	Suwandono
DEMOGRAPHICS								
Age	↑ 25%	↓	0	0	↑	0	-	-
Males	Males 9%	0	0	0	0	0	↑	-
CLINICAL INDICATORS								
Taste alteration	↑ 100%	-	-	-	-	-	-	-
Skin sensitivity	↑ 100%	-	-	-	-	-	-	-
Petechiae (scattered, spontaneous bleeding)/positive tourniquet test	↑ 75%	0	-	-	↑	0		↑
Liver size >1cm/hepatomegaly	↑ 74%	0	-	↑	-	-		-
Anorexia	↑ 74%	0	-	-	-	-		-
Lethary/Prostration	↑ 74%	-	↑	-	0	-		-
Rash (including macular rash)	↑ 65%	0	↑	↑	↑	0	↑	-
Arthralgia/joint pain	↑ 50%	↑	0	-	0	-		-
***Hemorrhagic signs	↑ 49%	0	↑	↑	0	0		-
Itching/Pruritis	↑ 46%	-	-	-	-	-		-
Cough/rhinitis/breathlessness/ coryza/runny nose	↓ 44%	↓	0	-	-	0		-
Vomiting	↑ 42%	0	0	-	-	0		0
Abdominal pain/abdominal tenderness/stomach ache	↑ 32%	↑	0	↑	-	0		0
Nausea	↑ 31%	0	-	-	0	0		0
Myalgia/muscle pain/backache	↑ 30%	↑	-	-	0	0	0	0
Sore throat/red pharynx	↑ 21%	0	-	-	-	-		0
Duration of fever[B]	↑ 20%	0	-	-	-	-		-
Headache/retro-orbital pain	↑ 16%	↓	0	-	↑	0	0	0
Diarrhea	↓ 16%	↓	0	-	-	-		-
Splenomegaly	0	0	0	-	-	-		-
LABORATORY INDICATORS								
Neutrophils/neutropenia	↓ 100%	-	↓	-	-	-		-
Hemoglobin	↑ 100%	-	-	-	-	-		-
Lymphocytes/lymphopenia	↓ 100%	-	↓	-	-	-		-
WBC/Leukocytosis/Leukopenia	↓ 89%	-	↓	↓	-	-	↓	↓
Platelets/Thrombocytopenia	↓ 83%	-	↓	↓	-	0	0	↓
AST/ALT	↑ 75%	-	-	-	-	-	0	-
PT	↓ 67%	-	-	-	-	-	0	-
Creatinine	↓ 65%	-	-	-	-	-	↓	-
Hematocrit	↑ 55%	-	0	-	-	0		↑
Total Protein	↓ 52%	-	-	-	-	-		-
Albumin	↑ 50%	-	-	-	-	-		-
Jaundice/Icterus/Bilirubin§	↓ 43%	0	-	-	-	-	↓	-
APTT	↑ 32%	-	-	-	-	-	0	-
Urea	0	-	-	-	-	-	0	-

Table 1-2 (*Continued*)

Symptoms	Children				Adults			
	Phuong (2004)	*Kalayanarooj	Karande[A]	Sawasdivorn	Chadwick	Wilder-Smith	McBride	Low[A]
DEMOGRAPHICS								
Age	↑	↑	0	0	0	0	-	↑
Males	0	0	0	0	0	↑	-	0
CLINICAL INDICATORS								
Taste alteration	-	-	-	-	-	-	↑	↑
Skin sensitivity	-	-	-	-	-	-	↑	↑
Petechiae (scattered, spontaneous bleeding)/positive tourniquet test	↑	↑	-	↑	↑	-	-	
Liver size >1cm/hepatomegaly	↑	-	-	-	↑	-	-	
Anorexia	↑	↑	-	-	-	-	-	↑
Lethary/Prostration	-	-	-	-	-	-	↑	↑
Rash (including macular rash)	-	-	0	0	↑	-	↑	↑
Arthralgia/joint pain	0	-	↑	0	-	-	↑	↑
***Hemorrhagic signs	0	0	0	0	↑	-	↑	↑
Itching/Pruritis	-	-	-	-	0	-	↑	
Cough/rhinitis/breathlessness/ coryza/runny nose	↓	-	0	-	0	-	↓	
Vomiting	↑	↑	0	-	0	-	↑	↑
Abdominal pain/abdominal tenderness/stomach ache	↑	0	0	-	0	-	0	-
Nausea	-	↑	-	-	0	-	-	↑
Myalgia/muscle pain/backache	0	-	-	0	↑	-	↑	0
Sore throat/red pharynx	↑	-	-	-	0	-	0	
Duration of fever[B]	↑	0	0	0	-	-	-	↑
Headache/retro-orbital pain	0	0	0	-	0	-	↑	↑
Diarrhea	0	-	0	-	0	-	0	0
Splenomegaly	↓	-	-	-	↑	-	-	
LABORATORY INDICATORS								
Neutrophils/neutropenia	-	↓	-	-	↓	↓	-	↓
Hemoglobin	-	-	-	-	↑	↑	-	
Lymphocytes/lymphopenia	-	-	-	-	↓	↓	-	↓
WBC/Leukocytosis/Leukopenia	-	↓	-	0	↓	↓	-	↓
Platelets/Thrombocytopenia	↓	↓	↓	-	↓	↓	-	↓
AST/ALT	-	↑	-	-	↑	↑	-	
PT	-	-	-	-	↓	↓	-	
Creatinine	-	-	-	-	↓	0	-	
Hematocrit	↑	0	↑	-	↑	↑	-	0
Total Protein	-	-	-	-	0	↓	-	
Albumin	-	0	-	-	↑	-	-	
Jaundice/Icterus/Bilirubin§	-	-	0	-	↓	0	-	
APTT	-	-	-	-	↑	0	-	
Urea	-	-	-	-	↓	↑	-	

Footnotes to Table 1-2

*Only analysis performed on day of presentation is shown

**Consistency score= |[∑(quality assessment %)(+1/-1/0)]/[∑(quality assessment % of studies measuring this variable)]|,

for example, anorexia: |[(.84)(0) + (.79)(1) + (.80)(1) + (.84)(1)]/[(.84) + (.79) + (.80) + (.84)]|= ↑74%

***Hemorrhagic signs: other than petechiae (bleeding gums, gingival bleeding, mucosal bleeding, vaginal bleeding, hematemesis, reported bleeding, bleeding manifestations, melena)

§=bilirubin is a laboratory measure that correlates with clinical measures of jaundice/icterus

↑= indicates positive association with dengue positive patients compared to patients with OFI

↓= indicates negative association with dengue positive patients compared to patients with OFI

0= indicates no significant association

-= not measured

[A=]Reported associations as relative risks or odds ratios

[B=]prior to enrollment except for Karande and Low which is during illness

Table 1-3 Signs, symptoms, and additional indicators reported in only one study but which showed a significant association between dengue and OFI

Study	Symptoms	Direction of association
McBride (1998)	Days of work lost Visited the doctor Hospitalized	↑
Chadwick (2006)	Pulse Temperature	↓
Chadwick (2006)	Skin flushing Islands of sparing	↑
Hammond (2005)	Chills	↑
Karande (2005)	Edema	↓
Phuong (2006)	Pallor	↑
Kalayanarooj (1997)	Absolute monocyte counts	↓
Bruce (2005)	Skin abrasions	↓
Low (2006)	Red eyes	↑

Table 1-4 Non-significant signs, symptoms, and additional indicators reported in one or more studies

Study	Symptoms
Phuong (2006)	Tender muscles on palpation, arthrites, dehydration, tender liver, constipation, altered consciousness, bruises, lymphadenitis, eschar, and vesicles
Bruce (2005)	Red eyes, eye irritation, eye pain, nuchal rigidity
Karande (2005)	Polyserositis, altered sensorium, convulsions, oliguria, respiratory rate, and hepatosplenomegaly
Deparis (1998)	Acute respiratory distress
Chadwick (2006)	Respiratory rate
Buchy (2005)	Conjunctival injection
Low (2006)	Swollen glands

Table 1-5 Studies with multivariable predictor models presented as odds ratios

Study	Predictors	OR (95% CI)
Wilder-Smith	Platelet count (10^9 platelets/L) <140	456 (37, 5917)
	AST (IU/L) >34	68 (6, 719)
	WBC (10^9 cells/L) <5	47 (4, 518)
Phuong	Petechiae	4.82 (2.71, 8.58)
(2004)	Hepatomegaly >1 cm	2.93 (1.14, 7.53)
	Admission after >3 days of illness	2.47 (1.38, 4.42)
	Hematocrit	1.13 (1.05, 1.22)
	Coryza	0.36 (0.16, 0.81)
	Sore throat	0.33 (0.14, 0.76)
Deparis	Macular rash	2.07 (1.53, 2.62)
	Pruritis	2.55 (2.31, 2.79)
	Low Platelet Count	1.002 (1.001, 1.005)
	Leukopenia	1.2 (1.06, 1.37)
Chadwick	Rash (patient reported)	9.13 (2.14, 38.94)
	Hemoglobin	1.52 (1.11, 2.06)
	WBC	0.43 (0.31, 0.59)
	Creatinine	0.73 (0.57, 0.93)
	Bilirubin	0.74 (0.59, 0.94)
	Prothrombin Time	0.44 (0.30, 0.65)

Table 1-6 Studies with multivariable models presented as positive predictive values

Study	Predictors	Positive Predictive Value
Sawasdivorn	Fever + Positive Tourniquet Test + Leukopenia	73%
McBride	Rash + Bleeding (gums, nose, vagina) + bone pain + Taste Alteration	73%
Karande	Arthralgia + Thrombocytopenia	100%

Supplemental Table 1-1

Characteristics of Excluded Studies

Authors	Year	Title	Reason for exclusion
Ali [41]	2006	Dengue fever in malaria endemic areas	Unavailable through UMass library
Zahur [42]	2001	Clinical spectrum of thrombocytopenia in adult population of Karachi	Unavailable through UMass library
Flannery [34]	2001	Referral pattern of leptospirosis cases during a large urban epidemic of dengue	No statistical comparison of dengue and non-dengue patients
Ageep [58]	2006	Clinical presentations and laboratory findings in suspected cases of dengue virus	No statistical comparison of dengue and non-dengue patients
Akram [59]	1998	Dengue virus infection among children with undifferentiated fever in Karachi	No statistical comparison of dengue and non-dengue patients
Baruah [60]	2006	Incidence of dengue in a tertiary care centre-Kasturba Hospital, Manipal	No statistical comparison of dengue and non-dengue patients
Barauh [61]	1996	Haemorrhagic manifestations associated with dengue virus infection in Nagaland	No statistical comparison of dengue and non-dengue patients
CDC-MMWR [27]	2007	Dengue hemorrhagic fever---U.S.-Mexico border, 2005	No statistical comparison of dengue and non-dengue patients
Cheng [62]	2004	Clinical deterioration in community acquired infection associated with lymphocyte upsurge in immunocompetent hosts	No statistical comparison of dengue and non-dengue patients
Deepak [63]	2006	Differential diagnosis of acute liver failure in India	No statistical comparison of dengue and non-dengue patients
Dietz [33]	1992	Diagnosis of measles by clinical case definition in dengue-endemic areas: implications for measles surveillance and control	No statistical comparison of dengue and non-dengue patients

Dietz [64]	1990	Epidemic dengue 1 in Brazil, 1986: Evaluation of a clinically based dengue surveillance system	No statistical comparison of dengue and non-dengue patients
Gupta [65]	2000	Gall bladder wall edema in serology proven pediatric dengue hemorrhagic fever: A useful diagnostic finding which may help in prognostication	No statistical comparison of dengue and non-dengue patients
Kalayanarooj [66]	1999	Can doctors make an accurate diagnosis of dengue infections in an early stage?	No statistical comparison of dengue and non-dengue patients
Kittigul [67]	2003	Dengue hemorrhagic fever: knowledge, attitude and practice in Ang Thong Province, Thailand	No statistical comparison of dengue and non-dengue patients
Kularatne [68]	2005	Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults: a descriptive study in Sri Lanka	No statistical comparison of dengue and non-dengue patients
Leelarasamee [69]	2004	Etiologies of acute undifferentiated febrile illness in Thailand	No statistical comparison of dengue and non-dengue patients
Peyerl-Hoffman [70]	2004	Serological investigation of the prevalence of anti-dengue IgM and IgG antibodies in Attapeu Providence, South Laos	No statistical comparison of dengue and non-dengue patients
Reynes [71]	1994	The first epidemic of dengue hemorrhagic fever in French Guiana	No statistical comparison of dengue and non-dengue patients
Rodier [72]	1996	Epidemic dengue 2 in the city of Djibouti 1991-1992	No statistical comparison of dengue and non-dengue patients
Anuradha [73]	1998	The 1996 outbreak of dengue hemorrhagic fever in Delhi, India	No other febrile illness comparison group
Chairulfatah [74]	1995	Clinical manifestations of dengue hemorrhagic fever in children in Bandung Indonesia	No other febrile illness comparison group
Domingues [75]	2006	Headache features in patients with dengue virus infection	No other febrile illness comparison group
Espinoza-Gomez [76]	2005	Clinical pattern of hospitalized patients during a dengue epidemic in Colima, Mexico	No other febrile illness comparison group

Kalayanarooj [77]	2005	Is dengue severity related to nutritional status	No other febrile illness comparison group
Neeraja [78]	2006	Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital	No other febrile illness comparison group
Monira [79]	2004	Clinical and laboratory observations associated with the 2000 dengue outbreak in Dhaka, Bangladesh	No other febrile illness comparison group
Ranjit [80]	2007	Early differentiation between dengue and septic shock by comparison of admission hemodynamic, clinical, and laboratory variables: A pilot study	No other febrile illness comparison group
Shah [81]	2005	Clinical and laboratory abnormalities due to dengue in hospitalized children in Mumbai in 2004	No other febrile illness comparison group
Fadilah [82]	1999	Quantation of T-lymphocytes subsets helps to distinguish dengue hemorrhagic fever from classic dengue fever during the acute febrile stage	Quality assessment=48%
Shah [83]	2005	Clinical and laboratory profile of dengue, leptospirosis, and malaria in children: a study from Mumbai	Quality assessment=30%
Watt [36]	2003	Differentiating dengue virus infection from scrub typhus in Thai adults with fever	Quality assessment=40%
Zavala-Velazquez [84]	1996	Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico	Quality assessment=36%
Ellis [85]	2006	Causes of dengue fever in adults on the Thai-Myanmar border	Limited number of dengue cases
Ashford [86]	2003	Outbreak of dengue fever in Palau, Western Pacific: risk factors for infection	Only compared environmental factors
Pancharoen [87]	2001	Dengue virus infection during infancy	Short report

Chapter II Research Design and Methods

This chapter provides an in-depth description of the research design and analytical methods used to address each of the three previously stated research objectives. Although the study design and statistical methods are broadly described in the “Methods” sections for each of the subsequent chapters, this chapter provides a more detailed description of these methods. First, this chapter gives a general description of the location/population of the two clinical study sites, including the two study hospitals, and then provides a detailed description of the study design and data collection protocol, including descriptions of the clinical laboratory variables used in each of the analyses.

The chapter concludes with a thorough explanation of all analytical methods used for each of the three research objectives.

IIA. Study sites

Queen Sirikit National Institute of Child Health (QSNICH) is a tertiary care, pediatric hospital sponsored by the Thai Ministry of Public Health. The hospital is located in Bangkok, Thailand, the nation’s capital. Bangkok has approximately 7 million residents, with a population of around 10 million during the daytime hours [88]. QSNICH is a 538-bed teaching hospital, which includes a 30-bed hemorrhagic fever ward. Patients seen at the outpatient ward or hemorrhagic fever ward with suspected dengue were eligible for the study.

Kamphaeng Phet Province is located in northwestern Thailand with a population of approximately 700,000. The city of Kamphaeng Phet is approximately 223 miles from Bangkok, with an approximate population of 44,000 [88]. Kamphaeng Phet Provincial Hospital (KPPPH), located in the city of Kamphaeng Phet, serves the entire province and is the referral center of all district hospitals in the province. KPPPH has a more rural catchment area than QSNICH. KPPPH is a government-sponsored hospital which serves patients of all age groups and has a separate pediatric ward.

IIB. Enrollment and data collection protocols

The clinical study protocol was previously reported by Kalayanarooj and colleagues [44]. The study enrolled Thai children ages 6 months to 15 years who presented to QSNICH or KPPPH with fever onset within 72 hours of presentation and oral temperature $\geq$ 38 degrees Celsius who did not have a specific identifiable cause of fever. Patients with hypotension, malnutrition, or history of chronic medical illness were excluded. Parental informed consent was obtained prior to study enrollment. On the day of enrollment (study day 1), patients were admitted to the hospital and a blood sample was obtained by the study nurse. Serological assays (IgM/IgG ELISA and hemagglutination inhibition assay) were performed on blood samples collected at enrollment and at convalescence. Viral isolation and RT-PCR (only 1997 and after) were performed using blood samples collected on day of enrollment. Patients with serologic and/or virologic evidence

of dengue were considered confirmed dengue cases. Patients were routinely observed and daily clinical and laboratory measurements were recorded by a study nurse using standardized data collection forms and reviewed by a physician for recording errors. On the day of defervescence, finger-stick hematocrits were measured every six hours for 18 hours in order to capture hemoconcentration. A right lateral decubitus chest x-ray was taken the day following defervescence to assess for pleural effusion. After completion of the case record, a final diagnosis of DF, DHF, or OFI was assigned by a single expert physician (who was not directly involved in patient care) based on review of the entire medical record.

IIB. 1 Clinical data

Clinical data was collected daily for each patient during hospitalization until discharge (24 hours after defervescence), including:

Vital signs (temperature, pulse, blood pressure, and respiratory rate) were obtained by a ward nurse every 3 to 6 hours. The interval was decreased to every 2 hours upon signs of clinical deterioration.

Weight was measured every morning after breakfast.

Hematocrits were measured daily. Finger-stick hematocrits were measured at the time of defervescence, defined as two temperature readings below 38˚C, and repeated every six hours for at least 3 measurements. Hematocrits were

measured by filling 2 capillary tubes simultaneously and recording the mean of the two.

Right lateral decubitus chest x-rays were taken on patients with stable vital signs the day following defervescence and were evaluated by a hospital radiologist. The chest x-ray was used to measure the amount of pleural effusion, which is an indicator of plasma leakage. The pleural effusion index was calculated as follows: PEI=100 x (maximum width of right pleural effusion)/(maximum width of right hemithorax).

IIB. 2 Laboratory data

Blood samples for research were obtained each morning the patient was in the study up to a maximum of five consecutive samples. Blood samples were used for daily complete blood count (hemoglobin and platelets), WBC count, differential WBC count, including neutrophils, eosinophils, basophils, lymphocytes, monocytes, atypical lymphocytes, bands, other cell types, and liver function tests (AST, ALT, and albumin). All serologic and virologic testing was performed at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand.

Table 2-1 gives an example of when clinical and laboratory data were collected for a typical patient in the study. In this example, the subject presented to the hospital on the third day of illness and was enrolled in the study (Study day 1). He/she was followed in the hospital until 24 hours after defervescence, and

then had a follow-up outpatient visit 5 days after discharge. Throughout the hospitalization phase, clinical data, blood samples, and CBC/WBC/Liver panel were obtained daily. A chest x-ray to measure pleural effusion was taken on the day after defervescence.

Each patient had a scheduled follow-up visit approximately 5-8 days after defervescence in which clinical and laboratory data were collected. We did not use any follow-up data in our analyses as we were looking for early indicators of disease and the dynamics of these indicators throughout hospitalization.

Table 2-2 lists the clinical laboratory variables used, including their definition, units, normal range, and how the variable was utilized in each chapter. Each variable was utilized differently according to the research question. For example, chapter III used longitudinal models to describe how each variable changes across time during each patient's hospitalization. Chapters IV and V were cross-sectional analyses of the data. Chapter IV investigated how each variable's greatest deviation (maximum or minimum values) correlated with the physician's final diagnosis and provides classification models that do not require indicators of plasma leakage. Chapter V investigated clinical variables at presentation to identify patients according to their final diagnosis or severity of illness that subsequently ensued.

IIC. Data sources

IIC. 1 Data management

Upon study enrollment, each patient received a study identification number according to the order in which they were enrolled in the study. Any references to individual subjects in these analyses are made using the study number. Copies of laboratory test results, x-rays results, and pathology reports were included in the study record using the standard reporting formats of the testing facilities. One month after enrollment, the patient record was reviewed for completeness and data were entered into an electronic database. The original files are located and stored at AFRIMS under lock and key and only used for study analyses by authorized individuals. Electronic files were exported from FoxPro as dBASE (DBF) files and received on CD from Thailand. The files used for these analyses were stored on a password-protected shared network drive with restricted access.

IIC. 2 Data processing steps

The DBF data file was converted to a Stata Intercooled version 9 (Stata Corporation, College Station, TX) dataset using StatTransfer version 8 software. The DBF file was also converted to an Excel file for use in SPSS AnswerTree version 3.1 for Chapter V.

Data cleaning steps were taken to establish separate analytical datasets for each aim and contact was maintained with study personnel in Thailand for

any database issues or recording errors found in the data. For example, albumin was recorded differently for the first year of the study, in units of g/L, not g/dl. To make these data comparable over years, values were converted to g/dl by dividing all values where albumin≥10; however, albumin was eventually dropped from all analyses. All changes/updates to the database were made to both the analytical datasets as well as an original data file, and were annotated in Stata DO files.

IID. Summary of dataset

Table 2-3 provides a summary of the study sample by the number of patients with a final diagnosis of DF, DHF, or OFI in each hospital from 1994-2007, where KPPPH is from 1994-97 only (note: the descriptive tables of the study sample vary for each aim according to different exclusion criteria applied for each aim). There were a total of 1384 patients enrolled in the study, of which 1311 had a final diagnosis of DF, DHF, or OFI (73 patients had an unknown/missing final diagnosis or were classified as having a non-viral illness and were excluded from all analyses). Among those with a final diagnosis from both hospitals, 630 had a dengue illness (394 DF; 236 DHF) and 681 had OFI. The majority of patients (86.5%) were enrolled at QSNICH. There were 722 males and 589 females, with an average age of 7.9 years (95% CI: 7.7, 8.1). The average number of days ill at fever day+1 was 6 days (range: 2-12 days).

IIE. Detailed analytical methods

IIE.1 Analytic approach to research objective 1

The analyses for Chapter III were used to describe the patterns of clinical laboratory variables known to be associated with dengue, and how these variables change throughout an illness episode for DHF, DF, and OFI, starting with the day of presentation (within 72 hours of fever onset) through the day after defervescence. The primary analytical methods used for this aim were lowess smoothing curves and population-average (marginal) models with a first order autoregressive correlation matrix.

IIE. 1.1 Lowess smoothing curves

Lowess smoothing curves are often used to assess the bivariate associations of two variables in the data and may be used to visually assess adherence to the assumption of linearity used in normal least-squares regression analysis. Lowess is an abbreviation for *locally weighted scatterplot smoothing*. The technique fits a weighted low-order polynomial for each data point x using a subset of the entire dataset that surrounds x, where data points closer to x contribute more to the estimate of x (weighted more) than data points further away from x [89, 90]. Each of the resulting low-order polynomials is then used to calculate the lowess regression function.

The weighting function used in this technique is the tri-cube weight function, where the weight for a given data point is a function of the distance from

the data point x being estimated [90]. The tri-cubic function is given by $w(x) = (1 - |d|^3)^3$ where d = scaled distance between the weighted data point and the data point being estimated. The scaled distance is between 0 and 1 where the scaled distance of the point being estimated =0 and the maximum data point in the subset of data =1. To calculate distance, a Euclidean distance measure is used and is defined as: $d_i = [(X_{i1} - X_{h1})^2 + (X_{i2} - X_{h2})^2]^{1/2}$, where (X_{h1}, X_{h2}) represent predictor variables to obtain a fitted value [90]. The bandwidth or subset of data used in each polynomial- to estimate each data point - can be adjusted and is represented as a proportion of the overall dataset. The bandwidth for all lowess analyses in this manuscript was the Stata default bandwidth of 0.8.

Lowess curves for these analyses were used to determine the type of function the data followed in order to structure an appropriate model. For example, in Figure 2-1, platelet count (dependent variable) among patients with DHF decreases over time (independent variable) but will eventually reach an inflection point and begin to recover; thus, the platelet count does not continue to decrease in a linear manner in these patients. When overlapping a linear function, quadratic function, and a lowess curve of platelet count and days of illness, the lowess curve closely approximates the quadratic curve, more than the linear curve; thus, a quadratic model was deemed the best function to fit with this model. When similar comparisons were done for other variables among each diagnosis, all variables followed a quadratic trend. Thus, quadratic longitudinal

models were used to assess the dynamics of clinical laboratory parameters throughout the hospitalization of subjects in this study.

IIE. 1.2 Longitudinal modeling

In general, the objective of longitudinal data analysis is to evaluate changes in the mean response over time and whether these changes are associated with specified covariates [91]. A key difference in longitudinal models, in which repeated measures are taken on the same individuals, is the violation of independence, which is an assumption in many simple linear regression models. When interpreting the variance of the mean response in changes over time for longitudinal models, the correlation between variances at different time points (covariance) must be considered. The covariance between responses at two different time points on the same subject can be defined as:

$$Cov = E\{(Y_{ij} - u_{ij})(Y_{ik} - u_{ik})\}$$

where Y_{ij} and Y_{ik} are the response variables for the i^{th} individual at timepoint j and k, respectively, and u_{ij} or u_{ik} is the mean response at those timepoints [91]. The covariance is used in the calculation of the variance of a longitudinal model to capture the correlation of response variables. When multiple subjects have n repeated measures, a variance-covariance matrix can be established and defined as:

$$\begin{bmatrix} Var(Y_{i1}) & Cov(Y_{i1}, Y_{i2}) & \dots & Cov(Y_{i1}, Y_{in}) \\ Cov(Y_{i2,} Y_{i1}) & Var(Y_{i2}) & \dots \ddots & Cov(Y_{i2}, Y_{in}) \\ \vdots & \vdots & & \\ Cov(Y_{in,} Y_{i1}) & Cov(Y_{in}, Y_{i2}) & \dots & Var(Y_{in}) \end{bmatrix}$$

where $Y_{i1,}Y_{i2,\dots,}Y_{in}$ is the response variable for i^{th}individual across 1, 2,… n repeated measures, respectively [91]. However, this definition assumes the variance and covariance is the same across all individuals.

