PREDICTIVE MODELS FOR DENGUE FEVER AND SEVERE DENGUE

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IN HONDURAS AND THE NATIONAL CLASSIFICATION AGREEMENT WITH

THE TWO MOST RECENT INTERNATIONAL GUIDELINES ON SEVERE DENGUE

By

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ABSTRACT

Dengue fever is the most important viral disease transmitted by arthropods worldwide.

Dengue is transmitted by the mosquito *Aedes aegypti* and its causal agent is the dengue virus

which comprises four serotypes; dengue virus is part of the Flaviviridae family.

Dengue fever can cause temporary disability affecting economic productivity and school

absenteeism. Severe dengue fever can cause increased morbidity and lead into a mortality of 1-

10 % of its cases depending on the medical preparedness for early diagnosis and treatment.

The severe form of dengue is mediated by an increase in capillary permeability that can

cause severe bleeding (e.g. gastrointestinal bleeding) and plasma leakage resulting in ascites and

pleural effusions.

Dengue is difficult to diagnose clinically because its manifestations are similar to other

febrile illnesses that are endemic to the same areas where dengue fever occurs such as malaria,

leptospirosis, hantavirus, chickunkunya and *S. typhi*. For dengue laboratory confirmation is

required with serology and viral isolation being the most common diagnostic methods used.

A limitation of the laboratory diagnosis for dengue is that it may take several days or

weeks for the results to be completed and reported. Modeling can help predict dengue and severe

dengue fever and allow for more rapid decision making about management.

Our studies on dengue and severe dengue were developed using data derived from a

cohort of patients from Honduras using epidemiological reports of suspected dengue cases from

2009-2010.

We developed two models. One was for dengue fever, to discriminate it from other

febrile illness. This model was built using logistic regression and its outcome was a positive

dengue test. Predictors included the following: retro-ocular pain, petechiae, and bleeding gums,

all associated with an increased risk of dengue fever , while epistaxis and pallor associated to

decreased risk. The model had a sensitivity of 86.2% and a specificity of 27.2%. The other

model was used to predict severe dengue cases in a cohort of confirmed dengue cases; logistic

regression analysis was used as well to construct it. The outcome for this model was plasma

leakage. The predictor variables ascites and platelets count less than 50,000/mm^3 were ssociated

with an increased risk while the presence of petechiae and headache were associated with a

decreased risk of severe dengue. The sensitivity of the model was 76.5% and the specificity was

70.3%

      We also addressed the issue of classification of severe dengue (previously known as

dengue hemorrhagic fever and dengue shock syndrome). We used the classification of cases

done by the Honduras Ministry of Health in 2009-2010 and calculated the level of agreement

between these and 2009 WHO guidelines. The kappa between the national classification and the

2009 WHO classification was 0.11 which is in a range of slight agreement.

      During the preparation of the thesis we discussed the application of methodological tools

for validation of the models, assessment of the accuracy and about the classification of severe

dengue the use of the agreement measures leading to calculation of the kappa value.

      There is need to improve the models and validate them in different populations and to

increase the knowledge of the recent 2009 WHO guidelines to improve the agreement with the

international norm.

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CHAPTER 1

Background information on dengue

Dengue fever is considered the most important arboviral disease worldwide (1). Dengue fever is currently endemic to most tropical and subtropical countries affecting mainly cities and other urbanized localities. The dengue virus is transmitted by mosquitoes of the *Aedes* genus, the most important being *Aedes aegypti* followed by *Aedes albopictus* , the latter of which has been reported in the southeastern United States, increasing the possibility for viral transmission in North America(2, 3).

The World Health Organization estimates that 250 million people are at risk of infection from dengue and 50 million contract dengue infection annually(4, 5). Dengue fever is currently endemic in South-east Asia, the Americas, the Eastern Mediterranean, Africa, and the Western Pacific with the largest number of reported cases corresponding to the Americas and Southeast Asia (4-7).