General assumptions can be made about the variance and covariance of a model. Repeated measures on the same subject may often be positively correlated, and these measures are usually assumed to have a higher correlation with decreased time separation between measurements [91]. In the models presented for these analyses, a first-order autoregressive model of the covariance was used. This type of covariance pattern has only two parameters and assumes that measurements are made at equally spaced intervals over time and error terms are dependent on the previous error term only [92]. The first-order autoregressive correlation is defined as:

$$Cov_{jk} = \sigma^2 \rho^{|j-k|}$$

where Cov_{jk} is the covariance between timepoints j and k, σ^2 is the variance, and $\rho^{|j-k|}$ is the autoregressive correlation parameter between time points j and k [92]. In this type of covariance structure, the correlation decreases exponentially across timepoints as seen here:

$$Cov = \sigma^2 \begin{bmatrix} 1 & \rho & \rho^2 & \dots & \rho^{n-1} \\ \rho & 1 & \rho & \dots & \rho^{n-2} \\ \rho^2 & \rho & 1 & \dots & \rho^{n-3} \\ . & . & . & \dots & . \\ \rho^{n-1} & \rho^{n-2} & \rho^{n-3} & \dots & 1 \end{bmatrix}$$ 92

Reasons for choosing this type of covariance structure were: 1) some subjects had only 2 timepoints and a valid covariance structure that depends on multiple timepoints for each patient could not be established, 2) there is clinical relevance of laboratory parameters following a pattern that is dependent on a previous measure, and 3) time intervals were approximately equally spaced at 24 hours, since blood samples were collected each morning.

Population-average models, also referred to as marginal models, were used to determine the mean change in clinical laboratory parameters among each diagnosis throughout hospitalization while adjusting for covariates of age, gender, hospital, and year of study enrollment. After modeling each diagnosis separately, interaction terms of diagnosis and day of illness were used to evaluate differences in clinical laboratory parameters between the diagnostic groups at each timepoint. Population-average models assume fitting of $E(Y_{ij}) = X_{ij}\beta$ using the identity function and the variance is a fixed scale parameter θ that depends only on the marginal mean. Assumptions about the correlation have minimal effect on the estimates of β so even if the first-order autoregressive covariance is incorrect, it will have minimal effects on the estimates of β.

However, it may have a significant impact on the variance of β and thus the significance tests.

Population-average models were chosen based on the structure of the data; some subjects had few data points and applying a random slope and intercept model using a quadratic fit would not be valid. Also, as noted above, specifying the correct covariance structure is not a necessity for these models. Furthermore, as detailed in chapter III, the models that were established tended to follow the same trajectory as the individual means at each timepoint.

IIE. 2 Analytical approach to research objective 2

This aim provides a cross-sectional evaluation of the most extreme values of specific indicators and how they correlated with the physician's final diagnosis. Multivariable binary logistic regression modeling was used to determine probability cutpoints that optimized sensitivity and specificity of DHF or severe dengue illness in the absence of standard diagnostic measures of plasma leakage, such as chest x-rays, ultrasounds, or hemoconcentration. This was done by collapsing each patient's clinical laboratory parameters obtained throughout their hospitalization into a maximum or minimum value (see Table 2-2). Additionally, kappa statistics were used to assess the agreement between the final multivariable logistic regression models, the physician's diagnosis, and the WHO classification of DHF versus DF.

IIE. 2.1 Univariable and multivariable logistic regression

Logistic regression is an important technique in the modeling of dichotomous dependent variables and is often used in epidemiological studies to state an individual's risk for developing a disease [93]. Logistic regression models can be defined in terms of probability of a dichotomous outcome as follows:

$$pr(Y = 1) = \frac{1}{1 + exp\left[-\left(\beta_0 + \sum_{j=1}^{k} \beta_j X_j\right)\right]}$$

where Y is coded as (0,1) and is expressed in the equation as the probability of $Y = 1$ [93]. This probability can also be expressed as the natural log odds of $Y = 1$ (logit):

$$logit[pr(Y = 1)] = log_e[odds(Y = 1)] = log_e\left[\frac{pr(Y = 1)}{1 - pr(Y = 1)}\right]$$

which is equivalent to:

$$logit[pr(Y = 1)] = \beta_0 + \sum_{j=1}^{k} \beta_j X_j$$

where the logit is simply a way to linearize the probability of an outcome [93].

For the analyses presented in this aim, multivariable logistic regression models were established that best distinguished between DF vs. DHF, DHF vs.

DF+OFI, any dengue vs. OFI, and severe dengue vs. non-severe dengue+OFI. An example model is given:

$$\hat{p} = \frac{e^{\widehat{\beta_0} + \widehat{\beta_1} x_1 + \cdots + \widehat{\beta_n} x_n}}{1 + e^{\widehat{\beta_0} + \widehat{\beta_1} x_1 + \cdots + \widehat{\beta_n} x_n}}$$

Where $\hat{p}$ is the estimated probability of outcome (i.e. diagnosis of DHF as opposed to DF) and $e^{\widehat{\beta_0} + \widehat{\beta_1} x_1 + \cdots + \widehat{\beta_n} x_n}$ is the odds of having the outcome of interest, for example, a physician's diagnosis of DHF, with n number of covariates in the model [93]. Models are expressed as odds ratios where unit increases (or decreases) in a certain independent variable of interest represent increases (or decreases) in the odds of having one diagnosis compared to another, such as DHF as opposed to DF.

IIE. 2.2 Model building process

The process of establishing each multivariable model was a manual stepwise procedure in which independent variables were added to the model in the order of the univariable analyses that produce the best area under curve (AUC) of the receiver operator characteristic (ROC) curve.

First, univariate logistic regression models were used to evaluate indicators that distinguished between the diagnostic groups of interest. For each outcome, univariate logistic regression was performed on each of the following variables: maximum tourniquet test, hematocrit, % monocytes, % lymphocytes,

% neutrophils, AST, and ALT, and minimum platelet count and WBC count (see Table 2-2). Lowess curves from univariable analyses were used assess the linear or nonlinear relationship between y and x, and used as evidence to determine the categorization for variables that had skewed distributions. If the linearity assumption appeared to hold true, then the variable was used as a continuous variable. Frequency tables were used to show the distribution of each categorized variable with the outcome.

Second, ROC curves of each univariate logistic regression model were produced and the AUC obtained. Significant ($\propto < .05$) variables from univariable analyses with the best AUC values were added in a manual forward selection process to establish the best multivariable models.

Finally, the optimal sensitivity and specificity for each model was determined and used to establish a probability cutpoint for each model. The optimal sensitivity and specificity was chosen based on a probability cutoff where the sum of sensitivity and specificity were maximized, the maximum % correctly classified was achieved, and sensitivity remained higher than specificity.

IIE. 2.3 Validation

All analyses for this aim were first performed using only the QSNICH data as a training dataset. Then, each multivariable model was applied to the KPPPH data as a validation of the models to test their performance in a different dataset from a different hospital with a different catchment area.

IIE. 2.4 Analysis of agreement

Kappa statistics were used to determine the amount of agreement between the final validated multivariable models of DHF vs. DF and DHF vs. All others to the physician's final diagnosis of DHF [94]. Additionally, these models were compared to the WHO diagnostic criteria for DHF. The physician's diagnosis of DHF was also compared to the WHO diagnostic criteria for DHF.

Kappa statistics are used to test the percentage of observed agreement to agreement expected by chance [94]. Using preliminary data, an example of Kappa statistic is given:

Physician diagnosis	WHO DHF	WHO DF	Total
+	125	43	168
-	16	134	150
Total	141	177	318

Kappa=(Observed agreement – Expected agreement)/(1- Expected agreement)

Observed agreement=(125 + 134)/318=.8145

Expected agreement =[(141*168) + (177*150)]/318^2=.4968

Kappa=(.8145-.4968)/(1-.4968)=63.14%

The use of Kappa statistics for this aim will demonstrate that the physicians did not strictly adhere to the WHO diagnostic criteria for DHF. This will also help to highlight which of the four criteria were in most agreement with physicians and which were in least agreement.

IIE. 3 Analytical approach to research objective 3

This aim applied the methodology of classification and regression tree analysis (CART) to establish classification trees based on data available at the day of enrollment only. In CART, the data are partitioned into different nodes based on an impurity function where patients within each partitioned node be will be as similar as possible in terms of the characteristics analyzed (in this case, clinical laboratory parameters) [95, 96, 97]. CART is a non-parametric method that establishes mutually exclusive subgroups within a sample based on shared characteristics that are associated with the outcome of interest [95]. CART is used with binary outcomes and the final outcome yields prevalence estimates of the outcome variable within each of the identified subgroups [96]. A simplified example of a CART output is presented in Figure 2-2.

All candidate independent variables were considered, as well as age, and the sample was split according to values (cut-points) of the independent variable with the largest difference between the impurity in the parent node and a

weighted average of the impurity between the two child nodes [96]. Each node represents the probability of having the dependent measure, for example a physician's final diagnosis of DF or DHF, within each grouping of independent variables, which can be categorical or continuous, and is given by a 2x2 table. For example, if a cut-off of AST is used to split the outcome variable into the 2x2 table below, we are left with conditional probabilities of being above or below the cut-point given the final diagnosis.

	DF	DHF	
AST<=50 Left node impurity	n_{11}	n_{12}	$n_{1.}$
AST>50 Right node impurity	n_{21}	n_{22}	$n_{2.}$
	$n_{.1}$	$n_{.2}$	

Which independent variables best 'split' the dependent variable is based on the impurity function. The impurity function used for this analysis was the Gini impurity function (Gini improvement measure), which uses the proportion of subjects with the dependent variable in a parent node and a weighted average of subjects in the resulting child nodes to calculate an impurity at each possible split [95, 96, 97]. Lemon et al outlined four steps used to calculated Gini improvement measure[96] :

$$1.\, Diversity\ index = 1 - \sum {p_{i/j}}^2 = 2p_{i/j}\left(1 - p_{i/j}\right)$$

where $p_{i/j}$ represents the probability the dependent variable is equal to i in the Node j. The second step involves calculating the diversity index for each of the two resulting child nodes. The third step calculates a weighted diversity index using the proportion of subjects from the parent node that are now in the resulting child node[96]:

3. $Weighted\ diverity\ index = [(p_l)(diversity\ index_l)] + [(p_r)(diversity\ index_r)]$

where $\mathrm{p_l}$ and p_r are the proportion of subjects included from the parent node that are in the left (l) and the right (r) child nodes, and $diversity\ index_l$ and $diversity\ index_r$ are the diversity index parameters for the resulting child nodes. The last step calculates the Gini improvement measure by taking the difference of the diversity index of the parent node and the weighted diversity index[96]:

4. $Gini\ improvement\ measure = Diversity\ index\ of\ parent\ node - Weighted\ diversity\ index$

Additionally, each tree can consider a cost-complexity measure, where complexity is the number of nodes in a tree. The quality of each tree can be penalized if it is too big (pruning). An example of a 'pruning' method is based on the Studentized log relative risk (slRR) as described in chapter 4 of Zhang et al [97]. Briefly, for each left and right node (2x2 table as discussed above) a slRR can be calculated by taking the log of the relative risk of each left and right node and dividing by the standard error of the log relative risk. The value of a slRR for a parent node is replaced by the maximum value of the slRR from any of its resulting offspring if the offspring slRR was greater than the parent node. Nodes

with a slRR less than the cut-off value of 1.96 (equivalent to alpha>0.05) are usually pruned.

However, for the analyzes presented in Chapter V, no pruning method was applied. Instead, stopping rules were used to keep the tree from being fully saturated (where the number of nodes in a tree approximates the number of subjects). The stopping rules were: 1) no terminal node could contain <5% of the original sample size, 2) no more than 5 levels per tree, and 3) a minimum improvement in impurity of .0001.

IIE. 3.1 K-fold validation

Validation of each tree was made by using the k-fold validation procedure, which establishes differences in the frequency counts of the nodes to estimate the selection bias caused by relative risk pruning (splits are made based on the impurity function that is closely related to the relative risk) [97]. For these analyses, the dataset was divided into five subpopulations of equal size, L_i, (i=1,2,3,4,5) where L_{-i} is the sample after removing one of the subpopulations. The L_{-i} was used to establish a split, yielding two 2x2 tables [97]:

Left Node	A(-1)	B(-1)
Right Node	C(-1)	D(-1)

Left Node	A(1)	B(1)
Right Node	C(1)	D(1)

The process was repeated for all of the subpopulations to estimate a selection bias for A and D cells by:

$$\frac{1}{4}\sum_{1}^{5} A_{(-1)} - \sum_{1}^{5} A_{(1)}$$

and

$$\frac{1}{4}\sum_{1}^{5} D_{(-1)} - \sum_{1}^{5} D_{(1)}$$

This bias was used to correct the frequency counts in the entire dataset and establish a relative risk [97]. A tree was accepted only if all nodes had a slRR, adjusted for selection bias, that was >=1.96 (alpha<=0.05). A final overall sensitivity and specificity was calculated for each tree.

This method has advantages over logistic regression techniques when applied to this type of scenario in resource-poor areas: 1) CART is a non-parametric method and is thus not bound to the assumptions of logistic regression, 2) calculating a probability based on a complicated logistic regression model would be difficult without the use of computers and, in resource-poor areas, this may not be feasible, 3) the CART method mimics a physician's way of thinking by ruling out certain diagnosis based on dichotomizing symptoms and does not require calculation of probabilities. Similar methodology has been used

in other studies of dengue, however, these studies have their limitations as discussed in chapter V [98, 99].

For this aim, the classification trees distinguish between the different categories of dengue disease severity among all patients enrolled with a suspected dengue illness. The categories of dengue disease severity used are: (1) dengue shock syndrome (DSS, as defined by WHO criteria); (2) DSS or PEI>15; (3) DSS or required intravenous fluid; (4) DSS or platelet count <=50,000 anytime during illness; (5) DSS or received fluid intervention (oral or intravenous) in any 24-hour period that exceeded maintenance volume + 5% volume deficit [100, 101]. Additional classification trees were used to evaluate differences between DHF vs. DF and severe dengue vs. non-severe dengue; however, the focus of this aim was on prediction of dengue disease severity and limiting trees to only those patients known to have dengue does not suit this purpose as confirmatory serology of dengue is not generally available at presentation.

IIF. Analytic software

The following software was used to perform the analyses presented: Stata Intercooled version 9 (StataCorp, College Station, TX) for chapters III and IV and SPSS AnswerTree 3.1 (SPSS AnswerTree, version 3.1, SPSS Inc., Chicago, IL) for chapter V. As mentioned in section 2C, the data files were received as dbf

files and were converted to the appropriate analytic dataset using Stat/Transfer (Stat/Transfer version 8 for Windows).

Table 2-1 Example of the schedule of measures for a typical patient

Study day	1	2	3	4	9
Day of illness	3	4	5	6	11
Fever day	-2	-1	0	1	6
Clinical data	+	+	+	+	+
Blood sample	+	+	+	+	+
CBC/WBC/Liver panel	+	+	+	+	+
Chest x-ray	-	-	-	+	-

Table 2-2 Clinical laboratory variables used in thesis, including definitions, units, normal ranges, and utilization for each research objective

Variable	Definition	Units	Normal range[a,b, c]	Variable Utilization		
				Research objective 1	Research objective 2	Research objective 3
Platelet count	Platelets (thrombocytes) are non-nucleated cell fragments that trigger substances for the formation of blood clots[d]	Cells/mm^3	200,000-500,000	Continuous (square root transformation)	Minimum (per 25,000 units)	Continuous
WBC count	White Blood Cells (leukocytes) are nucleated cells responsible for producing a wide range of immune responses[d]	Cells/mm^3	5,000-10,000	Continuous (natural log transformation)	Minimum (per 500 units)	Continuous
Hematocrit	The fractional contribution of erythrocytes (red blood cells) in the blood volume (height of erythrocyte column ÷ height of whole blood column)[d]	% packed blood volume	40-45	Continuous	Maximum	Continuous
% Monocytes	Leukocyte important in phagocytic defense[d]	% WBC differential	4-8	Continuous	Maximum (categorical)	Continuous
% Lymphocytes	Leukocytes made up of B and T lymphocytes and null cells that are important for direct and memory immune responses[d]	% WBC differential	20-40	Continuous	Maximum (categorical)	Continuous
% Neutrophils	Leukocytes important in phagocytosis[d]	% WBC differential	40-60	Continuous	Maximum (categorical)	Continuous
AST	Aspartate aminotransferase, liver enzyme that catalyzes the transfer of the amino group of aspartate to α-keto-glutarate to produce oxaloacetate and glutamate[e]	SI units (U/L)	15-40	Continuous (Box-Cox transformation)	Maximum (categorical)	Continuous
ALT	Alanine aminotransferase, liver enzyme that catalyzes the transfer of the amino group of alanine to α-keto-glutarate to produce pyruvate and glutamate[e]	SI units (U/L)	10-35	Continuous (Box-Cox transformation)	Maximum (categorical)	Continuous
Tourniquet Test	Measured by inflating blood pressure cuff to half systolic and dystolic pressure and holding for five minutes, then counting the number of petechiae (small red spots caused by weak capillary vessels)	# of petechiae/ square inch	N/A	N/A	Maximum (ordinal)	Ordinal

[a] Reference [102]

[b] Reference [103]

[c] Dr. Pra-on Supradish, personal communication from QSNICH

[d] Reference [104]

[e] Reference [105]

Table 2-3 Characteristics of subjects enrolled at the two study hospitals and who received a final diagnosis of DHF, DF, or OFI

Hospital/Diagnosis	N (%)	Gender		Age in years mean (range)	Days ill at presentation, median
		Males	Females		
QSNICH	1134 (86.5)	614	520	7.7 (0.5, 15.0)	2
DHF	182 (16.1)	105	77	8.8 (1.5, 14.9)	2
DF	330 (29.1)	177	153	8.5 (2.0, 15.0)	2
OFI	622 (54.8)	332	290	6.9 (0.5, 14.6)	2
KPPPH	177 (13.5)	108	69	8.8 (1.4, 14.9)	2
DHF	54 (30.5)	36	18	9.1 (2.8, 14.9)	2
DF	64 (36.2)	30	34	9.2 (2.7, 14.9)	2
OFI	59 (33.3)	42	17	8.0 (1.4, 14.3)	2
Total	1311	722	589	7.8 (0.5, 15.0)	2

Figure 2-1 Lowess smoothing curve overlapped with linear and quadratic functions of platelet count and illness day among patients with DHF

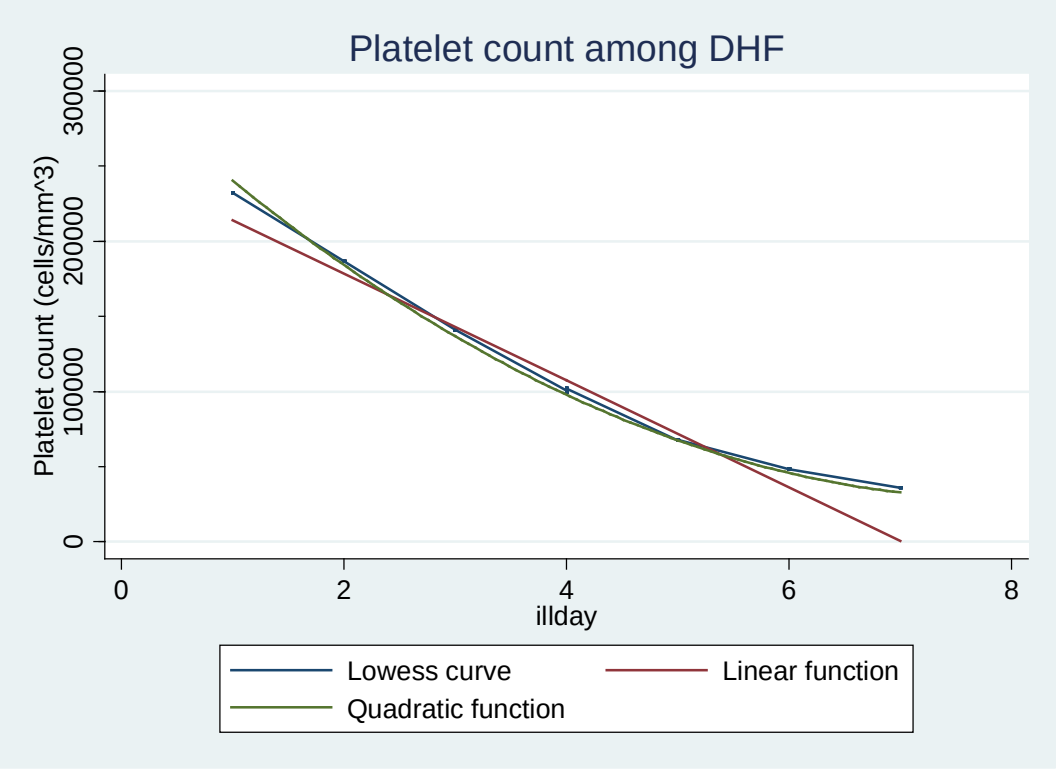
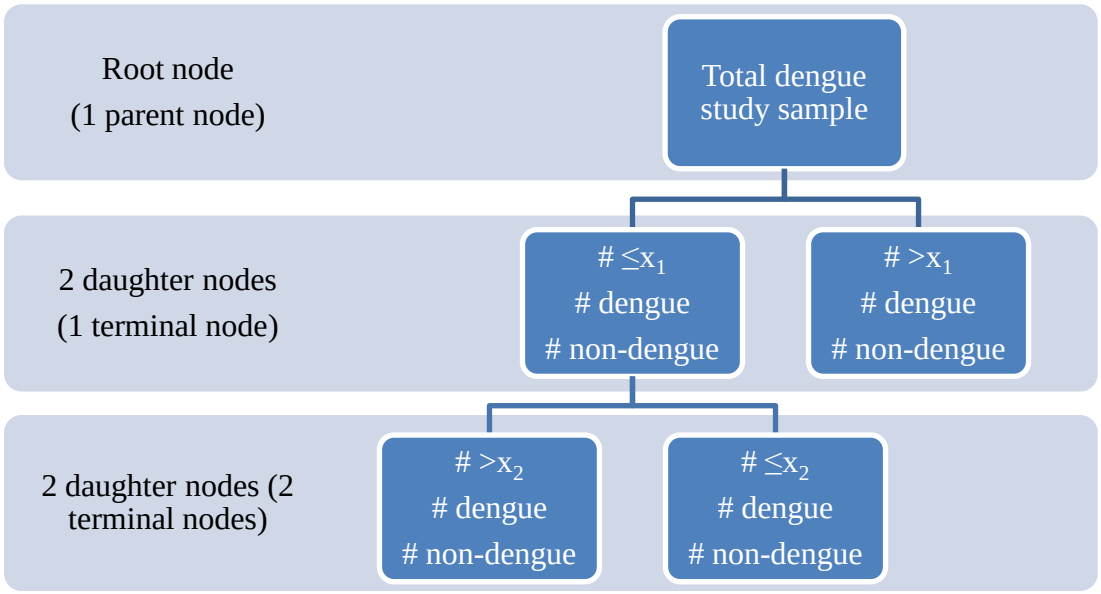


Figure 2-2 Example of a simplified CART analysis



Chapter III: Dynamics of clinical laboratory parameters distinguish among Thai pediatric patients with different dengue disease severity

Abstract

Background

Dengue is an emerging infectious disease which is endemic in tropical and subtropical areas. A quantitative, longitudinal description of dengue illnesses is needed to better understand the dynamics of patients with different grades of dengue illness severity.

Objectives

To describe the temporal dynamics of clinical laboratory parameters throughout the febrile phase among patients with suspected dengue infection.

Study Design

Clinical and laboratory data were collected from Thai children aged 6 months to 14 years who presented to a study hospital within the first 72 hours of illness. Final diagnoses- DF, DHF, or other febrile illness (OFI)- were assigned by an expert physician. Locally weighted scatterplot smoothing curves and population-average models were constructed for laboratory parameters among each diagnosis.

Results

Data were available for 1245 children enrolled from 1994-2007 (231 DHF, 388 DF, and 626 OFI). The median length of observation was five days for patients with dengue and four days for patients with OFI. Quadratic functions of platelet count, hematocrit, WBC count, % monocytes, % lymphocytes, % neutrophils, AST, and ALT were deemed the best fit by assessment of lowess curves. Our models showed lower platelet count and higher AST and ALT in patients with DHF compared to DF and OFI throughout hospitalization. Lower WBC count was observed among patients with dengue in the first four days of illness. Lower percent neutrophils and elevated hematocrit was seen among those with dengue in the later days of illness.

Conclusions

Clinical laboratory variables follow distinct patterns during illness between patients with DHF, DF, and OFI. The dynamics of these variables should help clinicians identify patients with impending DHF and better utilize limited hospital resources.

1. Background

Dengue is a major global health problem, causing an estimated 50-700 million infections annually and approximately 21,000 deaths [24]. Dengue illnesses contribute to a significant economic and public health burden in under-developed countries and are endemic in many resource-poor tropical and sub-tropical regions [28, 29, 31, 32].

Dengue viruses are transmitted through the bite of infected mosquitoes, typically *Aedes aegypti* or *Aedes albopictus*. After a 4-10 day incubation period, the initial clinical manifestations of patients with symptomatic dengue infection are similar to many other febrile illnesses (OFI). Patients with DF often have a self-limited, non-severe, febrile illness. However, patients with DHF develop severe symptoms, such as plasma leakage, that manifest in the latter stages of illness (critical phase), typically after the initial febrile phase subsides (defervescence).

Despite numerous publications on clinical indicators of dengue illnesses, there is limited published information comparing the day-to-day dynamics of clinical laboratory parameters in patients with dengue to patients with OFI or patients with DF to patients with DHF [39]. Early indicators or warning signs to identify patients with dengue and impending development of severe symptoms would help better utilize limited hospital resources in dengue-endemic regions.