Dengue virus is classified as part of the *Flaviviridae* family, which also includes yellow fever virus, chikunkunya and West Nile virus(8). Dengue virus comprises four distinct serotypes (1 to 4) with similar clinical manifestations in humans. The Dengue virus is a single-stranded, positive –sense RNA molecule with a diameter of 50 nm and constituted by three structural proteins (capsid, envelope, and membrane) and 7 nonstructural proteins(9).

Around 85% of dengue infections are asymptomatic and the remaining 15% evolve into febrile illness; approximately 500,000 dengue cases progress to life-threatening disease causing 20,000 to 25,000 deaths annually(6). Dengue is the leading cause of childhood death in many

countries in Southeast Asia (1). The clinical presentation includes fever and typically an influenza-like syndrome characterized by headache, retro-ocular, and joint pain, as well as rash and lymphadenopathy; known as classic dengue (5). The febrile phase may be followed by the condition known traditionally as dengue hemorrhagic fever (DHF) characterized by thrombocytopenia, pleural and abdominal effusions; and dengue shock syndrome (DSS) (DHF with evidence of systemic hypo perfusion) (7); both conditions are now classified as Severe dengue fever.

Following infection with the dengue virus, viremia occurs for up to 6 days after onset of fever (10). After that time the virus is no longer isolated since the virus and the infected cells are cleared(7, 11). The key characteristic of severe dengue is plasma leakage potentially due to pro-inflammatory cytokine-inflicted damage to the endothelium(12). Plasma leakage coincides with clearance of the viremia, suggesting that the tissue damage is related to the host response rather than to viral direct action (13-18).

Diagnosis of dengue

Dengue fever is difficult to diagnose clinically because it resembles other febrile illnesses that are prevalent in the same regions such as malaria, influenza, leptospirosis, hantavirus, chikunkunya and *S. typhi*. Studies in India and in South America have identified *P.falciparum* and *P.vivax* in cohorts of febrile patients tested for dengue(19-21). In Puerto Rico influenza has been reported among patients tested for dengue virus and Hantavirus cases have been reported in Brazil among patients tested for dengue(22, 23). Leptospirosis can also cause a febrile illness that mimics dengue or can co-infect dengue as has been reported in India, Barbados and Mexico (24-26). Several febrile illness were identified in a cohort of patients tested for dengue virus in Southeast Asia , the most frequent of them being chikunkunya and *S. typhi (27).*

Several laboratory methods have been developed for the confirmation of dengue. The most commonly used are: antigen detection, viral isolation, detection of dengue RNA RT-PCR, seroconversion with paired acute and convalescent phase sera by IgM capture ELISA or Inhibition ELISA. It is conventionally accepted that a >4 fold increase in antibodies titer in paired samples of acute and convalescent sera (By Inhibition ELISA) is evidence for dengue infection(6, 8).The most common techniques for dengue diagnostic are serology and viral isolation, in the first case the purpose is to identify anti dengue antibodies in the patients samples (usually serum) and the viral isolation looks for viral particles.

The method used for studies in this thesis was the Ig M MAC ELISA (M antibody-capture enzyme-linked immunosorbent) from Standard Diagnostic of Bio Venture Company (Gyeonggi-do, South Korea),which uses dengue-specific antigens from serotypes DEN 1 to 4 (28). For this assay, most of the antigens come from the envelope protein and capture antidengue IgM specific antibodies in serum.   The sensitivity of this assay has ranged in recent studies from 84.9% to 96.8% and specificity from 87.8% to 99.4% (28-30). One of the limitations is that it may cross-react with antibodies of other flaviviruses resulting in false positives. However, this is unlikely to pose a problem in Honduras where other flaviviruses such as yellow fever virus have not reported cases in the last 50 years, and chikunkunya, or West Nile virus have not been known to be circulating even when very recently the former has been reported in the Caribbean basin(31). A definite limitation is that the assay is not serotype specific. This diagnostic method can be used if patients are at least in the fifth day of clinical disease and optimally if the onset of infection is less than three months(32). An IgM/IgG ratio can be used to distinguish primary and secondary dengue virus infections; this concept has been applied by commercial producers(32). However,

for the purpose of studies in this thesis, where the distinction between primary and secondary infections is not critical, the results of the IgM assay alone is sufficient to establish diagnosis.

Testing individuals for dengue and reporting the results to the treating physician can be a lengthy process and the confirmation of dengue may require several days depending on the local health system. Immediate management of cases may therefore be based on clinical acumen and the use of available epidemiological information to help provide opportune care to patients and case reporting to epidemiological surveillance systems. Dengue models based on available information can be very useful in discriminating dengue cases from other febrile illness(32).

Classification of dengue cases is important to guarantee good clinical management, reporting of cases and adequate measures for dengue control. This has been the justification to develop the studies that will be presented in the following chapters, adding the fact that is important to know the divergences that national teams have when classifying the severity of dengue when compared to international criteria.

Predictive Modeling

Predictive models are important in the diagnosis of dengue since laboratory confirmation takes time (days or weeks) to be reported. Physician training to diagnose the clinical illness can vary according to specialty and years of experience, but is still limited given the presence of similar febrile diseases transmitted in the same geographic locations and season. Modeling can therefore be of assistance in predicting dengue and its severity.

In both cases (predicting dengue among several febrile illness, and predicting severe cases among a cohort of patients with clinical dengue) factors associated with the outcomes (dengue fever or severe dengue), the predictor variables, are sought.

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Previous studies in dengue fever and other febrile illness have identified a series of predictors of which some are clinical findings and others laboratory tests that are significantly associated with dengue fever (33-36). A similar approach has been developed to identify predictor variables for severe dengue (37, 38). Different models have been built depending on the variables included in the analysis and the method for their development. Most studies have determined the association of particular symptoms to the presence of dengue fever, or have assessed factors associated with progression of dengue fever into severe dengue (34, 37-39). An overview of predictive models is described in the following paragraphs

A predictive model is one where the model can accurately determine an outcome where independent variables form part of an equation(36, 40, 41). Use of such models may help infer outcomes such as dengue (given a presentation of a febrile illness in an endemic region) or severe dengue given known dengue infection. Multivariable analysis is the basis for the development of predictive models. Various types of models can be developed based on the particular method used and these can include linear regression, multiple logistic regression, proportional hazards, discriminant function analysis, as well as others.

Multiple logistic regression models are used in the prediction of dichotomous outcomes (y), and the dependent variable can take either of two possible values. Presence or absence of a disease, success or failure of a vaccine, positivity or negativity of a diagnostic test are examples of dichotomous outcomes, with y= 1 if the event occur and y=o if the event does not occur. The method assesses the relationship between independent variables and the probability of the event to occur.

$p = \Pr|y=1| \; x_1, x_2, \ldots, x_i$

For an independent variable x the model assumes that p can be described as a function of

x through the logistic function : $P = \frac{e^{\beta_0 + \beta_1 x}}{1 + e^{\beta_0 + \beta_1 x}}$ or $logit\ (p|x) = \frac{\beta_0 + \beta_1 x}{1 + \beta_0 + \beta_1 x}$

The prediction of dichotomous outcomes means that the error variance is not likely to follow a normal distribution but a logistic distribution. Formally, the model logistic regression model is that: $Log\left(\frac{p(x)}{1 - p(x)}\right) = \beta_0 + \beta_1 x$, where x is the explanatory variable

Logistic regression models the association of binary outcomes and exposure variables in terms of odd ratios:

Exposure odds ratios = Odds in exposed group/Odds in unexposed group.

In this way we have two model parameters: baseline odds and exposure odds ratio(40, 41). The baseline refers to when there is no exposure to the studied risk factor or predictor, we then assign a value of zero; the exposure to the risk factor is assigned a value of one. The exposure odds ratio expresses the effect of the exposure on the odds of disease (40, 41).

It may be valuable to compare results of one model with those of other methods of predictive modeling, to identify levels of accuracy of alternative models; and to find ways to identify subgroups of population at high risk of having the health condition that is dengue fever and severe dengue in a way that is accessible to clinicians in terms of their understanding. A good illustration of this is recursive partitioning. Recursive partitioning is a specific type of modeling and it is exemplified by the Classification and Regression Trees (CART), which can use continuous or categorical data and can generate binary outcomes(42). CART looks for identifying characteristics that enable a division or split of the study population into subgroups

i.e. with differing risk of a particular outcome. This method is a simple method for classification of the individuals in the study (see annexes 1 and 2).