2. Objectives

The objectives of this study were to describe the temporal dynamics of clinical laboratory parameters during the acute illness among patients with DF, DHF, and OFI using data obtained from a 12 year prospective pediatric cohort study conducted in Thailand.

3. Study Design

3.1 Study setting

This prospective longitudinal observational study was conducted at two hospitals in Thailand: 1) the Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok during the years 1994-97, 1999-2002, and 2004-07, and 2) Kamphaeng Phet Provincial Hospital (KPPPH) during the years 1994-97. The study methods have been described elsewhere [44]. In brief, children between the ages of six months and 15 years, presenting with temperatures ≥ 38.5˚C for ≤ 72 hours, and no localizing symptoms were eligible for the study. Exclusion criteria included: signs of shock at presentation, chronic disease, or an initial alternate non-dengue diagnosis. Children were admitted to the hospital and monitored throughout their hospital stay until 24 hours after their fever subsided. Written parental informed consent was obtained prior to enrollment. The study protocol was approved by the Institutional Review Boards of the Ministry of Public Health, Thailand, the U.S. Army, and the University of Massachusetts Medical School.

3.2 Clinical laboratory data

A blood sample was obtained on the day of enrollment and daily thereafter until one day following defervescence or for a maximum of five consecutive blood collections. Clinical laboratory studies included complete blood count and manual WBC differential. Serological assays (IgM/IgG ELISA and hemagglutination inhibition assay), viral isolation, and/or RT-PCR were used to confirm all dengue cases. Patients were observed daily and clinical and laboratory measurements were recorded using standardized data collection forms.

Beginning at defervescence (T<38˚C), finger-stick hematocrits were measured every six hours for 18 hours in order to capture hemoconcentration. A right lateral decubitus chest x-ray was taken the day following defervescence to assess for pleural effusion and a pleural effusion index (PEI) was measured as 100 x (maximum width of right pleural effusion)/(maximum width of right hemithorax). After completion of the case record and careful review of the medical record and laboratory results, a final diagnosis of DF, DHF, or OFI was assigned by an expert physician who was not directly involved in patient care.

3.3 Statistical analysis

Descriptive characteristics, such as diagnosis, age, gender, length of illness at presentation, and study hospital, were evaluated between the three diagnostic groups (DHF, DF, and OFI) using t-test for continuous variables with a

normal distribution or Wilcoxon rank-sum test for continuous variables with a skewed distribution, and Pearson's χ^2 for categorical variables.

Population average models (marginal models) were used to assess the temporal dynamics of clinical laboratory parameters across days of illness throughout the febrile phase until 24 hours after defervescence in subjects with DHF, DF, and OFI (Supplementary Figure 3-1). Parameters included in the models were platelet count, hematocrit, WBC count, % monocytes, % lymphocytes, % neutrophils, AST, and ALT. Some variables were transformed to achieve normality: 1) square root transformation for platelet count, 2) natural logarithm transformation for WBC count, and 3) Box-Cox transformation for AST and ALT (see Appendix A). Each variable was modeled separately for each diagnosis and adjusted for age, gender, hospital (QSNICH or KPPPH), and year of enrollment. Statistical interactions were used to evaluate differences between patients with the diagnoses under study across all illness days.

4. Results

4.1 Study sample characteristics

There were 1384 subjects enrolled in the study; 1311 of these patients had a diagnosis of DHF, DF, or OFI (presumed viral, non-dengue illness). For 65 of these subjects (55 with OFI, 6 with DF, and 4 with DHF), a day of defervescence could not be assigned and therefore they were excluded from the

analysis. Additionally, one subject was missing information on day of illness and was excluded from the analysis.

A total of 1245 subjects were used in this analysis (231 DHF, 388 DF, and 626 OFI). Table 3-1 summarizes the characteristics of these subjects. Among subjects with dengue, 82% had secondary infections; patients with DHF had a higher proportion of secondary infections compared to those with DF (Wilcoxon p-value= <.001, 93% of subjects with DHF and 75% of subjects with DF had secondary infections). The dominant dengue serotype was DENV1 (42%), followed by DENV3 (24%), DENV2 (21%), and DENV4 (13%). However, there were no differences between DHF and DF with regard to serotype (Kruskal-Wallis p-value=.14). Additionally, there were no differences in serotype among those with DHF grade I/II compared to DHF grade III/IV (Wilcoxon p-value=.71) The median day of illness at enrollment was two days for all three diagnostic groups. Defervescence occurred at a median of five days and four days after onset of illness in patients with dengue from QSNICH and KPPPH, respectively, but occurred on the third day of illness among patients with OFI (Wilcoxon p-value<.001). Table 3-2 shows the number of patients in each diagnostic group according to day of illness. Over 90% of subjects in all three diagnostic groups remained in the study until defervescence and over 80% remained until defervescence+24 hours. Only 13 patients with dengue (1 with DHF, 12 with DF) were not in the study until at least defervescence compared to 43 with OFI (χ^2 p<.001).

4.2 Population-average models

Lowess curves were used to determine the longitudinal functionality of the clinical laboratory parameters. Both linear and quadratic functions were used to model the data; the quadratic functions showed a closer fit to the lowess curves for all of the selected laboratory parameters. Data obtained after seven days of illness were not included in the models because very few patients remained febrile beyond seven days (Table 3-2).

Figure 3-1 shows graphs of the models for each variable among all three diagnostic groups after adjusting for age, gender, year of enrollment, and hospital (QSNICH or KPPPH); adjusted population-average values obtained from the models and their 95% confidence intervals are given in Supplementary Table 3-1. The structure of each population-average model is given in Supplementary Figure 3-1. Statistical evidence of significant interactions between diagnosis and day of illness with regards to the association with clinical laboratory outcomes is given in Table 3-3. Beginning on the second day of illness (median day of presentation), patients with DHF had lower platelet counts compared to patients with DF and OFI and this difference remained throughout hospitalization. Additionally, patients with DF had lower platelet counts throughout hospitalization compared to patients with OFI. By the third day of illness, patients with DHF had higher maximum daily hematocrit values compared to patients with DF and OFI, and patients with DF had higher values compared to patients with OFI. On the third, fourth, and fifth days of illness, subjects with DHF had lower % lymphocytes

compared to subjects with DF or OFI. Additionally, subjects with dengue had lower WBC counts than subjects with OFI during the first five days of illness, and subjects with DHF had lower WBC counts compared to subjects with DF on the fifth day of illness. However, by the sixth and seventh days of illness, there were no differences in WBC counts in subjects with dengue compared to patients with OFI. Patients with dengue had elevated AST and ALT levels throughout all days of observation compared to patients with OFI, and patients with DHF had higher levels compared to patients with DF from days two through six. In the later stages of hospitalization, days five through seven, patients with dengue had lower percent neutrophils compared to patients with OFI.

5. Discussion

Our analysis shows that, on average, patients with dengue illness follow divergent patterns in clinical laboratory parameters according to whether they eventually manifest as DHF or DF. Some variables, such as platelet count, AST and ALT, were different between the two groups as early as the second day of illness, which corresponded to the average day of presentation in the study cohort, and followed different slopes throughout the febrile and critical (defervescence) phases. Other variables, such as hematocrit values, were similar at presentation but diverged during the febrile phase. Elevated liver enzymes, increased hematocrit, and lower platelet counts have previously been proposed as potential indicators of impending plasma leakage [106, 107]. WBC

counts differed between patients with DHF and DF at presentation; however, they were not different between the two groups at the end of the febrile phase or during the critical phase of illness. In addition, WBC count, AST and ALT were able to distinguish patients with dengue from patients with OFI within the first 72 hours of illness. These variables could be useful predictors of dengue illness while awaiting confirmatory serology.

Plasma leakage is considered the hallmark of DHF; however, this indicator of severe dengue disease is not seen until around the time of defervescence [106]. Although significant differences in laboratory findings between DHF and OFI have been described, many studies have relied on single measurements and have not reported the timing of data collection during illness or did not analyze changes in laboratory parameters over the entire course of illness [39]. A strength of our study is the enrollment of subjects within the first three days of illness, and, consequently the availability of clinical data collected daily from each subject over an extended observation period. By highlighting the average trends in clinical laboratory parameters among patients who go on to develop plasma leakage, our study should help enhance the clinical management of patients with suspected dengue illness. Since these laboratory tests depend on only basic clinical laboratory infrastructure, these findings could be widely applied, even in resource-limited settings.

One criticism of population-average models is they assume that each individual follows the same pattern and do not allow for individual variability. Some patients used in the analysis did not have more than two or three days of observation so reliable estimates of individual variability could not be appropriately modeled with these data. However, there is a low amount of variability in the dataset, as indicated by the narrow 95% CI of the mean at each time point (Supplementary Figure 3-2). Additionally, our models tend to fall within the 95% confidence intervals of the actual means during the most common days of hospitalization (illness days 2-5). When the models do fall outside of the 95% CI of the actual mean, this is explained by the individual level data not being transformed to fit a normal distribution nor adjusted for covariates that could result in skewed distributions and invalid representation of the true population mean at each time point. Individuals with outlying values still tend to follow the same slope as that of the population-average model. Accordingly, in actual clinical practice, patients could be identified for aggressive clinical management based on when serial clinical data collection begins and the slopes of the clinical laboratory parameters that each patient follows thereafter.

One limitation of our study is the lack of validation of the results to other study populations, including different dengue-endemic regions and different age groups. Some inconsistencies in clinical and laboratory findings across different locations and age groups were noted in a systematic review of published studies [39]. However, this study used 12 years of systematically collected data to reflect

the clinical course of dengue in a Thai pediatric population where dengue is a significant health problem, so our findings are not subject to the same concerns as studies that reflect a single outbreak or a limited array of viral serotypes/strains. Another limitation is the exclusion of children who first presented later in the illness, who might differ from our study population; however, the patients in our study did manifest a broad spectrum of dengue disease, including substantial degrees of plasma leakage.

We provide population-average models using clinical laboratory data obtained prospectively from a well-defined cohort of pediatric patients in a dengue-endemic region. These models show the average trend in clinical laboratory data throughout the febrile and critical phases of illness in patients diagnosed with DHF, DF, and OFI. The average trends in these models could potentially be used by clinicians to help identify patients at the greatest risk for plasma leakage and better utilize limited hospital resources. This analytical approach can be applied to clinical datasets from other dengue-endemic regions and age groups to describe the average progression of dengue illness in different populations.

Table 3-1 Study sample characteristics for research objective 1

Hospital	N (%)	Gender		Age (years)		Median days ill	
		Males	Females	Mean	95% CI	At presentation	At defervescence (range)
QSNICH	1074 (86.3)	586	488	7.8	7.6, 8.0	2.0	4.0 (1, 10)
DHF	177	103	74	8.8	8.4, 9.3	2 .0	5.0 (1, 9)
DF	326	176	150	8.6	8.3, 8.9	2 .0	5.0 (1, 8)
OFI	571	307	264	7.0	6.7, 7.4	2.0	3.0 (1, 10)
KPPPH	171 (13.7)	105	66	8.9	8.5, 9.3	2.0	4.0 (1, 7)
DHF	54	36	18	9.1	8.3, 9.9	2.0	4.0 (2, 6)
DF	62	30	32	9.3	8.6, 10.0	2.0	4.0 (1, 7)
OFI	55	39	16	8.2	7.4, 9.0	2.0	3.0 (1, 7)
Total	1245	691	554	8.0	7.7, 8.2	2.0	4.0 (1, 7)

Table 3-2 Number of patients in the study with DHF, DF, or OFI at each day of illness

Day of illness*	Number of subjects (Number of subjects at defervescence +24 hours)		
	DHF	DF	OFI
1	58	133	300
2	136 (1)	248 (4)	492 (59)
3	229 (5)	379 (20)	521 (136)
4	225 (25)	363 (51)	361 (111)
5	199 (83)	309 (112)	220 (70)
6	112 (76)	173 (120)	146 (60)
7	33 (25)	43 (31)	84 (50)
8	5 (2)	8 (6)	31 (23)
9	3 (1)	1 (0)	8 (5)
10	2 (2)	--	1(0)
11	1 (1)	--	--
Total patients n=defervescence+24 hours (%) **	231 221 (95.7%)	388 345 (88.9%)	626 522 (83.4%)

* Only five consecutive blood draws were allowed for any given patient

** Represents the total number of patients remaining in the study up to 24 hours after defervescence.

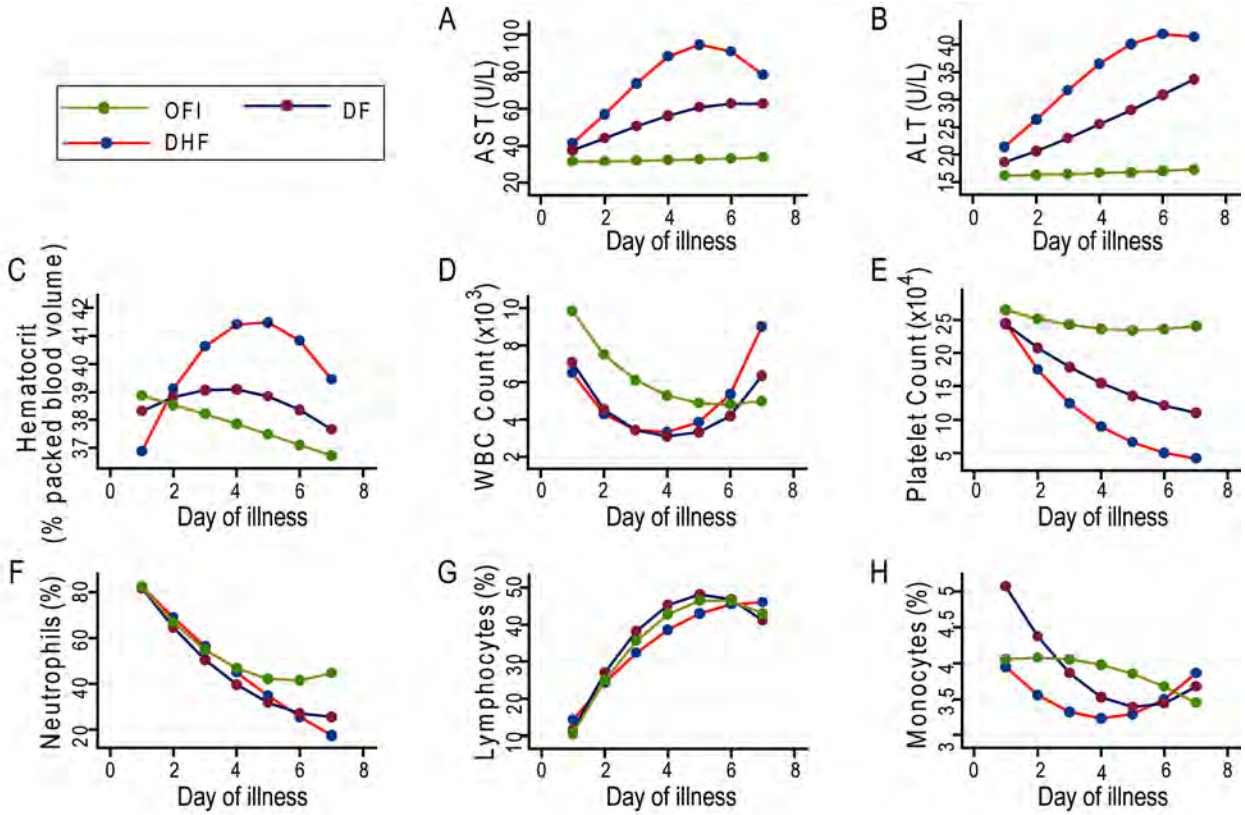
Table 3-3: P-values from adjusted population-average models indicating differences in association between clinical laboratory parameters and diagnosis according to day of illness

Day of Illness	Platelet count			WBC count			Hct			% Monocytes			% Lymphocytes			% Neutrophils		
	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI
1	.90	.02	.001	.12	<.001	<.001	<.001	<.001	.18	.03	.95	.002	.25	.25	.88	.85	.64	.74
2	<.001	<.001	<.001	.17	<.001	<.001	.27	.003	.03	.04	.10	.08	.02	.19	.15	.004	.04	.19
3	<.001	<.001	<.001	.98	<.001	<.001	<.001	<.001	<.001	.02	.003	.48	<.001	<.001	.02	<.001	.11	<.001
4	<.001	<.001	<.001	.04	<.001	<.001	<.001	<.001	<.001	.20	.004	.06	<.001	<.001	.02	<.001	.26	<.001
5	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.64	.07	.12	<.001	.002	.15	.01	<.001	<.001
6	<.001	<.001	<.001	<.001	.03	<.001	<.001	<.001	<.001	.98	.93	.85	.45	.48	.99	.52	<.001	<.001
7	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.01	.85	.39	.45	.90	.36	.39	.02	<.001	<.001

Table 3-3 (*Continued)*

Day of Illness	AST			ALT		
	DHF vs DF	DHF vs OFI	DF vs OFI	DHF vs DF	DHF vs OFI	DF vs OFI
1	.08	<.001	<.001	.04	<.001	.001
2	<.001	<.001	<.001	<.001	<.001	<.001
3	<.001	<.001	<.001	<.001	<.001	<.001
4	<.001	<.001	<.001	<.001	<.001	<.001
5	<.001	<.001	<.001	<.001	<.001	<.001
6	<.001	<.001	<.001	<.001	<.001	<.001
7	.01	<.001	<.001	.04	<.001	<.001

Figure 3-1 Adjusted population-average models for each clinical laboratory parameter among patients with dengue hemorrhagic fever (DHF), dengue fever (DF), or other febrile illnesses (OFI)



Supplemental Table 3-1: Population-average mean values with 95% CI for each clinical laboratory parameter among the diagnostic groups at each day of illness.

Day of Illness	Platelets	WBC	Hct	% Monocytes	% Lymphocytes	% Neutrophils
Illness Day 1						
DHF	244653 (226237, 263789)	6503 (5903, 7165)	36.8 (36.0, 37.6)	3.9 (3.2, 4.6)	14.4 (11.2, 17.6)	82.3 (78.4, 86.1)
DF	243686 (232122, 255532)	7073 (6656, 7517)	38.3 (37.8, 38.7)	5.0 (4.5, 5.6)	11.7 (9.3, 14.0)	81.7 (79.1, 84.3)
OFI	265438 (256338, 274698)	9879 (9428, 10351)	38.8 (38.5, 39.1)	4.0 (3.6, 4.4)	10.7 (8.8, 12.7)	82.4 (80.3, 84.4)
Illness Day 2						
DHF	174911 (164845, 185276)	4317 (4060, 4591)	39.1 (38.5, 40.0)	3.5 (3.1, 3.9)	24.4 (22.5, 26.3)	68.6 (66.3, 71.0)
DF	208406 (201101, 215841)	4510 (4332, 4696)	38.8 (38.4, 39.1)	4.3 (4.0, 4.6)	27.2 (25.8, 28.6)	64.4 (62.8, 66.0)
OFI	252267 (245818, 258801)	7547 (7279, 7826)	38.5 (38.3, 38.7)	4.0 (3.8, 4.2)	25.0 (23.7, 26.2)	66.7 (65.3, 68.1)
Illness Day 3						
DHF	124697 (117217, 132409)	3451 (3271, 3642)	40.5 (40.1, 41.0)	3.3 (2.9, 3.6)	32.4 (30.9, 34.1)	56.1 (54.1, 58.1)
DF	179336 (173053, 185732)	3419 (3294, 3548)	39.0 (38.7, 39.3)	3.8 (3.5, 4.1)	38.5 (37.2, 39.7)	50.3 (48.9, 51.7)
OFI	242972 (236529, 249502)	6138 (5917, 6368)	38.2 (37.9, 38.4)	4.0 (3.8, 4.2)	35.6 (34.3, 36.9)	54.8 (53.4, 56.2)
Illness Day 4						
DHF	89595 (83244, 96181)	3323 (3148, 3507)	41.3 (40.9, 41.8)	3.2 (2.8, 3.5)	38.7 (37.1, 40.3)	44.8 (42.7, 46.8)
DF	155665 (149726, 161719)	3080 (2966, 3199)	39.0 (38.7, 39.3)	3.5 (3.2, 3.8)	45.5 (44.2, 46.8)	39.3 (37.8, 40.8)
OFI	237351 (230996, 244343)	5314 (5110, 5527)	37.8 (37.6, 38.0)	3.9 (3.7, 4.2)	42.8 (41.4, 44.2)	46.6 (45.1, 48.2)
Illness Day 5						
DHF	65960 (60440, 71721)	3823 (3647, 4070)	41.4 (40.9, 41.9)	3.2 (2.9, 3.5)	43.0 (41.4, 44.6)	34.5 (32.5, 36.6)
DF	136680 (130950, 142533)	3299 (3172, 3430)	38.8 (38.5, 39.1)	3.3 (3.0, 3.6)	48.3 (46.9, 49.6)	31.5 (30.0, 33.0)
OFI	235279 (227328, 243368)	4898 (4684, 5123)	37.4 (37.2, 37.7)	3.8 (3.5, 4.1)	46.4 (44.7, 48.1)	42.2 (40.3, 44.0)
Illness Day 6						
DHF	50908 (44630, 57599)	5380 (5004, 5784)	40.8 (40.1, 41.4)	3.4 (3.0, 3.9)	45.4 (43.1, 47.8)	25.5 (22.6, 28.3)
DF	121773 (114752, 129002)	4200 (3987, 4423)	38.3 (37.9, 38.7)	3.4 (2.9, 3.8)	46.8 (44.9, 48.8)	26.8 (24.6, 29.0)
OFI	236711 (225430, 248268)	4806 (4521, 5110)	37.1 (36.7, 37.4)	3.6 (3.2, 4.1)	46.5 (44.0, 49.0)	41.4 (38.7, 44.2)
Illness Day 7						
DHF	42324 (33109, 52669)	9047 (8013, 10213)	39.4 (38.4, 40.3)	3.8 (2.9, 4.7)	46.0 (41.8, 50.2)	17.5 (12.5, 22.5)
DF	110432 (99778, 121627)	6355 (5836, 6920)	37.6 (37.0, 38.2)	3.6 (2.8, 4.4)	41.1 (37.7, 44.5)	25.2 (21.4, 29.1)
OFI	241678 (223942, 260092)	5020 (4575, 5509)	36.7 (36.1, 37.2)	3.4 (2.6, 4.2)	43.0 (38.9, 47.1)	44.4 (40.0, 48.9)

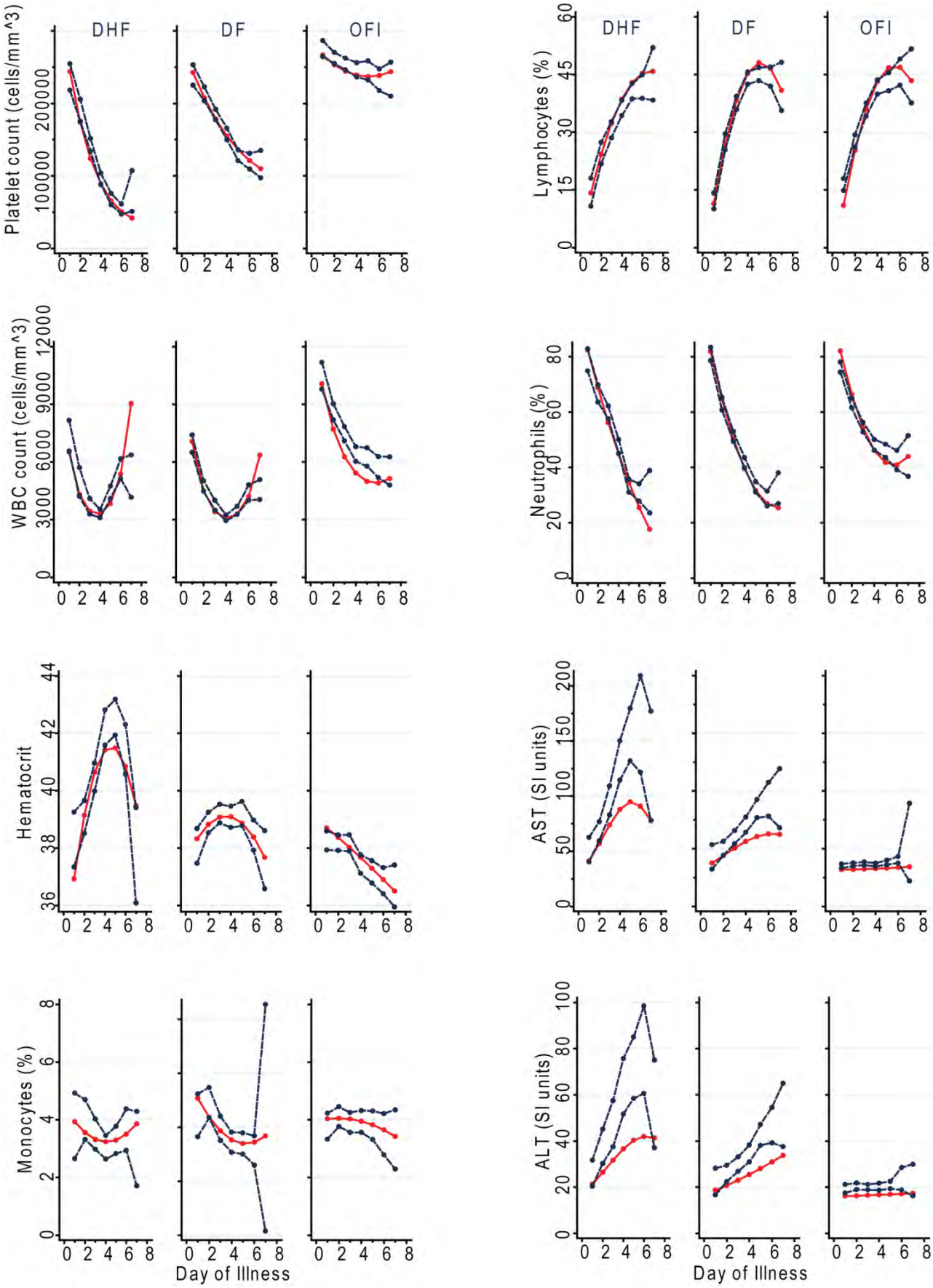
Supplementary Table 3-1 (*Continued*)

	AST	ALT
Illness Day 1		
DHF	41.8 (37.8, 46.5)	21.5 (19.3, 24.1)
DF	37.6 (35.5, 39.9)	18.7 (17.5, 20.0)
OFI	31.6 (30.5, 32.8)	16.2 (15.5, 16.9)
Illness Day 2		
DHF	57.4 (53.0, 62.4)	26.6 (24.3, 29.2)
DF	44.3 (42.3, 46.5)	20.8 (19.7, 22.0)
OFI	31.7 (30.9, 32.6)	16.3 (15.8, 16.8)
Illness Day 3		
DHF	74.5 (68.7, 81.1)	31.8 (29.1, 35.0)
DF	50.9 (48.6, 53.4)	23.1 (21.9, 24.4)
OFI	32.0 (31.1, 32.8)	16.5 (15.9, 17.0)
Illness Day 4		
DHF	89.0 (81.6, 97.5)	36.7 (33.3, 40.6)
DF	56.7 (54.0, 59.6)	25.6 (24.1, 27.1)
OFI	32.3 (31.4, 33.3)	16.7 (16.1, 17.3)
Illness Day 5		
DHF	95.8 (87.5, 105.3)	40.4 (36.5, 44.9)
DF	61.0 (57.9, 64.3)	28.2 (26.5, 30.0)
OFI	32.7 (31.7, 33.8)	16.9 (16.2, 17.6)
Illness Day 6		
DHF	91.9 (82.7, 102.6)	42.2 (37.6, 47.5)
DF	63.2 (59.4, 67.3)	31.0 (28.8, 33.4)
OFI	33.3 (31.9, 34.8)	17.1 (16.3, 18.0)
Illness Day 7		
DHF	79.1 (68.4, 92.5)	41.6 (35.6, 49.2)
DF	62.9 (57.5, 69.1)	33.9 (30.5, 37.8)
OFI	34.0 (31.9, 36.3)	17.4 (16.1, 18.8)

Supplementary Figure 3-1: Representative notation of population-average models used in this study

$$\mu_{ij} = \beta_0 + \beta_1(DF) + \beta_2(OFI) + \beta_3(Illday) + \beta_4(Illday^2) + \beta_5(DF^*Illday) + \beta_6(DF^*Illday^2)$$
$$+ \beta_7(OFI^*Illday) + \beta_8(OFI^*Illday^2) + \beta_9(Age) + \beta_{10}(Male) + \beta_{11}(Hospital)$$
$$+ \beta_{12}(1995) + \beta_{13}(1996) + \beta_{14}(1997) + \beta_{15}(1999) + \beta_{16}(2000) + \beta_{17}(2001)$$
$$+ \beta_{18}(2002) + \beta_{19}(2004) + \beta_{20}(2005) + \beta_{21}(2006) + \beta_{22}(2007)$$

Supplementary Figure 3-2: Comparisons between population-average models and 95% CI of the mean at each day of illness across eight clinical laboratory parameters for each diagnostic group


DHF
DF
OFI
Platelet count (cells/mm^3)
Lymphocytes (%)
WBC count (cells/mm^3)
Neutrophils (%)
Hematocrit
AST (SI units)
Monocytes (%)
ALT (SI units)
Day of Illness
Day of Illness

Appendix 3.A

Box-Cox transformation of AST:

(AST^-0.4262729-1)/-0.4262729

Box-Cox transformation of ALT:

(ALT^-0.4095955-1)/0.4095955

Chapter IV: Classification of dengue illness based on readily available laboratory data

Title: Classification of dengue illness based on readily available laboratory data

Authors: James A. Potts, Stephen J. Thomas, Anon Srikiatkhachorn, Pra-on Supradish, Ananda Nisalak, Suchitra Nimmannitya, Timothy P. Endy, Daniel H. Libraty, Robert V. Gibbons, Sharone Green*, Alan L. Rothman, Siripen Kalayanarooj.

Word count (abstract): 198

Word count (text): 3488

Footnotes page

ABBREVATIONS

ALT- alanine transaminase; AST- aspartate transaminase; AUC- area under the curve; CI- Confidence Interval; Corr. Class- Correctly Classified; DF- dengue fever; DHF- dengue hemorrhagic fever; DSS- dengue shock syndrome; DENV- dengue virus; Hct- hematocrit; KPPPH- Kamphaeng Phet Provincial Hospital; ln, natural logarithm; max- maximum; min- minimum; OFI- other febrile illness; pts- patients; PEI- pleural effusion index; PCR- polymerase chain reaction; $\hat{p}$, predicted probability; Prob. Cutoff- Probability Cutoff; QSNICH- Queen Sirikit National Institute of Child Health; ROC- receiver operator characteristic; WBC- white blood cell; WHO- World Health Organization.

Running head: Classification of dengue illness

Abstract

Reporting of dengue illness is largely based on clinical criteria, however, controversy surrounds WHO guidelines for the diagnosis of dengue hemorrhagic fever (DHF). The aim of this study was to examine dengue illness classification using only clinical laboratory data, without relying on X-ray, ultrasound findings, or calculation of percent hemoconcentration. We analyzed data from a prospective study of children who presented with acute febrile illness to two hospitals in Thailand. Multivariable logistic regression models were used to determine probability cutoffs that best distinguished: 1) DHF vs. dengue fever (DF), 2) DHF vs. DF + other febrile illness (OFI), 3) Dengue vs. OFI, 4) Severe dengue vs. non-severe dengue + OFI. Data from the second hospital were used as a validation set. A total of 1227 patients were included in the analysis (228 DHF, 386 DF, and 613 OFI). The sensitivity of the models ranged from 89.2% (dengue vs. OFI) to 79.6% (DHF vs. DF). The models demonstrated high sensitivity in the validation dataset. These models could be used to calculate a probability of DHF or severe dengue to classify patients based on readily available clinical laboratory data and will need to be validated in other dengue endemic regions.

Dengue is an emerging infectious disease throughout the world and is endemic in tropical and subtropical areas. Recent estimates are that 3.6 billion people (55% of the global population) are at risk of dengue infection and that 70-500 million dengue virus (DENV) infections occur annually, 2.1 million of which are severe dengue illnesses with ~21,000 deaths [24]. DENV is spread by mosquito vectors, usually *Aedes aegypti* or *Aedes albopictus*. Dengue illnesses are usually classified as two distinct entities: dengue fever (DF) and dengue hemorrhagic fever (DHF), with the most severe cases of DHF classified as dengue shock syndrome (DSS). Patients diagnosed with DF typically have a mild febrile illness with two or more of the following: headache, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia [108]. DHF is defined by four diagnostic criteria established by the WHO: fever, thrombocytopenia (platelet count <100,000 cells/mm^3), bleeding tendency (positive tourniquet test or spontaneous bleeding), and plasma leakage (evidence of pleural effusion, ascites or >20% hemoconcentration) [11]. Some patients with DF may exhibit severe illness, but do not meet all four WHO DHF criteria. Likewise, some patients meeting diagnostic criteria for DHF have relatively mild illness, with minimal evidence of plasma leakage and bleeding diathesis not requiring medical intervention.

Previous studies have shown limited agreement between a physician's diagnosis of severe dengue illness and strict adherence to the WHO definition of DHF, and have placed emphasis on a simpler definition of severe dengue illness

[16, 17, 57, 109]. Dengue endemic regions often have limited hospital resources and may not have the capability to perform chest x-rays or ultrasounds to detect pleural effusion or ascites, making it more difficult to fulfill the WHO criteria for the diagnosis of DHF. Changes in hematocrit may be influenced by early fluid resuscitation. In addition, baseline, convalescent, and/or reference hematocrit values are needed to demonstrate hemoconcentration; these values are often missing. Given these scenarios, patients with a severe dengue infection may be classified as DF, if WHO criteria are consistently applied, which may underestimate the global severity of dengue illness. Additionally, resource-poor areas lack essential laboratory support and may be unable to differentiate a DENV infection from other febrile illness (OFI). Previous studies suggest that other or additional indicators not in the WHO definition can distinguish patients with DHF from DF or patients with dengue from patients with OFI [39]. However, among the studies with multivariable models, all of the final models produced had limited generalizability and none of these models were statistically validated.

The aim of this study was to assess the value of laboratory measures physicians use to classify dengue illnesses. Using more readily available clinical laboratory measures, we developed, evaluated, and validated different models of dengue illness classification based on a large, prospectively collected dataset, and compared our models to the WHO classification system and an experienced physician’s diagnosis. We also assessed whether laboratory parameters alone could appropriately classify severe vs. milder dengue illnesses.

Material and methods

Study setting

A longitudinal observational study was conducted at two hospitals in Thailand: 1) the Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok during the years 1994-97, 1999-2002, and 2004-07, and 2) Kamphaeng Phet Provincial Hospital (KPPPH) during the years 1994-97. The study methods have been described elsewhere [44]. In brief, children between the ages of six months and 15 years, presenting with temperature ≥ 38.5˚C for ≤ 72 hours, and no localizing symptoms were eligible for the study. Exclusion criteria included: signs of shock at presentation, chronic disease, or an initial alternate non-dengue diagnosis. Children were admitted to the hospital and monitored throughout their hospital stay until 24 hours after their fever subsided. Written parental informed consent was obtained prior to enrollment. The study protocol was approved by the Institutional Review Boards of the Ministry of Public Health, Thailand, the U.S. Army, and the University of Massachusetts Medical School.

A blood sample was obtained on the day of enrollment and daily thereafter until one day following defervescence or for a maximum of five consecutive blood collections. Clinical laboratory studies included complete blood count and manual WBC differential, serum aspartate transaminase (AST) and serum alanine transaminase (ALT) levels. Serological assays (IgM/IgG ELISA and hemagglutination inhibition assay), viral isolation, and/or RT-PCR were used to

confirm all dengue cases. Patients were observed daily and clinical and laboratory measurements were recorded using standardized data collection forms.

On the day of defervescence, finger-stick hematocrits were measured every six hours for 18 hours in order to capture hemoconcentration. A right lateral decubitus chest x-ray was taken the day following defervescence and a pleural effusion index (PEI) was measured as 100 x (maximum width of right pleural effusion)/(maximum width of right hemithorax). After completion of the case record and careful review of the medical record and laboratory results, a final diagnosis of DF, DHF, or OFI was assigned by an expert physician, who was not directly involved in patient care.

Severe dengue definition

An additional category was constructed to classify patients with severe dengue vs. non-severe dengue. Patients with dengue were classified as having severe dengue if they met any of the following criteria: 1) final diagnosis of DHF grade 3 or 4 (i.e., DHF with shock); 2) significant pleural effusion (PEI>15); 3) required total fluid intervention (oral or intravenous) in any 24-hour period that exceeded maintenance volume + 5% volume deficit [100, 101]; or 4) required any intravenous fluid (IVF) throughout hospitalization (IVF was administered only under stringent circumstances, such as poor intake of oral fluids or signs of shock).

Statistical analysis

Descriptive characteristics, such as diagnosis, age, gender, length of illness at presentation, and which one of the two hospitals patients were admitted to were evaluated using t-tests for continuous variables and Pearson's χ^2 test for categorical variables.

Logistic regression models were constructed using data from QSNICH and validated using data from KPPPH. For each outcome (DF vs. DHF, DHF vs. all others, any dengue vs. OFI, and severe dengue vs. all others), univariate logistic regression was performed on the training dataset (QSNICH) for each of the following variables: maximum values for tourniquet test (number of petechiae per square inch), hematocrit, AST, ALT, % neutrophils, % lymphocytes, and % monocytes, and minimum platelet count and WBC count. Lowess curves were used to assess the distribution of the independent variables and determine the categorization for those with skewed distributions. If the linearity assumption held true, then the variable was used as a continuous variable.

Multivariable models were constructed for each outcome in a manual stepwise procedure based on the univariate indicators with the best area under the receiver operator characteristic (ROC) curve. For variables that were highly correlated, only the variable with the higher area under the curve (AUC) from the univariate analysis was used in the multivariable modeling. Variables that did not remain significant at the alpha=0.05 level were removed from the model and the

variable with the next highest area under the ROC was added into the model. The optimal sensitivity and specificity for each final multivariable model was chosen based on a probability cutoff where the sum of sensitivity and specificity were maximized, the maximum % correctly classified was achieved, and sensitivity remained higher than specificity.

Sensitivity and specificity for each model was also established in the test dataset using the same coefficients and probability cutoff as from the training dataset.

The % agreement between the physician's diagnosis of DHF or the probability of DHF obtained from the models and the WHO definition of DHF was evaluated using Kappa statistics. The optimal probability cutoff from the model was used to determine the proportion of patients that would be defined as DHF where each patient above the cutoff was considered DHF and each patient below the cutoff was considered not to have DHF.

Results

Characteristics of the Study Populations

There were 1384 patients enrolled in the study; 1311 of these patients had a diagnosis of DHF, DF, or OFI (presumed viral, non-dengue illness). For 65 of these patients (55 with OFI, 6 with DF, and 4 with DHF), a day of defervescence could not be assigned and they were excluded from the analysis. Some patients with OFI were discharged from the study prior to defervescence due to a negative PCR. An additional 19 patients failed to have information available on all clinical laboratory variables used in the analysis and were excluded.

There were 1227 patients included in the analysis (228 with DHF, 386 with DF, and 613 with OFI). Patients with DHF were classified by grade as follows: grade 1 (n=59), grade 2 (n=129), grade 3 (n=39), and grade 4 (n=1). There were 1058 patients who completed the study at QSNICH and 169 patients from KPPPH (Table 4-1). The number of patients included in each model is shown in Figure 4-1.

We compared the characteristics of subjects enrolled at QSNICH and those treated KPPPH (Table 4-1). A higher proportion of patients with DHF were enrolled at KPPPH compared to QSNICH (30.8% vs. 16.6%, P<0.001). Patients presenting to KPPPH were also older than those presenting to QSNICH (8.9 [95% CI: 8.4, 9.3] vs. 7.7 [95% CI: 7.5, 7.9], P<0.001). However, at QSNICH, patients with DHF presented later than patients with DF or OFI (2.3 vs. 2.1 and

1.7, respectively, P<0.001) and patients with DF presented later than patients with OFI (2.1 vs. 1.7, respectively, P<0.001). Additionally, at QSNICH, patients with DHF and patients with DF were older than patients with OFI (P<0.001). At KPPPH, a lower percentage of males was seen among patients with DF compared to patients with DHF or OFI (χ^2 P=0.03 and χ^2 P=0.02, respectively).

Univariate analysis

After univariate logistic regression modeling using the QSNICH data, clinical laboratory variables distinguishing each of the different diagnostic categories included minimum platelet count, maximum daily hematocrit (Hct), AST>100, maximum ALT, and a positive tourniquet test (>20 petechiae) (Table4-2). Unit decreases in minimum platelet count and increases in maximum Hct, AST, ALT, and having a positive tourniquet test were associated with having a more severe outcome. The AUC for these variables ranged from 0.92 to 0.54. Additional variables of declining age and decreases in minimum WBC count distinguished between all categories except DHF vs. DF and were associated with having a more severe outcome in each model. The maximum % lymphocytes and % neutrophils distinguished patients with DHF versus DF and those with dengue versus OFI.

Multivariable analysis

Table 4-3 shows the results of multivariable analysis using QSNICH data. Maximum AST and ALT were found to be highly correlated (Pearson correlation=0.86); therefore, we utilized only maximum AST levels in the modeling. Incremental decreases in minimum platelet count (one unit= 25,000 cells/mm^3) and a maximum AST>100 were found to be associated with a more severe outcome in all the final multivariable models. Additionally, increases in the maximum daily Hct were associated with having a diagnosis of DHF when compared to DF or DF+OFI. Increases in maximum % neutrophils, age, and incremental decreases in minimum WBC count (one unit=500 cells/mm^3) were associated with a diagnosis of DHF versus DF. All variables except maximum % lymphocytes and % neutrophils showed an association with having severe dengue illness compared to non-severe dengue or OFI. In addition to distinguishing patients with severe dengue, a tourniquet test of petechiae>=20 was also included in the model for distinguishing patients with serologically confirmed dengue from those with OFI. Younger patients experienced a higher odds of DHF or severe dengue after adjusting for all other variables in the model.

The probability cut-off for each multivariable model was defined as the probability that gave the highest % of patients correctly classified where the sum of sensitivity and specificity was maximized and sensitivity remained higher than specificity. Figure 4-2 illustrates the trade-off between sensitivity and specificity

for each probability cut-off for all of the multivariable models and shows the optimal cutpoint.

Validation of multivariable models

The frequency distribution of each diagnosis (DHF, DF, OFI) according to the optimal probability cut-off for each model is given in Figure 4-3, showing the distribution of diagnoses in both the training (QSNICH) and test (KPPPH) datasets. The sensitivity/specificity analysis, including AUC, positive and negative predictive values, and % correctly classified, and validations with the test data set are indicated in Table 4-4. When applying each model to the test data set, the sensitivity decreased by 2.7% (DHF vs. DF) to 8.9% (DHF vs. All Others). The specificity increased for each validation except Severe dengue vs. All Others, where specificity decreased and sensitivity increased. The % correctly classified decreased by 0.7% (DHF vs. DF) to 4.3% (Severe dengue vs. All Others). The final model distinguishing between patients with dengue and patients with OFI performed the best, giving the highest AUC, specificity, and % correctly classified in both the training and test datasets. Figure 4-4 shows the four validated multivariable models. Coefficients for the calculation of probability for each model were obtained by taking the natural logarithm of the odds ratio for each variable shown in Table 4-3.

Outside of a research setting, the entire spectrum of a patient's illness may not be available. Therefore, each model was applied to the test dataset

using data only at defervescence and at 24 hours after defervescence, the period of greatest risk for plasma leakage and when patients are most likely to require hospitalization. When using data from the test dataset obtained in these later stages of illness, the sensitivity for most models decreased. However, using data obtained only at 24 hours after defervescence, it remained moderately high for DHF vs. DF, increasing from 77% to 86%, and DHF vs. All others at 71%.

Classification from models compared to WHO and Physician diagnosis of DHF

Table 4-5 shows the % agreement and Kappa statistics between the model's classification of DHF, the physician's diagnosis of DHF, and the WHO diagnosis of DHF. When applying the optimal probability cutoffs, the % agreement between the model and the WHO classification of DHF compared to DF was 80.0% (κ=0.58, P<0.001). The % agreement between the model and the WHO definition of DHF improved to 86.2% when compared to all others (DF+OFI, κ=0.60, P<0.001). In both cases, the model had a higher % agreement with a WHO diagnosis of DHF than the physician's diagnosis of DHF.

The model of DHF vs. DF classified 42.0% (258/614) of patients with dengue as having DHF. The physician diagnosed 37.1% (228/614) of patients with dengue as DHF and strict adherence to the WHO definition would have diagnosed 33.1% (203/614) patients with dengue as DHF. The model of DHF vs. all others classified 51.6% (317/614) of patients with dengue as DHF. The model

of severe dengue vs. all others classified 49.2% (302/614) of patients with dengue as having a severe dengue illness, including 203 patients diagnosed by the physician as DF. Only 6.5% (40/614) of patients with dengue were diagnosed by the physician as DHF grade 3 or 4.

Discussion

In this study, we developed models using clinical laboratory indicators to find associations with an expert physician's final diagnosis and WHO criteria of DHF and DF. Although these models rely on laboratory results, these tests are part of standard clinical practice, and the models do not rely on more costly chest x-rays or other measures of capillary leakage. In addition, we established a category of severe dengue illness using indicators known to be associated with DSS. We found a large percentage of patients with dengue would be classified as having a severe dengue illness given their clinical laboratory values throughout their hospitalization. To our knowledge, our study involves the largest set of systematically collected data to address these questions.

An important aspect of this study is the use of a validation dataset. Previous studies have shown that strict adherence to WHO criteria does not identify all severe dengue disease [16, 17, 57, 109]. Some studies have used simpler definitions that may be suitable for identifying severe disease and are less confusing for physicians [13, 109, 110, 111, 112, 113]; however, these different definitions have not been previously validated. The validation dataset in our study involved a

different hospital, with a distinct and more rural catchment area (approximately 350 km northwest of Bangkok). Although patients at KPPPH were older, and more patients were diagnosed with DHF, our models were still a robust fit for these data with little change in the AUC when compared to the training dataset. KPPPH is also in a region where Japanese encephalitis (JE) virus is known to co-circulate and there is routine vaccination against JE [114, 115]. This diversity adds to the validation of the models presented here.

There is controversy surrounding the classification of DHF using the WHO definition. The previous WHO definition of DHF requires strict adherence to four criteria, which includes ambiguous definitions of bleeding tendency and hemoconcentration [11]. By comparing our models to an expert physician's diagnosis of DHF and the WHO definition of DHF, the ambiguity of components of the WHO definition of DHF is abrogated by finding other, objective, indicators that do not depend on convalescent visits or use of expensive tests in resource limited areas. Although the physician in our study used chest x-rays to determine the final diagnosis, our models show high sensitivity in distinguishing DHF from DF and DHF from all others without including chest x-ray or hemoconcentration findings. Our models did not show an improved % agreement over the physician's diagnosis or the WHO definition of DHF. However, this is not surprising when considering that the physician used PEI as an indicator for plasma leakage and the majority of patients diagnosed with DHF had evidence of pleural effusion. Nevertheless, our validated model of DHF vs. DF did show a

high % agreement with both the physician's diagnosis of DHF and the WHO definition (79.5% and 80.0%, respectively).

We showed high sensitivity and specificity in classifying patients with severe dengue defined by shock or need for fluid resuscitation. Some indicators used in the WHO definition of DHF are affected by early hydration, and detection of hemoconcentration and plasma leakage can be difficult [110]. We defined a less-subjective category of "severe dengue" which considered the amount of fluid resuscitation needed, and produced a model with high sensitivity and specificity without using a chest x-ray or hemoconcentration. Although early hydration can still affect the values of hematocrit used in our models, we have removed the requirement for baseline or convalescent hematocrits that may not always be available outside of a research setting. Furthermore, we applied our models to the validation dataset using data obtained at 24 hours after defervescence only and still achieved high sensitivity and specificity for DHF vs. DF and DHF vs. All others. This suggests that our models will be applicable outside of a research setting and perhaps generalizable to patients who present later in illness; however, further studies are needed to test the generalizability of these models in these study populations and in different geographic settings with different illness prevalence estimates. .

Bleeding tendency is often indicated by a positive tourniquet test, but the test method is not always harmonized among treatment centers (SJT-

unpublished data) and confusion arises as to which cutoff should be used to indicate a positive test [110]. We found that a cutoff >=20 petechiae yielded a higher AUC when compared to a cutoff of >=10 petechiae (data not shown). However, across all multivariable models, a positive tourniquet test showed an association only in patients with dengue compared to patients with OFI. This supports use of this indicator to identify patients with dengue, whereas the tourniquet test has performed less well for distinguishing DHF from DF [15, 44, 113, 116].

Minimum platelet count and maximum Hct were associated with DHF and are part of the WHO definition. Although platelet count and Hct are included in the model, not all patients diagnosed by physicians with DHF had a platelet count below 100,000 and most patients with DHF had plasma leakage detected by pleural effusion rather than by hemoconcentration. Our models have no thresholds for particular variables but instead use a combination of clinical laboratory variables to calculate a probability which can be used to classify patients.

The main limitation to our study is the exclusion of children who first presented during later stages of their illness. This study design limits the number of patients who developed severe dengue illness. Our study was also limited to Thailand's pediatric population. However, this reflects what is seen in Southeast Asia, where the majority of dengue cases are in the pediatric population.

Our study identified clinical indicators that could be used to calculate a patient's probability of DHF or severe dengue illness. From our models, patients with DF or mild dengue illness and OFI have a uniformly low probability of DHF or severe dengue. The probability calculated from our models could be used to classify patients when other indicators, such as a chest x-ray or convalescent sera, are unavailable. These models are not meant to guide clinical management but can be used for retrospective classification of dengue illness in the absence of standard indicators of plasma leakage. Such classification is not only important for research purposes but may enable greater accuracy of epidemiologic reporting of dengue disease severity in affected resource poor endemic regions.

ACKNOWLEDGEMENTS

Author affiliations: Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, Massachusetts (James A. Potts, Anon Srikiatkhachorn, Daniel H. Libraty, Sharone Green, Alan L. Rothman); Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand (Stephen J. Thomas, Ananda Nisalak, Robert V. Gibbons); Queen Sirikit National Institute of Child Health, Bangkok, Thailand (Pra-on Supradish, Suchitra Nimmannitya, Siripen Kalayanarooj); University of New York, Upstate Medical University, Syracuse, New York (Timothy P. Endy).

This work was supported by the National Institutes of Health (grant number NIH-P01AI34533); Centers for Disease Control and Prevention Office of the Director (grant number 1R36CK00123-01 to J.A.P.); and the Military Infectious Disease Research Program. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the U.S. Government. The authors have no conflicting financial interests.

Table 4-1 Study population characteristics

Hospital	N (%)	Gender		Age (years)		Days ill at presentation	
		Male	Female	Mean	95% CI	Mean	95% CI
QSNICH	1058 (86.2)	579	479	7.7 *	7.5, 7.9	2.0	1.9, 2.0
DHF	176 (16.6)[a]	102	74	8.8	8.3, 9.3	2.3	2.2, 2.4
DF	324 (30.6)[a]	175	149	8.6	8.2, 8.9	2.1	2.0, 2.2
OFI	558 (52.7)[a]	302	256	6.9	6.6, 7.1	1.7	1.7, 1.8
KPPPH	169 (13.8)	102	67	8.9*	8.4, 9.3	2.0	1.8, 2.0
DHF	52 (30.8)[b]	35	17	9.1	8.3, 9.9	2.0	1.8, 2.2
DF	62 (36.7)[b]	29	33	9.3	8.5, 10.0	2.2	
OFI	55 (32.5)[b]	38	17	8.3	7.5, 9.1	1.9	2.0, 2.4
							1.8, 2.1
Total	1227	681	546	7.9	7.7, 8.1	1.9	1.9, 2.0

Abbreviations: CI, Confidence Interval; DF, dengue fever; DHF, dengue hemorrhagic fever; OFI, other febrile illness; KPPPH, Kamphaeng Phet Provincial Hospital. QSNICH, Queen Sirikit National Institute of Child Health

* P<.001 for age between the QSNICH and KPPPH cohorts

[a] Percentage of diagnosis in QSNICH cohort

[b] Percentage of diagnosis in KPPPH cohort

Table 4-2 Univariate analysis of maximum and minimum values of clinical laboratory variables using the training QSNICH dataset

	DHF vs. DF			DHF vs. All Others			Dengue vs. OFI			Severe Dengue vs. All Others		
	Odds ratio	95% confidence interval	AUC	Odds ratio	95% confidence interval	AUC	Odds ratio	95% confidence interval	AUC	Odds ratio	95% confidence interval	AUC
Age (years)	1.03	0.97, 1.09	0.52	1.15	1.09, 1.21 **	0.62	1.21	1.16, 1.27 **	0.66	1.13	1.07, 1.20 **	0.60
Gender[a]	1.17	0.81, 1.70	0.52	1.17	0.84, 1.62	0.52	1.05	0.83, 1.34	0.51	1.22	0.86, 1.72	0.52
Min. Platelets[b]	0.46	0.39, 0.54 **	0.84	0.42	0.37, 0.48 **	0.92	0.54	0.50, 0.58 **	0.90	0.49	0.43, 0.55 **	0.90
Min. WBC count[c]	1.01	0.94, 1.09	0.52	0.75	0.70, 0.80 **	0.74	0.56	0.52, 0.60 **	0.90	0.72	0.66, 0.78 **	0.76
Max. Hct. (%)	1.29	1.21, 1.37 **	0.73	1.39	1.32, 1.47 **	0.79	1.24	1.19, 1.29 **	0.69	1.39	1.32, 1.47 **	0.78
Max. % Monocytes												
<=5[d]												
>5/<=10	0.85	0.57, 1.28		0.83	0.58, 1.19		0.97	0.70, 1.18		0.84	0.57, 1.24	
>10	0.67	0.39,1.14	0.54	0.87	0.54, 1.41	0.52	1.37	0.95, 1.96	0.53	1.25	0.78, 2.02	0.54
Max. % Lymphocytes												
<=40[d]												
>40/<=50	0.58	0.34, 1.01		1.18	0.76, 1.86		2.15	1.52, 3.04 **		1.88	1.16, 3.03 *	
>50/<=60	0.42	0.24, 0.73 *		1.08	0.67, 1.73		2.99	2.08, 4.29 **		1.37	0.81, 2.31	
>60	0.52	0.30, 0.88 *	0.58	1.14	0.74, 1.77	0.52	2.39	1.70, 3.34 **	0.61	1.32	0.80, 2.15	0.56

Max. % Neutrophils												
<=60[d]												
>60/<=75	2.04	1.23, 3.38 *		1.46	0.93, 2.31		0.70	0.49, 0.99 *		1.21	0.75, 1.97	
>75	1.61	1.001, 2.58	0.57	0.92	0.60, 1.42	0.55	0.45	0.33, 0.62 **	0.58	0.92	0.59, 1.44	0.53
Max. AST[e]												
<=100[d]												
>100	4.61	3.12, 6.83 **	0.68	14.10	9.72, 20.45 **	0.77	39.36	20.54, 75.41**	0.70	10.71	7.3, 15.64 **	0.75
Max. ALT[e]												
<=50[d]												
>50/<=100	3.02	1.87, 4.87 **		6.97	4.49, 10.83 **		8.71	5.09, 14.88 **		4.98	3.11, 7.98 **	
>100	3.28	1.99, 5.43 **	0.63	9.05	5.61, 14.59 **	0.70	21.68	9.35, 50.27 **	0.65	12.47	7.66, 20.28 **	0.71
Max. Tourniquet Test[f]												
<=20[d]												
>20	1.59	1.01, 2.50 *	0.54	6.02	4.03, 9.00 **	0.70	10.22	7.70, 13.57 **	0.76	6.27	4.06, 9.70 **	0.70

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DF, dengue fever; DHF, dengue hemorrhagic fever; Hct, hematocrit; max, maximum; min, minimum; OFI, other febrile illness; QSNICH, Queen Sirikit National Institute of Child Health; WBC, white blood cell

*P<0.05

**P<0.001

[a] Reference group is females

[b] One unit = 25,000 cells/mm^3

[c] One unit = 500 cells/mm^3

[d] Reference group

[e] Units/dL

[f] Number of petechiae per square inch

Table 4-3 Multivariable models among the training QSNICH dataset

	DHF vs. DF		DHF vs. All Others		Dengue vs. OFI		Severe Dengue vs. All Others (non-severe dengue + OFI)	
	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval
Age (years)	0.90	0.83, 0.99 *					0.88	0.81, 0.96 **
Min. Platelets[a]	0.53	0.45, 0.62 **	0.51	0.44, 0.59 **	0.68	0.63, 0.74 **	0.61	0.53, 0.69 **
Min.WBC count[b]	1.19	1.05, 1.34 *			0.68	0.63, 0.74 **	0.88	0.80, 0.98 **
Max. Hct. (%)	1.19	1.10, 1.29 **	1.17	1.09, 1.25 **			1.23	1.14, 1.32 **
Max.% Neutrophils								
<=60[c]								
>60/<=75								
>75	2.10	1.10, 3.99 *			0.56	0.31, 0.99 *		
	1.89	1.03, 3.49 *			0.55	0.33, 0.93 *		
Max. AST[d]								
<=100[c]								
>100	2.32	1.43, 3.78 **	3.40	2.14, 5.40 **	8.99	4.07, 19.85 **	2.31	1.45, 3.68 **
Max. Tourniquet Test[e]								
<=20[a]								
>20								
					3.80	2.51, 5.75 **		

Abbreviations: AST, aspartate aminotransferase; DF, dengue fever; DHF, dengue hemorrhagic fever; Hct, hematocrit; max, maximum; min, minimum; OFI, other febrile illness; QSNICH, Queen Sirikit National Institute of Child Health; WBC, white blood cell

*P<0.05

**P<0.001

[a] One unit = 25,000 cells/mm^3

[b] One unit = 500 cells/mm^3

[c] Reference group

[d] Units/dL

[e] Number of petechiae per square inch

Table 4-4 Validation of training QSNICH multivariable models to the KPPPH test dataset using the optimal probability cutoff

	DHF vs. DF		DHF vs. All Others		Dengue vs. OFI		Severe Dengue vs. All Others	
	QSNICH	KPPPH	QSNICH	KPPPH	QSNICH	KPPPH	QSNICH	KPPPH
AUC	0.87	0.86	0.94	0.91	0.96	0.93	0.92	0.86
Sensitivity	79.6	76.9	85.8	76.9	89.2	81.6	83.0	83.3
Specificity	79.3	80.6	85.4	85.5	88.4	90.9	82.3	76.7
(+) predictive value	68.0	76.9	53.9	70.2	87.1	94.9	44.3	49.2
(-) predictive value	87.8	80.6	96.8	89.3	90.1	70.4	96.6	94.4
% Corr. Class	79.6	78.9	85.4	82.8	88.7	84.6	82.4	78.1

Abbreviations: AUC, area under the curve; Corr. Class, Correctly Classified; DF, dengue fever; DHF, dengue hemorrhagic fever; KPPPH, Kamphaeng Phet Provincial Hospital; OFI, other febrile illness; QSNICH, Queen Sirikit National Institute of Child Health

Table 4-5 Comparison of % agreement and Kappa statistics between the final models, WHO DHF criteria, and the physician's final diagnosis

	Model vs. WHO			Model vs. Physician			Physician vs. WHO		
	% Agreement	Kappa	P-value	% Agreement	Kappa	P-value	% Agreement	Kappa	P-value
DHF vs. All Others									
Prob. cutoff = 0.19	86.2	0.60	<0.001	85.5	0.59	<0.001	92.4	0.74	<0.001
DHF vs. DF									
Prob. cutoff = 0. 35	80.0	0.58	<0.001	79.5	0.57	<0.001	84.6	0.66	<0.001

Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; Prob. Cutoff, Probability cutoff; WHO, World Health Organization

Figure 4-1 Flow chart of study

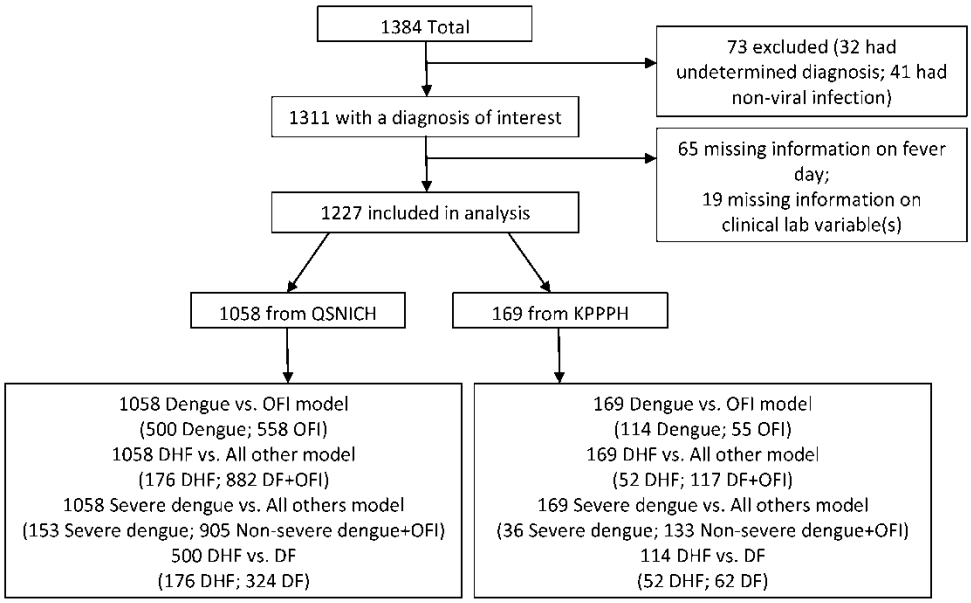


Figure 4-2 Sensitivity and specificity of multivariable logistic regression models from the training dataset

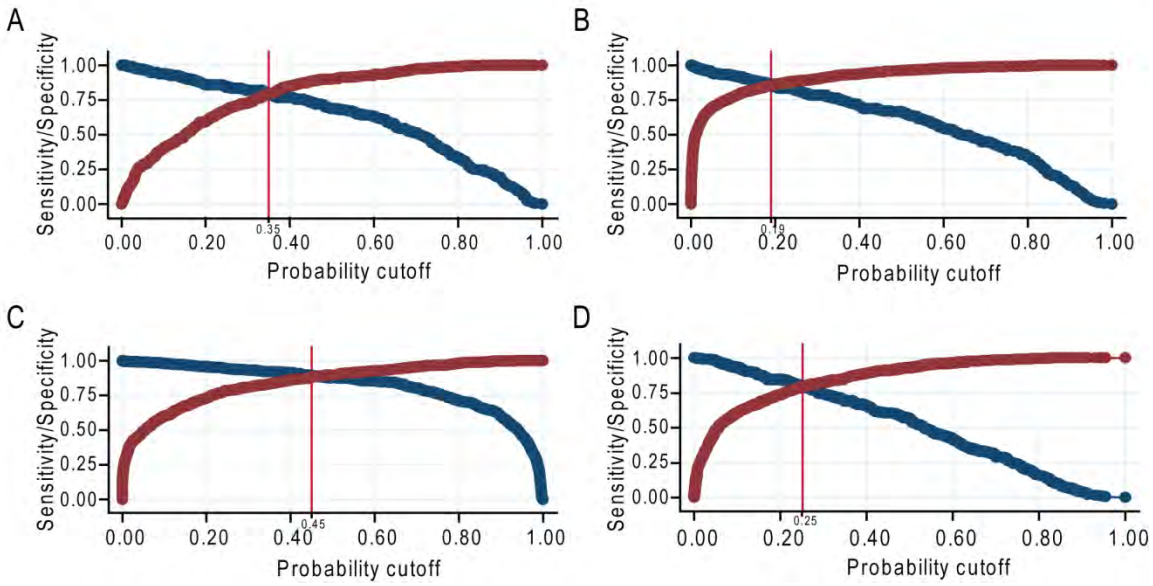


Figure 4-3 Distribution of calculated probabilities among each diagnosis for each model

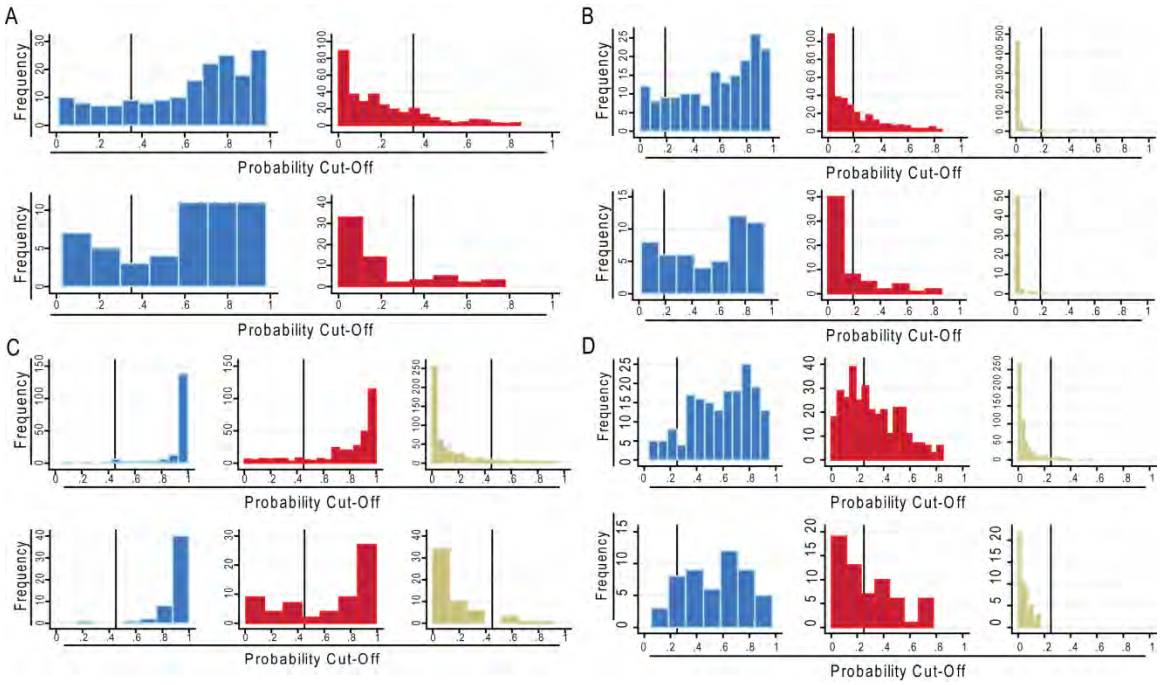


Figure 4-4 Validated multivariable probability models for classifying patients with dengue

(A) Equation 1: DHF vs. DF

$$ln\frac{\hat{p}}{1-\hat{p}} = -6.85 - .74(Neuts_{>60\&\leq 75}) + .64(Neuts_{>75}) - .10(Age)$$
$$- .64(Platelets/25{,}000) + .84(AST_{>100}) + .18(Hct)$$
$$+ .17(WBC/500)$$

(B) Equation 2: DHF vs. All others

$$ln\frac{\hat{p}}{1-\hat{p}} = -5.82 - .67(Platelets/25{,}000) + 1.22(AST_{>100}) + .16(Hct)$$

(C) Equation 3: Dengue vs. OFI

$$ln\frac{\hat{p}}{1-\hat{p}} = 4.61 - .39(Platelets/25{,}000) - .38(WBC/500) + 2.20(AST_{>100})$$
$$+ 1.33(PositiveTT) - .59(Neuts_{>60\&\leq 75}) - .60(Neuts_{>75})$$

(D) Equation 4: Severe dengue vs. All others

$$ln\frac{\hat{p}}{1-\hat{p}} = -6.79 - .50\left(\frac{Platelets}{25{,}000}\right) - .12\left(\frac{WBC}{500}\right) + .21(Hct) + .83(AST_{>100})$$
$$- .13(Age)$$

ABBREVIATIONS

DENV- dengue virus; DF- dengue fever; DHF- dengue hemorrhagic fever; DSS- dengue shock syndrome; OFI- other febrile illness; QSNICH- Queen Sirikit National Institute of Child Health; KPPPH- Kamphaeng Phet Provincial Hospital; PEI- pleural effusion index; AST- aspartate transaminase; ALT- alanine transaminase; Hct- hematocrit; WBC- white blood cell; ROC- receiver operator characteristic; AUC- area under the curve; PCR- polymerase chain reaction; min- minimum; max- maximum; pts- patients.

FIGURE LEGENDS

Figure 4-1. Flow chart of study. Boxes show the total number of patients enrolled in the study, reasons for exclusion from the analysis, and the number of patients from the training dataset (QSNICH) and the test dataset (KPPPH) used in each model. Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; KPPPH, Kamphaeng Phet Provincial Hospital; OFI, other febrile illness; QSNICH, Queen Sirikit National Institute of Child Health

Figure 4-2. Sensitivity and specificity of multivariable logistic regression models from the training dataset. Sensitivity is indicated by the solid blue line; specificity is indicated by the solid red line; the optimal probability cutoff is indicated by a solid vertical line. (A) DHF vs. DF with an optimal probability cutoff=0.35; (B) DHF vs. All others with an optimal probability cutoff=0.19; (C) Dengue vs. OFI with an optimal probability cutoff=0.45; (D) Severe dengue vs. All

others with an optimal probability cutoff=0.17. Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; OFI, other febrile illness.

Figure 4-3. Distribution of calculated probabilities among each diagnosis for each model. Blue=DHF; Red=DF; Gold=OFI. In each section, the top panel represents the distribution of probabilities in the training dataset (QSNICH) and the bottom panel represents the distribution of probabilities in the test dataset (KPPPH). (A) DHF vs. DF where the optimal probability cutoff=0.35; (B) DHF vs. All others where the optimal probability cutoff=0.19; (C) Dengue vs. OFI where the optimal probability cutoff=0.45; (D) Severe dengue vs. All others where the optimal probability cutoff=0.25. Abbreviations: DF, dengue fever; DHF, dengue hemorrhagic fever; KPPPH, Kamphaeng Phet Provincial Hospital; OFI, other febrile illness; QSNICH, Queen Sirikit National Institute of Child Health.

Figure 4-4. Validated multivariable probability models for classifying patients with dengue. (A) DHF vs. DF; (B) DHF vs. All others; (C) Dengue vs. OFI; (D) Severe dengue vs. All others.

*Note: Categorical variables (% neutrophils, % monocytes, positive tourniquet test, AST, and gender) are 0/1.

Abbreviations: AST, aspartate aminotransferase; DF, dengue fever; DHF, dengue hemorrhagic fever; Hct, hematocrit; ln, natural logarithm; Neuts, % neutrophils; WBC, white blood cell.

Chapter V: Classification of dengue disease severity among pediatric Thai patients using early clinical laboratory indicators

James A. Potts[1], Alan L. Rothman[1], Anon Srikiatkhachorn[1], Robert V. Gibbons[2], Stephen Thomas[2], Pra-on Supradish[3], Stephenie C. Lemon[4], Daniel H. Libraty[1], Sharone Green[1*], Siripen Kalayanarooj[3]

[1] University of Massachusetts Medical School, Worcester, Massachusetts, Department of Immunology and Virology

[2] Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

[3] Queen Sirikit National Institute of Child Health, Bangkok, Thailand

[4] University of Massachusetts Medical School, Worcester, Massachusetts, Department of Preventative and Behavioral Medicine

[*] Corresponding Author

Abstract

Background: Dengue virus is endemic in tropical and sub-tropical resource-poor countries. Dengue illness can range from a nonspecific febrile illness to severe disease, Dengue Shock Syndrome (DSS), in which patients develop circulatory failure. Earlier diagnosis of severe dengue illnesses would have a substantial impact on the allocation of health resources in endemic countries.

Methods and findings: We compared clinical laboratory findings collected within 72 hours of fever onset from children presenting to one of two hospitals in Thailand. Classification and regression tree analysis was used to develop diagnostic algorithms using different categories of dengue disease severity to distinguish between patients at elevated risk for developing a severe dengue illness and those at low risk.

A diagnostic algorithm using WBC count, percent monocytes, platelet count, and hematocrit achieved 97% sensitivity to identify patients who went on to develop DSS while correctly excluding 48% of non-severe cases. Addition of an indicator of severe plasma leakage to the WHO definition led to 99% sensitivity using WBC count, percent neutrophils, AST, platelet count, and age.

Conclusions: This study identified two easily applicable diagnostic algorithms using early clinical indicators obtained within the first 72 hours of illness onset. The algorithms have high sensitivity to distinguish patients at

elevated risk for developing severe dengue illness from patients at low risk, which included patients with mild dengue and other non-dengue febrile illnesses. Although these algorithms need to be validated in other populations, this study highlights the potential usefulness of specific clinical indicators early during the course of illness.

Author summary

Patients with severe dengue illness typically develop complications in the later stages of illness, making early clinical management of all patients with suspected dengue infection difficult. An early prediction tool to identify which patients will have a severe dengue illness will improve the utilization of limited hospital resources in dengue endemic regions. We performed classification and regression tree (CART) analysis to establish predictive algorithms of severe dengue illness. Using a Thai hospital pediatric cohort of patients presenting within the first 72 hours of a suspected dengue illness, we developed diagnostic decision algorithms using simple clinical laboratory data obtained on the day of clinical presentation. These algorithms correctly classified almost all patients who developed a severe dengue illness while excluding upwards of 50% of patients with mild dengue or other febrile illnesses. Our algorithms utilized white blood cell counts, percent white blood cell differentials, platelet counts, elevated aspartate aminotransferase, hematocrit, and patient's age. If these algorithms can be validated in other regions and age groups, they will help in the clinical management of patients with suspected dengue illness who present within the first three days of fever onset.

Background

Dengue fever (DF) and dengue hemorrhagic fever (DHF), the more severe form of dengue illness, are re-emerging viral diseases [10]. Dengue is endemic in resource-poor countries in tropical and subtropical areas. Dengue viruses are transmitted through the bite of an infected mosquito [7]. Illnesses caused by dengue viruses can range from a nonspecific febrile illness, as in most DF cases, to more severe illness with bleeding, thrombocytopenia, and plasma leakage, in cases of DHF [11]. DHF with circulatory failure defines DHF grades 3 and 4, also termed dengue shock syndrome (DSS) [11]. However, strict adherence to WHO criteria for diagnosis of DHF has been difficult and some researchers have established different categories of severe dengue illnesses [13, 16, 110, 111].

Dengue has a substantial economic impact in developing countries [28, 30]. Individuals and families are impacted by lost wages, cost of seeking care, cost of treatment, missed school, and extended effects of recovery [28, 29, 30, 31, 32]. Prevention and control strategies have been poorly implemented or unsustained and thus largely ineffective [20, 117].

Currently, there is no licensed vaccine or anti-viral against dengue. The treatment for patients with suspected dengue is supportive care consisting of rehydration and anti-pyretics [11]. Patients with suspected dengue are often hospitalized for close monitoring. Plasma leakage occurs around the time of

defervescence. Prior to this critical phase, it has proven difficult to differentiate mild vs. severe dengue illness. Ideally, only severe cases of DF and DHF should be hospitalized. However, there are no diagnostic/prognostic tools available to distinguish severe dengue from non-severe dengue or other febrile illness (OFI) during the early stages of illness. Such tools could improve clinical practice by decreasing the number of un-necessary hospitalizations, improving utilization of limited hospital resources to treat more severely ill patients, improving outcomes of severely ill patients by administering needed care earlier, and improving the capability of physicians in developing or rural areas to make a more accurate early diagnosis.

We conducted a prospective study of Thai children with acute febrile illness, consistent with dengue, enrolled from an early stage of illness onset [44]. We applied classification and regression tree (CART) analysis to this dataset to distinguish patients with severe dengue illness from those with mild dengue illness and OFI. CART analysis is a non-parametric analytic tool that has many advantages over logistic regression models [96, 118]. CART was used to establish a diagnostic decision tree using clinical laboratory variables and patient characteristics collected at presentation.

Methods

Study Setting. A longitudinal observational study was conducted at two hospitals in Thailand: 1) the Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok during 1994-97, 1999-2002, and 2004-07, and 2) the Kamphaeng Phet Provincial Hospital (KPPPH) during 1994-97. The study methods have been described in detail elsewhere [44]. In brief, children between the ages of six months and 15 years presenting with temperature ≥ 38˚C for no more than 72 hours and no localizing symptoms were eligible for enrollment with parental consent. Exclusion criteria included: signs of shock at presentation, chronic illness, or an initial alternate non-dengue diagnosis. Subjects were admitted to the hospital and monitored until 24 hours after defervescence. The study protocol was approved by the Institutional Review Boards of all participating institutions.

A blood sample was obtained on the day of enrollment and daily thereafter until discharge or for a maximum of five consecutive blood collections. Serological assays (IgM/IgG ELISA and hemagglutination inhibition assay), virus isolation, and/or RT-PCR were used to confirm all dengue cases. Patients were observed and daily clinical and laboratory measurements were recorded using standardized data collection forms.

After defervescence (2 consecutive temperatures below 38˚C), serial finger-stick hematocrits were measured to capture hemoconcentration. A right

lateral decubitus chest x-ray was taken the day following defervescence and a pleural effusion index (PEI) was measured as 100 x (maximum width of right pleural effusion)/(maximum width of right hemithorax). After completion of the case record, an expert physician, who was not directly involved in patient care, assigned a final diagnosis of DF, DHF, or OFI based upon chart review following WHO guidelines [11].

Categories of Dengue Illness Severity. Since not all DHF cases are severe, and not all DF cases are mild, we applied several different categories of dengue disease severity using data from each patient's entire hospital course : 1) dengue shock syndrome (DSS, as defined by WHO criteria); 2) DSS or PEI>15; 3) DSS or required intravenous fluid; 4) DSS or platelet count <=50,000 anytime during illness; 5) DSS or received fluid intervention (oral or intravenous) in any 24-hour period that exceeded maintenance volume + 5% volume deficit [100, 101].

Clinical Laboratory Variables and Patient Characteristics. The input variables used for establishing each tree were platelet count, hematocrit, WBC count, percent monocytes, percent lymphocytes, percent neutrophils, AST, ALT, tourniquet test (+/-), age, and gender, all of which were obtained on the day of presentation.

Statistical Analysis. Descriptive characteristics of the study sample were compared using t-tests and Pearson's χ^2. CART analysis was performed using SPSS Answer Tree 3.0 software (see Supplementary Methods) [119]. Age, gender,

and clinical laboratory data on the day of presentation were used to establish diagnostic decision trees to distinguish patients with severe dengue illness from those with non-severe illness or OFI. Stopping rules were: 1) no terminal node could contain <5% of the original sample size, 2) no more than 5 levels per tree, and 3) a minimum improvement in impurity of .0001.

Additional analyses were performed to examine differences in diagnostic trees according to the day of presentation among the low risk, non-severe group. The final trees selected were those that had minimum misclassification of severe dengue illness in low risk nodes (high sensitivity) and maximum correct classification of non-severe dengue and OFI in low risk nodes (high specificity). In each terminal node, patients were classified as low risk or elevated risk of severe dengue illness where optimal sensitivity could be achieved. For all analyses, sensitivity was weighted more heavily than specificity by using misclassification cost ratio of 1:10 severe dengue vs. non-severe. Each tree was validated using the k-fold cross validation method [95, 97]. We used k=5 in our analysis.

Results

Study Sample. In total, 1384 patients were enrolled in the study. Of these, 1311 had a final diagnosis of DHF, DF, or OFI. Of the remaining 73 patients, 32 had an undetermined diagnosis due to lack of convalescent blood sample for serology, and 41 had a presumed non-viral infection. An additional 81 patients were missing one or more variables of interest on the day of presentation and were excluded from the analysis.

Table 5-1 describes the 1230 patients included in the analysis. Among these, 208 had a final physician diagnosis of DHF (53 grade 1, 118 grade 2, 36 grade 3, 1 grade 4), 374 had DF, and 648 had OFI. Secondary infections accounted for 81.9% of all dengue infections (74.6% of DF cases and 95.2% of DHF cases). The most prevalent serotype of dengue infections was DENV1 (40.7%). Table 5-2 indicates the number of patients with severe dengue based on different definitions.

Classification Tree for Dengue Shock Syndrome. Trees were generated for each of the five categories of severe dengue illness. As summarized in Table 5-2, and shown in Figure 5-1 (Tree 1), the tree that provided the best discrimination on the day of presentation categorized severe dengue as DSS. The initial splitting variable in the tree is WBC count; other variables in the tree include percent monocytes, platelet count, and hematocrit. The tree resulted in five terminal nodes, of which three are considered low risk and two are considered elevated risk. The three low risk nodes are 1) WBC>8500, 2)

WBC<=8500 and percent monocytes >9.0, and 3) WBC<=8500, percent monocytes<=9.0, platelet count >160200, and hematocrit>40%. The two nodes considered elevated risk of severe dengue were 1) WBC<=8500, percent monocytes <=9.0, and platelet count <=160200 (64.9% of patients with severe dengue) and 2) WBC<=8500, percent monocytes<=9.0, platelet count >160200, and Hct<=40 (32.4% of patients with severe dengue).

A total of 576 (48.3%) patients with non-severe dengue were classified correctly in the low risk group at the cost of misclassifying one patient who later manifested DHF grade 3. The initial splitting variable correctly classified 384 (32%) of the patients with non-severe dengue. The patients that were correctly classified as low risk included 63.7% of all OFI, 32.1% of all DF, 41.5% of all DHF grade 1, and 17.8% of all DHF grade 2. Patients with non-severe dengue illness were more likely than patients with OFI to be classified as elevated risk of severe dengue (70.1% of non-severe dengue versus 36.3% of OFI).

Among the 617 (51.7%) patients with non-severe illness that were classified as elevated risk, the median day of presentation was 72 hours after illness onset and the average length of hospital stay was 6.8 days; patients with non-severe dengue that were correctly classified had a median day of presentation of 48 hours after illness onset and an average length of hospital stay of 7.3 days. To assess differences according to the day of presentation, the tree was applied using data from patients with non-severe illness at 72 hours

among patients who were still febrile. In this group of low risk patients, the percent correctly classified as low risk decreased slightly from 48% to 44% (data not shown).

Classification Tree using DSS or PEI>15. Figure 5-2 shows a diagnostic decision tree in which severe disease was defined as DHF grade 3 or 4 or PEI>15 (Tree 2). This disease categorization added nine patients with DHF grade 1 and 37 patients with DHF grade 2. No patients diagnosed with OFI or DF had a PEI>15. For this tree, the initial splitting variable was WBC count; other variables in the tree include AST, percent neutrophils, platelet count, and age. There are eight terminal nodes, of which five are considered low risk and three are considered elevated risk. The five low risk nodes are 1) WBC>13700, 2) WBC<=13700, AST 36-50, and platelet count >282000, 3) WBC<=13700, AST 36-50, platelet count <=282000, and age<=6.75, 4) WBC<=13700, AST<=35, and percent neutrophils<=68%, and 5) WBC<=13700, AST<=35, percent neutrophils>68%, and platelet count>291000. The three elevated risk nodes are 1) WBC<=13700, AST>50 (72.3% of patients with severe dengue), 2) WBC<=13700, AST 36-50, platelet count <=282000, and age>6.75 (16.9% of patients with severe dengue), and 3) WBC<=13700, AST<=35, percent neutrophils>68%, and platelet count<=291000 (9.6% of patients with severe dengue).

This tree correctly classified 505 (44%) patients with non-severe dengue at the cost of misclassifying one patient with severe dengue. The misclassified patient was diagnosed with DHF grade 2 and had PEI of 25.8. All patients with DHF grade 3 or grade 4 were correctly classified in this tree as elevated risk of severe dengue. Among the 505 patients correctly classified as low risk of severe dengue, 380 were OFI (58.6% of OFI), 105 were DF (28.1% of DF), and 20 were DHF grade 1 or 2 (16.0% of non-severe DHF). Patients with non-severe dengue illness were more likely than patients with OFI to be classified as elevated risk. When the tree was applied using data from patients with non-severe illness at 72 hours, the percent of non-severe cases correctly classified as low risk increased from 44% to 50%.

Classification Tree using Other Categories of Dengue Disease Severity. We assessed the generalizability of our trees using other categories of dengue disease severity (Table 5-2). For example, when applying the tree that was generated using DSS as the only criterion for dengue disease severity (Tree 1) to different categories of severity, the percentage of patients with a severe dengue illness that were misclassified as low risk ranged from 12.5% to 17.6% and the percentage of patients with non-severe illness that were correctly classified ranged from 48.6% to 52.1%. All additional trees (Trees 3-5) had moderate specificity but limited sensitivity (Table 5-2), with a misclassification of severe dengue as low risk ranging from 34.9% to 42.6% and a correct classification of

non-severe illness ranging from 72.1% to 81.5%. Each tree shared the same initial splitting variable of WBC count (data not shown).

Discussion

Early diagnosis of severe dengue illness not only has the potential to reduce morbidity and mortality, but could also reduce the economic impact of dengue illness by decreasing the duration of hospitalization and the number of patients who will develop shock. We identified two diagnostic algorithms using early clinical laboratory indicators and patient characteristics that could distinguish patients with severe dengue from those with non-severe dengue or other febrile illnesses within the first 72 hours of illness.

When applying these trees to other (broader) categories of disease severity, a high sensitivity was still achieved. Previous studies have shown that modified definitions of dengue disease severity have better agreement with a treating physician's assessment when compared to strict adherence to WHO criteria [13, 14, 15, 16]. For any classification of dengue disease severity utilized, a high proportion of patients with non-severe dengue or other febrile illness were correctly classified as low risk of severe dengue (Table 5-2). These data suggest that patients classified as 'elevated risk' of severe dengue based on these algorithms should be treated and managed more aggressively; in comparison, our data suggest that patients classified as 'low risk' of severe dengue could be safely managed on an outpatient basis.

The single patient with severe dengue that was misclassified in Tree 1 presented within the first 24 hours of illness, had an initial WBC count of 13700, and was diagnosed with DHF grade 3. Five other patients with severe dengue in Tree 1 also presented within the first 24 hours and yet were correctly classified as elevated risk. When we further investigated the effect of day of presentation by using day 3 data from all non-severe cases, we found that day of presentation had little effect on the sensitivity of Trees 1 and 2 (within the first 72 hours); Tree 1 still correctly classified 44% of the non-severe cases as low risk of severe dengue infection and, in Tree 2, the percent correctly classified as low risk increased from 44% to 50%.

Many of the variables used in our decision algorithms have been shown to distinguish between patients with dengue and patients with OFI in other settings [39]. Trees 1 and 2 have an initial splitting variable of WBC count, which reinforces the reported utility of this variable in distinguishing severe dengue illness within the first days of illness [39, 120, 121, 122, 123]. Both trees included nodes using platelet count as the splitting variable. Thrombocytopenia is a hallmark of severe dengue disease, although it frequently occurs in DF as well [11]. Platelet counts are able to distinguish between patients with dengue and OFI [39, 122]. However, when producing a tree using a minimum platelet count of <=50,000 as part of the categorization of severity (Severity category 4), the tree misclassified 42.5% of patients with severe dengue (Table 5-2). These data suggest that

thrombocytopenia is not a specific marker for severe disease in the early febrile phase of dengue illness.

One criticism of CART analysis is that the cutoff values may not be clinically meaningful. However, when we re-defined the cutoff values for Trees 1 and 2 the results maintained high sensitivity. For example, in Tree 1 when we rounded platelet count to 160,000, the results remained the same. In Tree 2, when we rounded the cutoffs of platelet count to 290,000 and 280,000, percent neutrophils to 70%, and age to 7, the tree correctly classified 45.9% of the non-severe cases while still achieving 94.0% sensitivity for severe cases. Interestingly, many of the cutoff lab values in our decision trees fall within the 'normal' range; this suggests that established 'normal' ranges for routine laboratory tests have low sensitivity to detect clinically relevant changes.

Tanner and colleagues published an analysis establishing dengue decision trees; however, their analysis was based on only three WHO-defined DHF cases and it was unclear if these three cases met other objective criteria for severity [99]. In contrast, our study has 37 cases of more severe WHO-defined DSS and 171 cases of DHF grade 1 or 2. We also applied other criteria that could classify patients with dengue as having severe illness. Their study included a platelet count of <50,000 as part of the definition of severe dengue, and the resulting tree was limited in its sensitivity (82.6%) [99]. Although the tree had a high specificity, sensitivity is a more important clinical consideration in the detection of

severe disease. A more recent decision tree study by Lee and colleagues found a history of clinical bleeding, serum urea, and serum protein to distinguish between patients with DF and patients with DHF; however, both studies have limited clinical utility as a predictive algorithm for patients with severe dengue because virologic confirmation of dengue infection is not known at presentation [98, 99]. Our study identifies those with severe dengue illness among all suspected dengue cases.

Our study is subject to some limitations. First, our study included only pediatric patients from two hospitals in Thailand. However, because the majority of dengue cases in Thailand and other regions of Southeast Asia are children, our findings are clinically relevant [8, 11, 21]. Further validation using datasets in other dengue endemic regions is needed to establish the clinical utility of our algorithms in other populations. Additionally, because our study enrolled patients only during the initial 72 hours of illness, we cannot make any conclusions regarding the sensitivity and specificity of these classification trees at later time points in illness.

We provide two decision tree algorithms using 12 years of systematically collected clinical data from a well-defined cohort of pediatric patients in a dengue-endemic region. Our algorithms have minimal misclassification of WHO-defined DSS cases among all patients with suspected dengue infection who present within the first 72 hours of illness. These algorithms also have minimal

misclassification of other severe dengue illnesses using different categorizations of severity. A robust, validated decision algorithm can be easily implemented in resource limited settings to identify patients who are at risk for developing a more severe dengue illness and limit the number of unneeded hospitalizations.

Table 5-1 Study sample characteristics, in the total sample and by final diagnosis

	Age (mean, 95% CI, years)	Gender (m:f ratio)	Days ill at presentation mean (median)	Length of observational period (24 hours after defervescence) mean(median)*
DHF				
Grade 1 (n=53)	8.4 (7.4, 9.4)	2.3	2.0 (2)	6.2 (6)
Grade 2 (n=118)	9.1 (8.5, 9.6)	1.3	2.3 (2)	6.4 (6)
Grade 3/4 (n=37) **	8.5 (7.6, 9.4)	0.9	2.4 (2)	7.3 (7)***
DF (n=374)	8.6 (8.4, 8.9)	1.1	2.1 (2)	6.3 (6)
OFI (n=648)	7.1 (6.8, 7.3)	1.2	1.8 (2)	5.3 (5)

* Includes only those patients who remained in the study until the end of the observational period (50 DHF grade1; 110 DHF grade 2; all DHF grade 3 and 4; 327 DF; 495 OFI)

** Only 1 patient had DHF grade 4; this subject was combined with DHF grade 3 for analysis

*** DHF grade 3 or 4 had longer observational periods when compared to patients with DHF grade 1 and 2, DF, or OFI ($p<.001$).

Table 5-2 CART analysis using different categories of severe dengue illness*

Tree	Outcome variable for tree	Outcome variable for evaluation of tree	% Misclassified severe dengue (# classified as low risk/total severe)	% Correctly classified non-severe (# classified as low risk/total non-severe)
1	Severity Category 1	Severity Category 1	2.7% (1/37)	48.3% (576/1193)
		Severity Category 2	16.9% (14/83)	49.1% (563/1147)
		Severity Category 3	16.8% (25/149)	51.1% (552/1081)
		Severity Category 4	12.5% (20/160)	52.1% (557/1070)
		Severity Category 5	17.6% (12/68)	48.6% (565/1162)
2	Severity Category 2	Severity Category 1	0.0% (0/37)	42.2% (504/1193)
		Severity Category 2	1.2% (1/83)	44.0% (505/1147)
		Severity Category 3	9.4% (14/149)	45.5% (492/1081)
		Severity Category 4	5.0% (8/160)	46.5% (498/1070)
		Severity Category 5	8.8% (6/68)	43.0% (500/1162)
3	Severity Category 3	Severity Category 3	34.9% (52/149)	72.1% (779/1081)
4	Severity Category 4	Severity Category 4	42.5% (68/160)	81.5% (872/1070)
5	Severity Category 5	Severity Category 5	42.6% (29/68)	77.3.0% (898/1162)

* Severity Category 1: DSS (DHF grade 3 or 4)

Severity Category 2: DSS or PEI>15

Severity Category 3: DSS or required intravenous fluid resuscitation during hospitalization

Severity Category 4: DSS or had min platelet count <=50,000 during hospitalization

Severity Category 5: DSS or received fluid intervention (oral or intravenous) >5% volume deficit above maintenance

Figure 5-1 Decision tree using DSS as 'severe dengue'

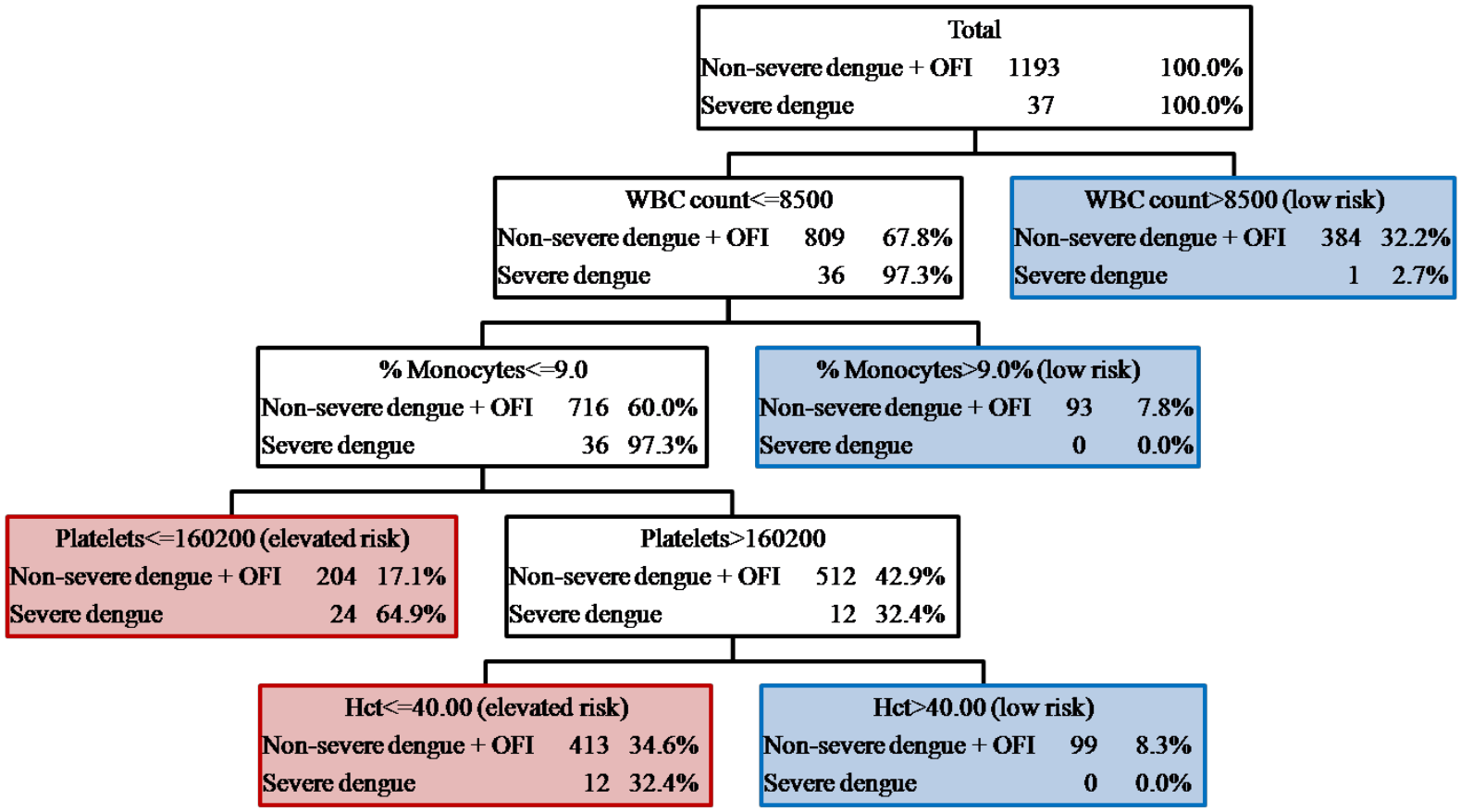
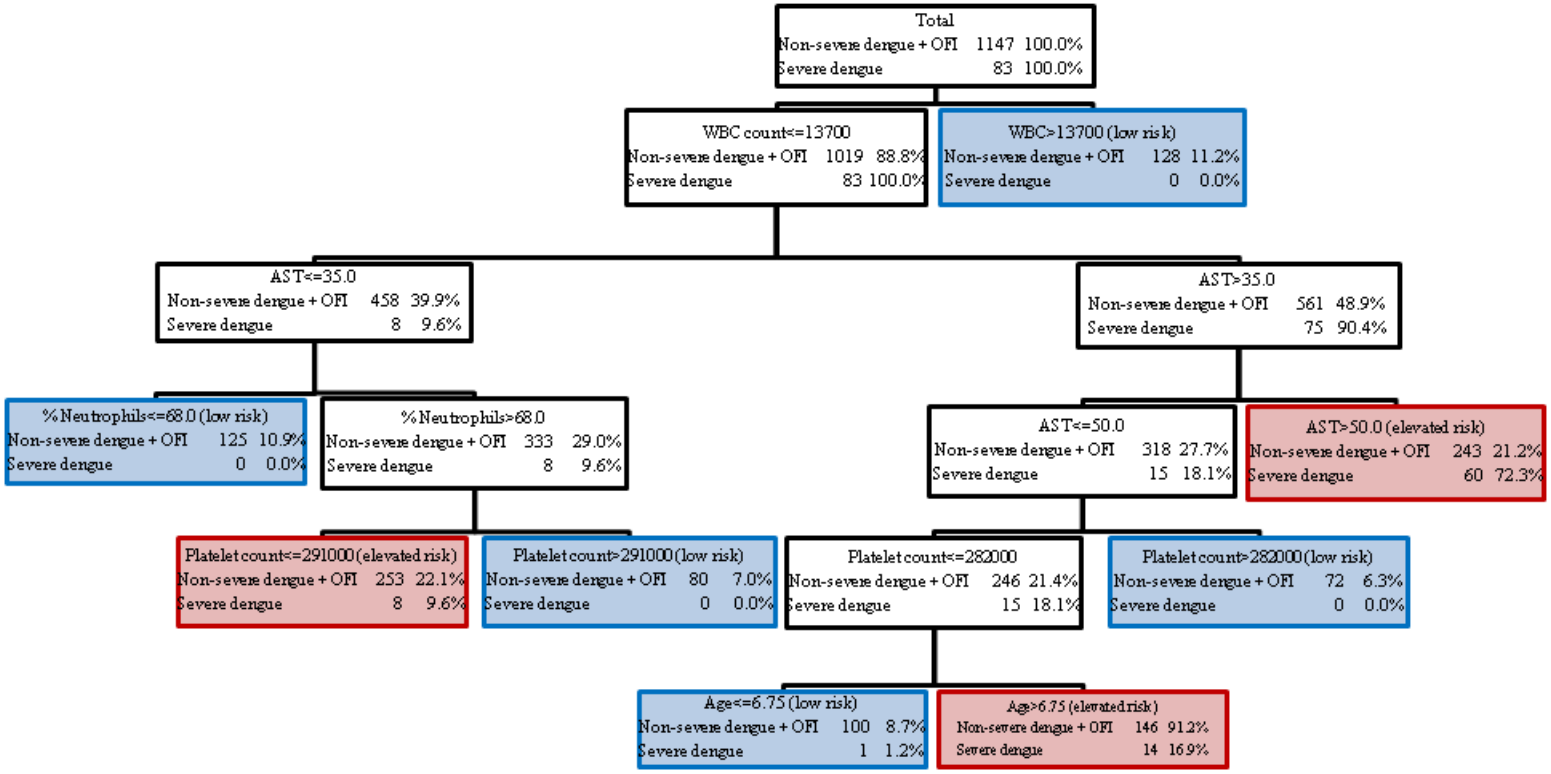


Figure 5-2 Decision tree using DSS or dengue+PEI>15 as 'severe dengue'



Supplementary Methods

Classification and Regression Tree Analysis

CART is a non-parametric statistical technique that is used to partition data into mutually exclusive and exhaustive subgroups (nodes) where patients within each partitioned node are as homogenous as possible in terms of the outcome measure of interest (diagnosis) [95]. The data is partitioned according to the independent variable that provides the greatest difference in an impurity function with respect to the outcome measure. At each point in the process, the root (parent nodes) split into two child nodes. The Gini impurity function was used for this analysis, which uses the proportion of the dependent variable in the parent nodes and a weighted average of patients in the resulting child nodes to calculate impurity at each split [95, 96, 97]. The data continue to be split until at least one stopping rule is met. The final nodes, terminal nodes, are mutually exclusive and exhaustive.

For validation of trees, the dataset was divided into five subsets where each subset acts as a validation dataset while the remaining subsets are used as a training dataset. This process is repeated five times until each subset has acted as the validation dataset. All CART analyses were performed using SPSS Answer Tree 3.0 software [119].

Figure legends

Figure 5-1: CART algorithm #1 for identifying patients who subsequently developed severe dengue (defined as WHO criteria for dengue shock syndrome, DSS) using clinical laboratory data obtained within the first three days of illness. Each node is shown with the selected splitting variable, the number of patients with severe/non-severe or OFI, and the proportion of each from the parent node. Terminal nodes are marked as ‘elevated risk’ of severe dengue illness, outlined in red, and ‘low risk’ of severe dengue, outlined in blue.

Figure 5-2: CART algorithm #2 for identifying patients who subsequently developed severe dengue (defined as WHO criteria for dengue shock syndrome, DSS, or dengue with significant pleural effusion) using clinical laboratory data obtained within the first three days of illness. Pleural effusion index (PEI) >15 was used as the criterion for significant pleural effusion. Each node is shown with the selected splitting variable, the number of patients with severe/non-severe or OFI, and the proportion of each from the parent node. Terminal nodes are marked as ‘elevated risk’ of severe dengue illness, outlined in red, and ‘low risk’ of severe dengue, outlined in blue.

Chapter VI Conclusions

The objective of this dissertation was to advance the knowledge and improve the clinical understanding of dengue illness. Using the available data, we applied various statistical methods to elucidate the clinical course of dengue illness and how the different disease manifestations compare with each other and other febrile illnesses.

Dynamics of clinical laboratory parameters distinguish among Thai pediatric patients with different dengue disease severity

The research objective of this study (Chapter III) was to describe the temporal dynamics of clinical laboratory parameters throughout the febrile phase of patients with suspected dengue illness. Using population average models, we established mathematical functions of these parameters for each diagnostic group (DHF, DF, OFI). We identified differences in the population-average means as well as differences in the slopes among the various diagnostic groups. These indicators could be used to evaluate trends in several clinical laboratory parameters in patients with suspected dengue illness and identify those subjects whose parameters follow the trends of patients who eventually develop DHF. These patients could be managed more aggressively in order to reduce the likelihood of shock resulting from severe plasma leakage.

These findings contribute to the existing literature (presented in Chapter I) by applying longitudinal techniques to evaluate changes over the course of

illness in patients with dengue. Many of the recent studies did not evaluate changes over time, or did not apply appropriate statistical methodology, to do so. However, these findings should be validated in other populations and age groups.

The development of plasma leakage is considered to be the hallmark feature of DHF. There are several ways to effectively measure plasma leakage which have been previously validated [106, 124]. However, patients with DHF don't develop plasma leakage until later in the course of illness, typically around the time of defervescence. Until defervescence, patients with DHF have similar clinical symptoms as patients with DF or OFI and therefore can be hard to distinguish these from other groups. This analysis has allowed us to assess which variables may be useful in identifying patients with DHF. Furthermore, the analysis shows when in the course of illness these variables might be most useful to distinguish subjects with a particular diagnosis (DHF, DF, or OFI).

Classification of dengue illness based on readily available laboratory data

The goal of this research objective (Chapter IV) was to establish and validate logistic regression models that can be used to classify subjects with dengue without the need for expensive or unobtainable measurements of plasma leakage (e.g., chest X-ray or hemoconcentration). The study design utilized two different hospitals, which allowed us to establish our models using data from one hospital and then validate this model using data from the other hospital.

For patients with dengue to be classified as DHF, they must meet all four WHO criteria for DHF: 1) fever, 2) hemorrhagic manifestations (positive tourniquet test, epistaxis, hematemesis and/or melena), 3) thrombocytopenia (100,000/mm^3 or less), and 4) evidence of plasma leakage (pleural effusion, ascites, or hemoconcentration>20% [11]. There is, however, controversy surrounding this classification system. In typical clinical care in resource-poor dengue-endemic countries, patients may not meet the criteria for plasma leakage or thrombocytopenia simply because of a failure to perform the relevant laboratory and radiographic tests. Strict adherence to the classification criteria may therefore lead to underreporting of DHF, leaving a false impression of the burden of disease. Lastly, dengue disease may not be dichotomous but rather cover a spectrum of disease severity in which some patients with DF are severely ill and some patients with DHF have mild illness.

The purpose of the models presented in chapter IV was to provide classification criteria for patients with dengue illness in resource-poor areas where routine measures of plasma leakage are not always available. For example, Clark, et al estimated the total direct cost to the family of a child with a symptomatic dengue episode in Thailand in 2005 to be 1,026 Baht (US$24) [29]. This estimate did not include the cost of travel to and from the hospital; many patients in rural areas of Thailand may have to travel long distances to obtain an appropriate level of treatment. The cost of a chest x-ray in a government-sponsored pediatric hospital in Thailand is approximately 150 Baht and the cost

of a chest ultrasound is approximately 1500 Baht (Dr. Pra-on Supradish, personal communication). Although Thailand has government sponsored healthcare, many families choose private care [29]. For these reasons, subjects may not be willing to pay for a chest x-ray if it is unnecessary nor be willing to return for a convalescent hematocrit.

The main findings of this research objective are presented in the logistic regression models shown in Fig 4.4. When these models were validated, they maintained a high sensitivity and percentage of subjects correctly classified as DHF, DF, or OFI when compared to a physician's diagnosis using chest x-ray or hemoconcentration to make a diagnosis (Table 4-4). Additionally, the simplicity of these models allow for the easy classification of patients with suspected or confirmed dengue illness in the absence of a measure of plasma leakage. These models can be used to classify patients as DHF based on readily available laboratory data during illness and still achieve close to the same sensitivity and specificity as a physician using chest X-ray findings and other WHO criteria to make a diagnosis of DHF. Our classification models can be helpful for researchers/epidemiologists trying to monitor dengue outbreaks and conduct dengue disease surveillance in resource poor areas.

Additionally, these logistic regression models do not require particular variables to be dichotomized (i.e. platelets<100,000) but instead allow for a combination of variables to calculate a patient's probability of having dengue. For

example, a subject previously classified as having DF due to a platelet count of 101,000 can be classified as DHF using the model because other variables beside platelet count contribute to the calculation of this probability.

An important future direction for this research would be to test these logistic regression models in different populations. Patients presenting later in the course of illness may not have received clinical care during the febrile phase of their illness, which could result in more abnormal clinical laboratory values than seen among subjects in this study. This could result in different probability cutpoints for these patients. Additionally, it would be of interest to obtain inter-rater reliability among physicians diagnosing the same patients and make direct comparisons to our classification models.

Prediction of dengue disease severity among pediatric Thai patients using early clinical laboratory indicators

The purpose of this analysis (Chapter V) was to establish a predictive diagnostic tool that clinicians can use to identify subjects who present with a suspected dengue infection who will eventually develop severe dengue disease. First, we defined five categories of dengue disease severity: 1) dengue shock syndrome (DSS, as defined by WHO criteria); 2) DSS or PEI>15; 3) DSS or required intravenous fluid; 4) DSS or platelet count <=50,000 anytime during illness; 5) DSS or received fluid intervention (oral or intravenous) in any 24-hour period that exceeded maintenance volume + 5% volume deficit [100, 101]. We then

established diagnostic trees for each category. Lastly, we applied each tree to all of the categories and calculated the sensitivity and specificity.

We found that subjects diagnosed as DHF grade 3 or 4 could be identified with high sensitivity based on four clinical laboratory values collected on the day of enrollment (Fig 5.1). Only one subject with DSS was misclassified as “low risk” in this tree. Additionally, when we defined severe dengue as either DSS or dengue with PEI>15, a decision tree using five clinical laboratory values identified all but one subject classified as having severe dengue (Fig 5.2). When these two algorithms were applied to different categories of dengue disease severity, the sensitivity in identifying those subjects classified as severe remained high.

These two algorithms yield promising results in allowing clinicians to easily identify and better manage patients with impending severe dengue disease. Potentially, clinicians could triage patients identified as “high risk” and begin aggressive fluid replacement therapy. Patients identified as “low risk” could be managed on an outpatient basis.

CART analysis provides easily interpretable results and has many advantages over more complicated analytical approaches with complex output. Additional analytical techniques were applied to this data set for comparison to the CART analyses presented in chapter V:

1) Various logistic regression models, using the same diagnostic outcomes, were established and optimal probability cut-points were selected for each

model. The probability cutpoints were selected to achieve the same sensitivity as the CART analysis to compare the performance of logistic regression models to CART analysis. For example, Tree 1 misclassified one subject with DSS, so the probability cutpoint selected for the logistic regression model was where one subject with DSS was misclassified. For this logistic regression model, the percent of subjects correctly classified was 34.4% compared to 48.3% from the CART analysis shown in Figure 5-1. In this example, a higher percentage of correctly classified subjects was achieved in the CART analysis compared to the logistic regression model while maintaining the same sensitivity in both approaches.

2) Boosted-CART (boostrap aggregation CART) analysis was performed using Stata [125]. Boosted-CART analysis is a modification of CART that uses a weighted-average that is applied to subjects who are misclassified; it is often applied to CART analyses that yield only 2 nodes [119, 126]. When this technique was applied to our data (Figure 6-1), the results were similar to the CART analyses presented in chapter V in which WBC count explained the majority of variation in the model (44.6%).
3) Chi-squared automatic interaction detector (CHAID) is a type of homogenous classification analysis, similar to CART, that produces trees which can have more than two categories at each branch (not binary). This type of analytical tool, used by Tanner et al [99], uses an F test for continuous target variables and chi-squared test if the target variable is

categorical [119]. This technique was applied to our dataset; however, the resulting tree (Figure 6-2) was not as robust as the CART analyses shown in chapter V. A modification of CHAID called *exhaustive* CHAID combines categories of statistically different nodes found in CHAID and computes adjusted p-values to find a stronger association with the target variable which yields a better split [119]. *Exhaustive* CHAID was not used with our dataset and further exploration of this analytical technique may be of interest.

Study Strengths and limitations

Limitations:

The major limitation affecting all three studies is that the data set was limited to a pediatric cohort from Thailand. This means that the results may not be generalizable to other age groups (e.g., adults) or to other dengue endemic populations. However, for research objective 2 (Chapter IV), we stratified the data set by hospital to create separate training and validation datasets. There were differences between the cohorts from the two; KPPPH had a higher proportion of DHF than QSNICH, study participants from KPPPH were older, KPPPH is a more rural area, and there is co-circulation of Japanese encephalitis (JE) in Kamphaeng Phet but not in Bangkok. However, this validation method (training and test datasets) could not be applied to the analyses in Chapter V due

to the small numbers of patients with severe dengue. Instead, the K-fold validation procedure was used for these analyses and is described in Chapter V.

Another limitation is that the physician's final diagnoses were assigned by a single expert clinician. Therefore, the statistical models may not show equivalent sensitivity and specificity when compared to diagnoses assigned by other physicians. It is likely that there would not be complete concordance in diagnoses assigned by different physicians, even with an established guideline provided by the WHO, as there was imperfect agreement between our expert physician's diagnosis of DHF and the WHO criteria for DHF (Chapter IV). Although the overwhelming majority of subjects diagnosed as DHF by the physician also met the WHO criteria for DHF, there was not complete agreement. This is also evident in the literature where researchers have established their own criteria for diagnosing a patient with dengue that they felt was severe but failed to meet all four WHO criteria for DHF [13, 16, 110, 111].

The criteria for enrollment in this study excluded patients who were severely ill at presentation. This was done to capture patients in the earlier phases of illness in order to establish descriptive and predictive models of dengue disease. However, this also limited the number of patients in our study with severe outcomes. Future studies should validate our findings in patients who present later in the course of illness with severe disease.

Different DENV serotypes can cause a slightly different spectrum of disease [4, 6]. Furthermore, the prominent serotype differs each year [5]. Changes in the health care system in Thailand, such as the 30 baht universal health coverage program implemented in 2001, affected our enrollment so that more patients were enrolled in the early years of the study than in subsequent years [127]. The 30 baht healthcare system expands government-funded healthcare to uninsured individuals and cost each patient no more than 30 baht ($0.84 US) per outpatient or inpatient visit [127]. However, models were adjusted for year of enrollment where necessary (Chapters III and IV).

Strengths:

This study used 12 years of systematically collected data from a diverse pediatric cohort in a dengue endemic region. Subjects were followed from the first three days of illness until 24 hours after their fever subsided allowing for a longitudinal description of dengue illness with little missing data (>85% of subjects had no missing data). Based on the systematic review of the literature presented in chapter I, very few studies have evaluated clinical laboratory parameters over time. Our unique study design allowed us to utilize appropriate statistical methodology to analyze trends and establish classification and predictive algorithms using clinical laboratory parameters among patients with a suspected dengue illness.

Additionally, the study design utilized patients hospitalized at two pediatric medical centers. This enabled us to test the generalizability of models established in chapter IV and address limitations in the existing literature. The characteristics of the subjects were different between the two hospitals, thus allowing for a more robust validation of our models. To our knowledge, no other studies have utilized a validation approach to test their models [39].

Another strength of our study is the involvement of international experts in the field of dengue research. The physician providing the final diagnoses of subjects, Dr. Suchitra Nimmannitya, is an internationally-known dengue expert and made substantial contributions to the establishment of the original WHO guidelines for dengue illness. This limits the variability of diagnosis between hospitals and across study years.

Final conclusions

The studies presented make substantial contributions to the existing literature regarding the description, classification, and prognostication of dengue illness from the perspective of the clinical laboratory. Additionally, these studies utilized analytical approaches that can be used by others and offer new ideas for further exploration of clinical laboratory data among patients with suspected dengue illness. These studies provide promising results of predictive algorithms and classification tools that might be used in resource-poor dengue endemic regions to improve hospital resource utilization in the management of dengue

outbreaks. The priorities for future research should focus on overcoming some of the limitations in these analyses; for example, validation in other populations and age groups, and additional testing with different diagnosing physicians.

In addition to advancing the research presented and to overcome some of its limitations, a focus of future research should be placed on prevention and awareness of dengue. Lack of sustained vector control is one of the reasons for the resurgence of dengue, as well as other viruses carried by the *Aedes aegypti* mosquito such as yellow fever and chikungunya [128]. The very successful efforts to eliminate yellow fever, led by William Gorgas during the building of the Panama Canal, focused on eliminating breeding sites of *Aedes aegypti* mosquitoes [129]. The efforts led by Fred Soper eliminated yellow fever and dengue transmission from most of Central and South America in the 1950s and 1960s [129]. However, these efforts were not sustained because of competing resources as there was no longer a perceived need [18]. This ultimately helped lead to the resurgence of *Aedes aegypti* populations in the Americas in the 1970s, and, eventually, the hyperendemicity of dengue serotypes in the 1980s, causing outbreaks of severe disease into the 21st century. Also, control of dengue and yellow fever was merged with malaria control, and the government response to the resurgence in dengue outbreaks was to use ultra low volume insecticide sprays, which have proved to be ineffective against the *Aedes aegypti* mosquito [130]. A successful vector-response program would be one that can be sustained, mimics previous successes, and has active community involvement

coordinated through public health officials. Community-wide elimination of Aedes mosquito breeding sites is vital; however, community responses are typically highest only during or after an outbreak [19].

Other causes for the resurgence in dengue illnesses are rapid urbanization and globalization. Gubler found a positive correlation between global population growth and the incidence of DF/DHF, particularly in urban areas (see Figure 6-3) [128, 131]. Rapid and unplanned urbanization has led to a lack of water resources in densely populated areas. This has caused individuals to store rainwater in uncovered barrels, thus providing a habitat for Aedes mosquitoes to lay eggs. Urbanization has also led to inadequate sewer and waste management systems, which has been shown to increase *Aedes aegypti* populations [20].

Globalization has caused a disease that was once confined to tropical areas and travelers to be widespread throughout the world. Modern transportation allows individuals who may be infected with dengue virus to travel from country to country in a matter of hours, well within the incubation period of the virus. Travelers from non-endemic areas who visit endemic areas and then return home can initiate autochthonous dengue transmission, which can cause large outbreaks in highly susceptible populations [26, 132]. This also increases the risk of establishing hyperendemicity and its association with severe dengue illness.

Another cause for the resurgence in dengue illnesses is climate instability. Increases in temperature lead to storage of water in open containers, especially in tropical resource-poor areas, increasing the number of potential *Aedes aegypti* breeding sites. One study conducted in Veracruz, Mexico used autoregressive models to assess the association between the El Nino Southern Oscillation cycle and the number of reported dengue cases from 1995-2002 [133]. They found that increases in sea-surface temperature, minimum weekly temperature, and rainfall were associated with increases in the reported number of dengue cases.

The Centers for Disease Control and Prevention (CDC) have recently updated the National Notifiable Infectious Disease List to include dengue fever [134]. With the resurgence and global spread of dengue and given the concerns over the WHO criteria for DHF, proposed revisions to the WHO criteria have recently been published [135]. The new WHO guidelines for classification of dengue illnesses have only two categories, “dengue” and “severe dengue,” and thus have a reduced emphasis on the DHF vs. DF classification [135]. However, data supporting the new classification scheme have not been peer-reviewed or validated against the existing literature. The updated WHO guidelines state that the clinical key to effective dengue disease management is early recognition and understanding of the clinical phases of the disease, which is crucial for identification of dengue outbreaks [135]. This study contributes to finding useful solutions to early recognition of dengue, improves the clinical knowledge of how

dengue illnesses progress throughout the febrile and critical phases, and provides recommendations for future research.

Figure 6-1 Boosted CART analysis comparing dengue and OFI

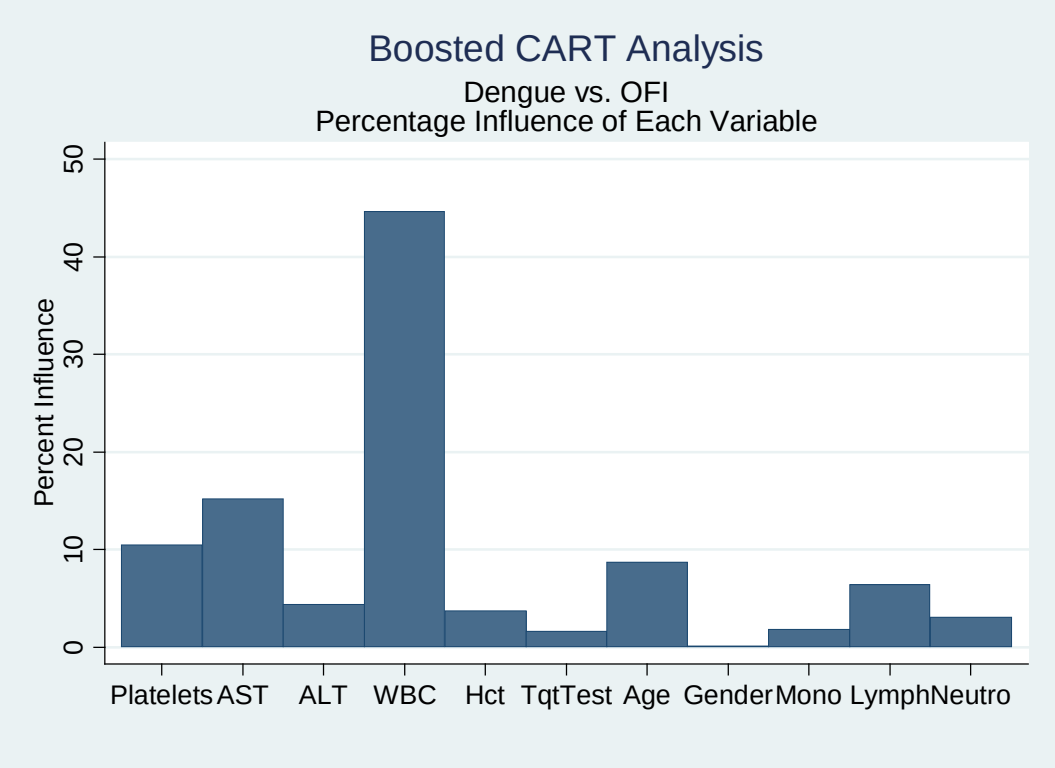


Figure 6-2 Chi-squared automatic interaction detector (CHAID) tree defining DSS as 'severe dengue'

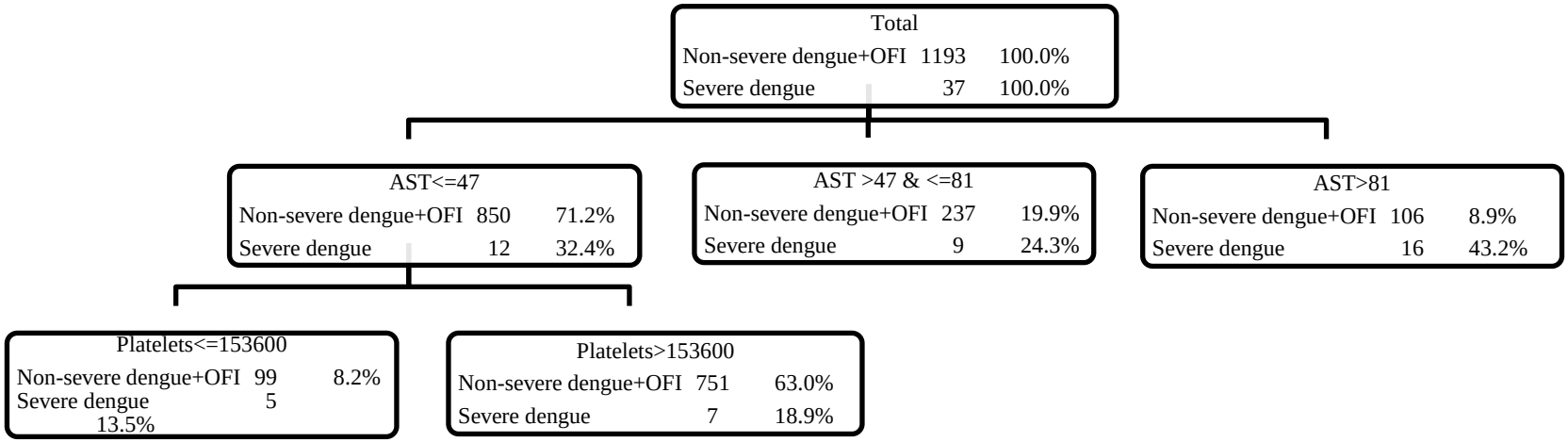
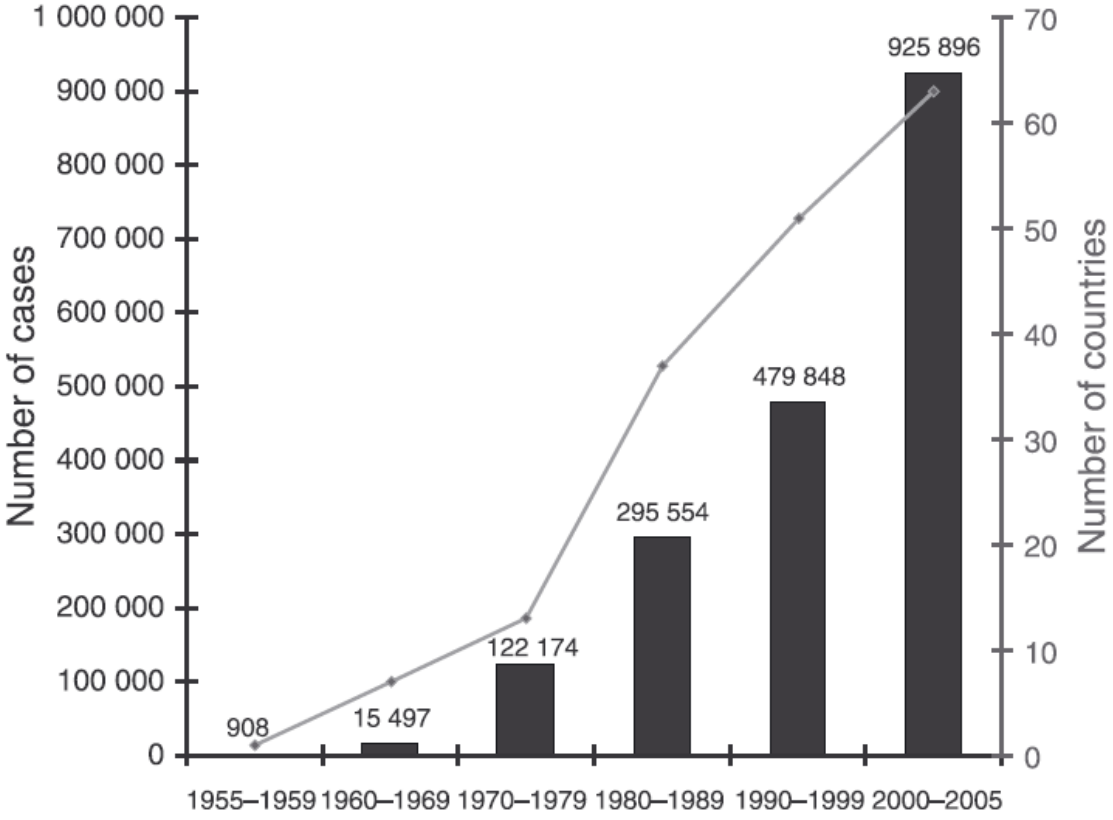


Figure 6-3 Average annual number of DF/DHF cases reported to the WHO and of countries reporting dengue

(courtesy of Farrar, et al [131])



Bibliography

1. Nelson KE, Williams CM, 2207. Infectious disease epidemiology: Theory and practice. Boston: Jones and Bartlett.

2. Henchal EA, Putnak JR, 1990. The dengue viruses. Clin Microbiol Rev 3: 376-96.

3. Rothman AL, 2003. Immunology and immunopathogenesis of dengue disease. Advances in Virus Research 60: 397-419.

4. Thomas L, Verlaeten O, Cabie A, Kaidomar S, Moravie V, Martial J, Najioullah F, Plumelle Y, Fonteau C, Dussart P, Cesaire R, 2008. Influence of the dengue serotype, previous dengue infection, and plasma viral load on clinical presentation and outcome during a dengue-2 and dengue-4 co-epidemic. Am J Trop Med Hyg 78: 990-8.

5. Nisalak A, Endy TP, Nimmannitya S, Kalayanarooj S, Thisayakorn U, Scott RM, Burke DS, Hoke CH, Innis BL, Vaughn DW, 2003. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. Am J Trop Med Hyg 68: 191-202.

6. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, Nisalak A, 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect

Dis 181: 2-9.

7. Halstead SB, 1988. Pathogenesis of dengue: challenges to molecular biology. Science 239: 476-81.

8. Gubler DJ, 1998. Dengue and dengue hemorrhagic fever. Clinical Microbiology Reviews 11: 480-96.

9. Morens DM, 2009. Dengue Fever and dengue hemorrhagic Fever. Pediatric Infectious Disease Journal 28: 635-6.

10. Morens DM, Fauci AS, 2008. Dengue and hemorrhagic fever: a potential threat to public health in the United States. JAMA 299: 214-6.

11. World Health Organization, 1997. Dengue haemorrhagic fever: diagnosis, treatment, prevention, and control. Geneva.

12. Nimmannitya S, 1987. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health 18: 392-7.

13. Harris E, Videa E, Perez L, Sandoval E, Tellez Y, Perez ML, Cuadra R, Rocha J, Idiaquez W, Alonso RE, Delgado MA, Campo LA, Acevedo F, Gonzalez A, Amador JJ, Balmaseda A, 2000. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. American Journal of Tropical Medicine and Hygiene 63: 5-11.

14. Murgue B, Deparis X, Chungue E, Cassar O, Roche C, 1999. Dengue: an evaluation of dengue severity in French Polynesia based on an

analysis of 403 laboratory-confirmed cases. Tropical Medicine and International Health 4: 765-73.

15. Phuong CX, Nhan NT, Kneen R, Thuy PT, van Thien C, Nga NT, Thuy TT, Solomon T, Stepniewska K, Wills B, 2004. Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the world health organization classification system helpful? American Journal of Tropical Medicine and Hygiene 70: 172-9.

16. Setiati TE, Mairuhu AT, Koraka P, Supriatna M, Mac Gillavry MR, Brandjes DP, Osterhaus AD, van der Meer JW, van Gorp EC, Soemantri A, 2007. Dengue disease severity in Indonesian children: an evaluation of the World Health Organization classification system. BMC Infect Dis 7: 22.

17. Srikiatkhachorn A, Gibbons RV, Green S, Libraty DH, Mammen MP, Thomas SJ, Endy TP, Vaughn DW, Nisalak A, Ennis FA, Rothman AL, Nimmannitaya S, Kalayanarooj S, 2010. Dengue hemorrhagic fever: the sensitivity and specificity of the world health organization definition for identification of severe cases of dengue in Thailand, 1994-2005. Clin Infect Dis 50(8): 1135-43.

18. Gubler D, 2005. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. Rev Panam Salud Publica 17: 221-4.

19. Koenraadt CJ, Tuiten W, Sithiprasasna R, Kijchalao U, Jones JW, Scott TW, 2006. Dengue knowledge and practices and their impact on Aedes aegypti populations in Kamphaeng Phet, Thailand. Am J Trop Med Hyg 74: 692-700.

20. Gubler DJ, Clark GG, 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerging Infectious Diseases 1: 55-7.

21. Halstead SB, 1992. The XXth century dengue pandemic: need for surveillance and research. World Health Statistics Quarterly. Rapport Trimestriel de Statistiques Sanitaires Mondiales 45: 292-8.

22. Guzman MG, Kouri GP, Bravo J, Soler M, Vazquez S, Morier L, 1990. Dengue hemorrhagic fever in Cuba, 1981: a retrospective seroepidemiologic study. Am J Trop Med Hyg 42: 179-84.

23. Carrington CV, Foster JE, Pybus OG, Bennett SN, Holmes EC, 2005. Invasion and maintenance of dengue virus type 2 and type 4 in the Americas. J Virol 79: 14680-7.

24. Beatty M, 2009. Global burden of dengue: Pediatric Dengue Vaccine Initiative. http://www.pdvi.org/about_dengue/GBD.asp (unpublished data).

25. Freedman DO, Weld LH, Kozarsky PE, Fisk T, Robins R, von Sonnenburg F, Keystone JS, Pandey P, Cetron MS, 2006. Spectrum of disease and relation to place of exposure among ill returned travelers. N

Engl J Med 354: 119-30.

26. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, Elm J, Tom T, Reiter P, Rigau-Perez JG, Hayes JM, Mills K, Napier M, Clark GG, Gubler DJ, 2005. Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 11: 742-9.

27. Center for Disease Control and Prevention, 2007. Dengue hemorrhagic fever--U.S.-Mexico border, 2005. MMWR Morb Mortal Wkly Rep 56: 785-9.

28. Anderson KB, Chunsuttiwat S, Nisalak A, Mammen MP, Libraty DH, Rothman AL, Green S, Vaughn DW, Ennis FA, Endy TP, 2007. Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study. Lancet 369: 1452-9.

29. Clark DV, Mammen MP, Jr., Nisalak A, Puthimethee V, Endy TP, 2005. Economic impact of dengue fever/dengue hemorrhagic fever in Thailand at the family and population levels. American Journal of Tropical Medicine and Hygiene 72: 786-91.

30. Suaya J, Shepard D, Armien B, Caram M, Castillo L, Chantha N, Garrido F, Kongsin S, Lum L, Montoya R, Sah B, Siqueira J, Sughayyar R, Tyo K, 2007. Multi-country study of costs of dengue among ambulatory and hospitalized patients. The American Journal of Tropical Medicine and Hygiene 77: 55.

31. Suaya JA, Shepard DS, Siqueira JB, Martelli CT, Lum LC, Tan LH,

Kongsin S, Jiamton S, Garrido F, Montoya R, Armien B, Huy R, Castillo L, Caram M, Sah BK, Sughayyar R, Tyo KR, Halstead SB, 2009. Cost of Dengue cases in eight countries in the Americas and Asia: a prospective study. American Journal of Tropical Medicine and Hygiene 80: 846-55.

32. Meltzer MI, Rigau-Perez JG, Clark GG, Reiter P, Gubler DJ, 1998. Using disability-adjusted life years to assess the economic impact of dengue in Puerto Rico: 1984-1994. American Journal of Tropical Medicine and Hygiene 59: 265-71.

33. Dietz VJ, Nieburg P, Gubler DJ, Gomez I, 1992. Diagnosis of measles by clinical case definition in dengue-endemic areas: implications for measles surveillance and control. Bull World Health Organ 70: 745-50.

34. Flannery B, Pereira MM, Velloso LdF, Carvalho CC, De Codes LG, Orrico GS, Dourado CM, Riley LW, Reis MG, Ko AI, 2001. Referral pattern of leptospirosis cases during a large urban epidemic of dengue. Am J Trop Med Hyg 65: 657-63.

35. Karande S, Gandhi D, Kulkarni M, Bharadwaj R, Pol S, Thakare J, De A, 2005. Concurrent outbreak of leptospirosis and dengue in Mumbai, India, 2002. J Trop Pediatr 51: 174-81.

36. Watt G, Jongsakul K, Chouriyagune C, Paris R, 2003. Differentiating dengue virus infection from scrub typhus in Thai adults with fever.

Am J Trop Med Hyg 68: 536-8.

37. Wilder-Smith A, Earnest A, Paton NI, 2004. Use of simple laboratory features to distinguish the early stage of severe acute respiratory syndrome from dengue fever. Clin Infect Dis 39: 1818-23.

38. Schwartz E, Mileguir F, Grossman Z, Mendelson E, 2000. Evaluation of ELISA-based sero-diagnosis of dengue fever in travelers. Journal of Clinical Virology 19: 169-73.

39. Potts JA, Rothman AL, 2008. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Tropical Medicine and International Health 13: 1328-40.

40. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, 2007. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Annals of Internal Medicine 147: 573-7.

41. Ali N, Nadeem A, Anwar M, Tariq WU, Chotani RA, 2006. Dengue fever in malaria endemic areas. J Coll Physicians Surg Pak 16: 340-2.

42. Zahur ur R, Maqbool A, Azhar M, Mehmood A, 2001. Clinical spectrum of thrombocytopenia in adult population of Karachi. JCPSP, Journal of the College of Physicians and Surgeons Pakistan. College of Physicians and Surgeons Pakistan, Karachi, Pakistan 11: 603-605.

43. Low JG, Ooi EE, Tolfvenstam T, Leo YS, Hibberd ML, Ng LC, Lai YL, Yap GS, Li CS, Vasudevan SG, Ong A, 2006. Early Dengue infection and outcome study (EDEN) - study design and preliminary findings. Ann Acad Med Singapore 35: 783-9.

44. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, Ennis FA, 1997. Early clinical and laboratory indicators of acute dengue illness. Journal of Infectious Diseases 176: 313-21.

45. Phuong HL, de Vries PJ, Nga TT, Giao PT, Hung le Q, Binh TQ, Nam NV, Nagelkerke N, Kager PA, 2006. Dengue as a cause of acute undifferentiated fever in Vietnam. BMC Infect Dis 6: 123.

46. Suwandono A, Kosasih H, Nurhayati, Kusriastuti R, Harun S, Ma'roef C, Wuryadi S, Herianto B, Yuwono D, Porter KR, Beckett CG, Blair PJ, 2006. Four dengue virus serotypes found circulating during an outbreak of dengue fever and dengue haemorrhagic fever in Jakarta, Indonesia, during 2004. Trans R Soc Trop Med Hyg 100: 855-62.

47. Chadwick D, Arch B, Wilder-Smith A, Paton N, 2006. Distinguishing dengue fever from other infections on the basis of simple clinical and laboratory features: application of logistic regression analysis. J Clin Virol 35: 147-53.

48. Deparis X, Murgue B, Roche C, Cassar O, Chungue E, 1998. Changing clinical and biological manifestations of dengue during the dengue-2 epidemic in French Polynesia in 1996/97--description and analysis in a prospective study. Trop Med Int Health 3: 859-65.

49. Sawasdivorn S, Vibulvattanakit S, Sasavatpakdee M, Iamsirithavorn S, 2001. Efficacy of clinical diagnosis of dengue fever in paediatric age groups as determined by WHO case definition 1997 in Thailand. Dengue Bulletin. World Health Organization Regional Office for South East Asia, New Delhi, India 25: 56-64.

50. Bruce MG, Sanders EJ, Leake JA, Zaidel O, Bragg SL, Aye T, Shutt KA, Deseda CC, Rigau-Perez JG, Tappero JW, Perkins BA, Spiegel RA, Ashford DA, 2005. Leptospirosis among patients presenting with dengue-like illness in Puerto Rico. Acta Trop 96: 36-46.

51. Buchy P, Vo VL, Bui KT, Trinh TX, Glaziou P, Le TT, Le VL, Bui TC, 2005. Secondary dengue virus type 4 infections in Vietnam. Southeast Asian J Trop Med Public Health 36: 178-85.

52. Hammond SN, Balmaseda A, Perez L, Tellez Y, Saborio SI, Mercado JC, Videa E, Rodriguez Y, Perez MA, Cuadra R, Solano S, Rocha J, Idiaquez W, Gonzalez A, Harris E, 2005. Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. Am J Trop Med Hyg 73: 1063-70.

53. McBride WJ, Mullner H, LaBrooy JT, Wronski I, 1998. The 1993 dengue 2

epidemic in Charters Towers, North Queensland: clinical features and public health impact. Epidemiol Infect 121: 151-6.

54. Nunes-Araujo FR, Ferreira MS, Nishioka SD, 2003. Dengue fever in Brazilian adults and children: assessment of clinical findings and their validity for diagnosis. Ann Trop Med Parasitol 97: 415-9.

55. Cardier JE, Marino E, Romano E, Taylor P, Liprandi F, Bosch N, Rothman AL, 2005. Proinflammatory factors present in sera from patients with acute dengue infection induce activation and apoptosis of human microvascular endothelial cells: possible role of TNF-alpha in endothelial cell damage in dengue. Cytokine 30: 359-65.

56. La Russa VF, Innis BL, 1995. Mechanisms of dengue virus-induced bone marrow suppression. Baillieres Clinical Haematology 8: 249-70.

57. Bandyopadhyay S, Lum LC, Kroeger A, 2006. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue haemorrhagic fever. Tropical Medicine and International Health 11: 1238-55.

58. Ageep AK, Malik AA, Elkarsani MS, 2006. Clinical presentations and laboratory findings in suspected cases of dengue virus. Saudi Med J 27: 1711-3.

59. Akram DS, Igarashi A, Takasu T, 1998. Dengue virus infection among children with undifferentiated fever in Karachi. Indian J Pediatr 65: 735-40.

60. Baruah J, Shiv A, Kumar GA, 2006. Incidence of dengue in a tertiary care centre - Kasturba Hospital, Manipal. Indian Journal of Pathology & Microbiology. Indian Association of Pathologists & Microbiologists, Chandigarh, India 49: 462-463.

61. Baruah HC, Mohapatra PK, Kire M, Pegu DK, Mahanta J, 1996. Haemorrhagic manifestations associated with dengue virus infection in Nagaland. Journal of Communicable Diseases 28: 301-303.

62. Cheng VC, Wu AK, Hung IF, Tang BS, Lee RA, Lau SK, Woo PC, Yuen KY, 2004. Clinical deterioration in community acquired infections associated with lymphocyte upsurge in immunocompetent hosts. Scand J Infect Dis 36: 743-51.

63. Deepak NA, Patel ND, 2006. Differential diagnosis of acute liver failure in India. Ann Hepatol 5: 150-6.

64. Dietz VJ, Gubler DJ, Rigau-Perez JG, Pinheiro F, Schatzmayr HG, Bailey R, Gunn RA, 1990. Epidemic dengue 1 in Brazil, 1986: evaluation of a clinically based dengue surveillance system. Am J Epidemiol 131: 693-701.

65. Gupta S, Singh SK, Taneja V, Goulatia RK, Bhagat A, Puliyel JM, 2000. Gall bladder wall edema in serology proven pediatric dengue hemorrhagic fever: a useful diagnostic finding which may help in prognostication. Journal of Tropical Pediatrics 46: 179-181.

66. Kalayanarooj S, Nimmannitya S, Suntayakorn S, Vaughn DW, Nisalak A, Green S, Chansiriwongs V, Rothman A, Ennis FA, 1999. Can doctors make an accurate diagnosis of dengue infections at an early stage? Dengue Bulletin. World Health Organization Regional Office for South East Asia, New Delhi, India 23: 1-9.

67. Kittigul L, Suankeow K, Sujirarat D, Yoksan S, 2003. Dengue hemorrhagic fever: knowledge, attitude and practice in Ang Thong Province, Thailand. Southeast Asian J Trop Med Public Health 34: 385-92.

68. Kularatne SA, Gawarammana IB, Kumarasiri PR, 2005. Epidemiology, clinical features, laboratory investigations and early diagnosis of dengue fever in adults: a descriptive study in Sri Lanka. Southeast Asian J Trop Med Public Health 36: 686-92.

69. Leelarasamee A, Chupaprawan C, Chenchittikul M, Udompanthurat S, 2004. Etiologies of acute undifferentiated febrile illness in Thailand. J Med Assoc Thai 87: 464-72.

70. Peyerl-Hoffmann G, Schwobel B, Jordan S, Vamisaveth V, Phetsouvanh R, Christophel EM, Phompida S, Sonnenburg FV, Jelinek T, 2004. Serological investigation of the prevalence of anti-dengue IgM and IgG antibodies in Attapeu Province, South Laos. Clinical Microbiology and Infection. Blackwell Publishing, Oxford, UK 10: 181-184.

71. Reynes JM, Laurent A, Deubel V, Telliam E, Moreau JP, 1994. The first

epidemic of dengue hemorrhagic fever in French Guiana. American Journal of Tropical Medicine and Hygiene 51: 545-553.

72. Rodier GR, Gubler DJ, Cope SE, Cropp CB, Soliman AK, Polycarpe D, Abdourhaman MA, Parra JP, Maslin J, Arthur RR, 1996. Epidemic dengue 2 in the city of Djibouti 1991-1992. Trans R Soc Trop Med Hyg 90: 237-40.

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

74. Chairulfatah A, Setiabudi D, Ridad A, Colebunders R, 1995. Clinical manifestations of dengue haemorrhagic fever in children in Bandung, Indonesia. Ann Soc Belg Med Trop 75: 291-5.

75. Domingues RB, Kuster GW, Onuki de Castro FL, Souza VA, Levi JE, Pannuti CS, 2006. Headache features in patients with dengue virus infection. Cephalalgia 26: 879-82.

76. Espinoza-Gomez F, Diaz-Duenas P, Torres-Lepe C, Cedillo-Nakay RA, Newton-Sanchez OA, 2005. Clinical pattern of hospitalized patients during a dengue epidemic in Colima, Mexico. Dengue Bulletin. World Health Organization Regional Office for South East Asia, New Delhi, India 29: 8-17.

77. Kalayanarooj S, Nimmannitya S, 2005. Is dengue severity related to nutritional status? Southeast Asian J Trop Med Public Health 36:

378-84.

78. Neeraja M, Lakshmi V, Teja VD, Umabala P, Subbalakshmi MV, 2006. Serodiagnosis of dengue virus infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol 24: 280-2.

79. Monira P, Shahina T, Ali MM, Mamun KZ, Islam MN, 2004. Clinical and laboratory observations associated with the 2000 dengue outbreak in Dhaka, Bangladesh. Dengue Bulletin. World Health Organization Regional Office for South East Asia, New Delhi, India 28: 96-106.

80. Ranjit S, Kissoon N, Gandhi D, Dayal A, Rajeshwari N, Kamath SR, 2007. Early differentiation between dengue and septic shock by comparison of admission hemodynamic, clinical, and laboratory variables: a pilot study. Pediatr Emerg Care 23: 368-75.

81. Shah I, Katira B, 2007. Clinical and laboratory profile of dengue, leptospirosis and malaria in children: a study from Mumbai. Arch Dis Child 92: 561.

82. Fadilah SA, Sahrir S, Raymond AA, Cheong SK, Aziz JA, Sivagengei K, 1999. Quantitation of T lymphocyte subsets helps to distinguish dengue hemorrhagic fever from classic dengue fever during the acute febrile stage. Southeast Asian J Trop Med Public Health 30: 710-7.

83. Ira S, Bhushan K, 2005. Clinical and laboratory abnormalities due to dengue in hospitalized children in Mumbai in 2004. Dengue

Bulletin. World Health Organization Regional Office for South East Asia, New Delhi, India 29: 90-96.

84. Zavala-Velazquez JE, Yu XJ, Walker DH, 1996. Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico. Am J Trop Med Hyg 55: 157-9.

85. Ellis RD, Fukuda MM, McDaniel P, Welch K, Nisalak A, Murray CK, Gray MR, Uthaimongkol N, Buathong N, Sriwichai S, Phasuk R, Yingyuen K, Mathavarat C, Miller RS, 2006. Causes of fever in adults on the Thai-Myanmar border. Am J Trop Med Hyg 74: 108-13.

86. Ashford DA, Savage HM, Hajjeh RA, McReady J, Bartholomew DM, Spiegel RA, Vorndam V, Clark GG, Gubler DG, 2003. Outbreak of dengue fever in Palau, Western Pacific: risk factors for infection. Am J Trop Med Hyg 69: 135-40.

87. Pancharoen C, Thisyakorn U, 2001. Dengue virus infection during infancy. Transactions of the Royal Society of Tropical Medicine and Hygiene. Royal Society of Tropical Medicine and Hygiene, London, UK 95: 307-308.

88. National Statistical Office, 2009. Thailand: Thomas Brinkhoff.

89. Cleveland W, 1979. Robust locally weighted regression and smoothing scatterplots. Journal of the American Statistical Association 74: 829-836.

90. Cleveland WS, Devlin S.J., 1988. Locally weighted regression: an approach to regression analysis by local fitting. Journal of the American Statistical Association 83: 596-610.

91. Fitzmaurice GM, Laird NM, Ware JH, 2004. Applied longitudinal analysis. Hoboken, NJ: John Wiley and Sons, Inc.

92. Hedeker D, Gibbons RD, 2006. Longitudinal data analysis. Hoboken, NJ: Wiley-Interscience.

93. Kleinbaum D, Kupper L, Muller K, Nizam A, 1998. Applied regression analysis and other multivariable methods. Pacific Grove: Duxbury Press.

94. Landis JR, Koch GG, 1977. The measurement of observer agreement for categorical data. Biometrics 33: 159-74.

95. Breiman L, Friedman JH, Olshen RA, Stone CJ, 1984. Classification and regression trees. Belmont: Wadsworth, Inc.

96. Lemon SC, Roy J, Clark MA, Friedmann PD, Rakowski W, 2003. Classification and regression tree analysis in public health: methodological review and comparison with logistic regression. Annals of Behavioral Medicine 26: 172-81.

97. Zhang H, Singer B, 1999. Statistics for Biology and Health: Recursive partitioning in the health sciences. New York: Springer.

98. Lee VJ, Lye DC, Sun Y, Leo YS, 2009. Decision tree algorithm in deciding hospitalization for adult patients with dengue haemorrhagic fever in

Singapore. Tropical Medicine and International Health.

99. Tanner L, Schreiber M, Low JG, Ong A, Tolfvenstam T, Lai YL, Ng LC, Leo YS, Thi Puong L, Vasudevan SG, Simmons CP, Hibberd ML, Ooi EE, 2008. Decision tree algorithms predict the diagnosis and outcome of dengue Fever in the early phase of illness. PLoS Negl Trop Dis 2: e196.

100. Holliday MA, Segar WE, 1957. The maintenance need for water in parenteral fluid therapy. Pediatrics 19: 823-32.

101. Johns Hopkins Hospital, 2009. The Harriet Lane Handbook: a manual for pediatric house officers: The Harriet Lane Service, Children's Medical and Surgical Center of The Johns Hopkins Hospital.

102. Hyde RM, 2000. Immunology. Baltimore, MD: Lippincott Williams and Wilkins.

103. Tietz NW, 1983. Clinical guide to laboratory tests. Company WBS, ed. Philadelphia.

104. Germann WJ and Stanfield SC, 2002. Principles of Human Physiology. San Francisco: Benjamin Cummings.

105. Berg JM, Tymoczko JL, Stryer L, 2002. Biochemistry. New York: WH Freeman and Company.

106. Srikiatkhachorn A, 2009. Plasma leakage in dengue haemorrhagic fever. Thromb Haemost 102: 1042-9.

107. Srikiatkhachorn A, Green S, Markers of dengue disease severity. Curr Top

Microbiol Immunol 338: 67-82.

108. Nimmannitya S, Thisyakorn U, Hemsrichart V, 1987. Dengue haemorrhagic fever with unusual manifestations. Southeast Asian J Trop Med Public Health 18: 398-406.

109. Balmaseda A, Hammond SN, Perez MA, Cuadra R, Solano S, Rocha J, Idiaquez W, Harris E, 2005. Short report: assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. American Journal of Tropical Medicine and Hygiene 73: 1059-62.

110. Rigau-Perez JG, 2006. Severe dengue: the need for new case definitions. Lancet Infect Dis 6: 297-302.

111. Rigau-Perez JG, Bonilla GL, 1999. An evaluation of modified case definitions for the detection of dengue hemorrhagic fever. Puerto Rico Association of Epidemiologists. Puerto Rico Health Sciences Journal 18: 347-52.

112. Thangaratham PS, Tyagi BK, 2007. Indian perspective on the need for new case definitions of severe dengue. Lancet Infect Dis 7: 81-2.

113. Wills B, Tran VN, Nguyen TH, Truong TT, Tran TN, Nguyen MD, Tran VD, Nguyen VV, Dinh TT, Farrar J, 2009. Hemostatic changes in Vietnamese children with mild dengue correlate with the severity of vascular leakage rather than bleeding. American Journal of Tropical Medicine and Hygiene 81: 638-44.

114. A-Nuegoonpipat N, Panthuyosri N, Anantapreecha S, Chanama S, Sa-Ngasang A, Sawanpanyalert P, Kurane I, 2008. Cross-reactive IgM responses in patients with dengue or Japanese encephalitis. Journal of Clinical Virology 42: 75-7.

115. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH, 1989. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. American Journal of Tropical Medicine and Hygiene 40: 418-27.

116. Cao XT, Ngo TN, Wills B, Kneen R, Nguyen TT, Ta TT, Tran TT, Doan TK, Solomon T, Simpson JA, White NJ, Farrar JJ, 2002. Evaluation of the World Health Organization standard tourniquet test and a modified tourniquet test in the diagnosis of dengue infection in Viet Nam. Tropical Medicine and International Health 7: 125-32.

117. Koenraadt CJ, Aldstadt J, Kijchalao U, Sithiprasasna R, Getis A, Jones JW, Scott TW, 2008. Spatial and temporal patterns in pupal and adult production of the dengue vector Aedes aegypti in Kamphaeng Phet, Thailand. American Journal of Tropical Medicine and Hygiene 79: 230-8.

118. Lewis R, 2000. An introduction to Classification and Regression Tree (CART) analysis. Annual Meeting of the Society for Academic Emergency Medicine. San Francisco, CA.

119. SPSS, 2001. AnswerTree 3.0 User's Guide. Chicago, IL: SPSS, Inc.

120. Abbasi A, Butt N, Sheikh QH, Bhutto AR, Munir SM, Ahmed SM, 2009. Clinical features, diagnostic techniques and management of dual dengue and malaria infection. J Coll Physicians Surg Pak 19: 25-9.

121. Butt N, Abbassi A, Munir SM, Ahmad SM, Sheikh QH, 2008. Haematological and biochemical indicators for the early diagnosis of dengue viral infection. J Coll Physicians Surg Pak 18: 282-5.

122. Libraty DH, Myint KS, Murray CK, Gibbons RV, Mammen MP, Endy TP, Li W, Vaughn DW, Nisalak A, Kalayanarooj S, Hospenthal DR, Green S, Rothman AL, Ennis FA, 2007. A comparative study of leptospirosis and dengue in thai children. PLoS Negl Trop Dis 1: e111.

123. Oishi K, Saito M, Mapua CA, Natividad FF, 2007. Dengue illness: clinical features and pathogenesis. J Infect Chemother 13: 125-33.

124. Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W, Wongtapradit L, Nithipanya N, Kalayanarooj S, Nisalak A, Thomas SJ, Gibbons RV, Mammen MP, Jr., Libraty DH, Ennis FA, Rothman AL, Green S, 2007. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. Pediatr Infect Dis J 26: 283-90; discussion 291-2.

125. Schonlau M, 2005. Boosted regression (boosting): An introductory tutorial and a Stata plugin. The Stata Journal 5: 330-354.

126. Sutton CD, 2005. Classification and regression trees, bagging, and boosting. Handbook of Statistics 24: 11-329.

127. Damrongplasit K, Melnick GA, 2009. Early results from Thailand's 30 Baht Health Reform: something to smile about. Health Aff (Millwood) 28: w457-66.

128. Gubler DJ, 2002. The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res 33: 330-42.

129. Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R, 2008. Defining challenges and proposing solutions for control of the virus vector Aedes aegypti. PLoS Med 5: e68.

130. Newton EA, Reiter P, 1992. A model of the transmission of dengue fever with an evaluation of the impact of ultra-low volume (ULV) insecticide applications on dengue epidemics. Am J Trop Med Hyg 47: 709-20.

131. Farrar J, Focks D, Gubler D, Barrera R, Guzman MG, Simmons C, Kalayanarooj S, Lum L, McCall PJ, Lloyd L, Horstick O, Dayal-Drager R, Nathan MB, Kroeger A, 2007. Towards a global dengue research agenda. Trop Med Int Health 12: 695-9.

132. Hayes JM, Rigau-Perez JG, Reiter P, Effler PV, Pang L, Vorndam V, Hinten SR, Mark KE, Myers MF, Street K, Bergau L, Meyer C, Amador M, Napier M, Clark GG, Biggerstaff BJ, Gubler DJ, 2006. Risk factors for infection during a dengue-1 outbreak in Maui,

Hawaii, 2001. Trans R Soc Trop Med Hyg 100: 559-66.

133. Hurtado-Diaz M, Riojas-Rodriguez H, Rothenberg SJ, Gomez-Dantes H, Cifuentes E, 2007. Short communication: impact of climate variability on the incidence of dengue in Mexico. Trop Med Int Health 12: 1327-37.

134. Center for Disease Control and Prevention, 2010. Notice to readers: Changes to the National Notifiable Infectious Disease List and data presentation---Jan 2010. Morbidity and Mortality Weekly Report 59: 11.

135. World Health Organization, 2009. Dengue: Guidelines for diagnosis, treatment, prevention, and control. Geneva: World Health Organization/Special Programme for Research and Training in Tropical Diseases.