It is obviously important to identify the optimal model to describe the relationship between predictors and the outcome. This can be done either through an exploratory approach (what model best describes the characteristics of the data) or a confirmatory approach (testing to know which one best fit the data)(43, 44).

Model Validation

Once a model is developed it is important to validate it in two stages, the first is internal validation to assess if the model is valid in subsets of the population where it was developed. The second is external validation, which refers to testing the model in a different population than the first but where the occurrence of the same event or outcome is assessed. Models need to be compared to identify their relative accuracy predicting the same outcome. The basic challenge for model development is that it requires the identification of relevant predictors that are related to the process under study and once is developed it has to be able to discriminate between individuals with the condition and those where that condition is absent. It should be able to identify the strength of association comparing the frequency of disease in the absence of a predictor and those where it is present (44).

The issue of discriminating between positive individuals (cases) and negative ones lead us to consider the need to classify cases for the purposes of a medical institution, a country or for international organizations like World Health Organization (WHO) using case definitions, algorithms and experts panels. Protocols for classification of cases take effort and the development of statistical and computer techniques as those described in modeling. The

implementation of classification schemes requires the adoption by local teams of guidelines designed by international health organizations like the World Health Organization. A limitation for a rapid adoption can be the difference in the levels of expertise and access to technological resources and the knowledge of previous reviews for classifying diseases like dengue (45-47).

There are a number of strategies for calculating how exact our classifications are compared to observed results, these include accuracy calculations when compared to a gold standard, and measurement of agreement among different classifications done by experts, among which the kappa coefficient is most widely used(48, 49).

Classification of severe dengue

In addition to diagnostic challenges on clinical grounds, the classification of cases of dengue as severe has been controversial.   Classifying severe dengue cases is important to the understanding of the epidemiology of dengue both regionally as well as for national and international surveillance.   Following a standard definition may also allow for a more uniform case management. Prior to 2009, the definition of DHF was characterized by fever, hemorrhagic tendency, thrombocytopenia and plasma leakage. Hemorrhagic tendencies were defined by at least one of the following: a positive tourniquet test, petechiae, ecchymosis, or purpura; bleeding from the mucosa, gastrointestinal tract, infection sites or other locations; hematemesis or melena. Thrombocytopenia was defined as platelets 100,000 cells per mm^3 or less. Evidence of plasma leakage was defined by at least one of the following: a rise in hematocrit following volume replacement treatment equal to or greater than 20% of baseline; signs of plasma leakage such as pleural effusion, ascites and hypoproteinemia. Progression of the disease can lead to dengue shock syndrome which is defined as rapid and weak pulse, narrow pulse pressure, hypotension for age, cold clammy skin and restlessness(45).

In 2009, new classification criteria for severe dengue were published by the World Health Organization(45-47). To reduce the complexity of the previous classification, the definition of severe dengue was simplified by limiting the definition to severe plasma leakage, severe bleeding manifestations, severe organ impairment in individuals with fever of 2-7 days of evolution, and living in or traveling to dengue endemic areas(45). This definition put emphasis on the history of dengue and the presence (residing or visiting) of the person in an endemic area; the clinical components are considered together (optimal) or any of them in the presence of the first two criteria are used in the definition). However, in many jurisdictions, there is a departure from the WHO criteria, where local committees decide upon case classification. As a consequence, it may be difficult to compare severity in various regions due to local interpretations or alterations of the WHO criteria. It is therefore important to document what the level of agreement is between WHO and local classifications.(50).

Accuracy studies

Sensitivity and specificity studies

A key task in clinical practice is to confirm or refute the presence of disease or support the diagnostic process. Usually these terms refer to the use of laboratory tests and epidemiological definitions. An accurate method is one that identifies patients with a given disease and is also able to correctly identify those who are disease free, these two characteristics are known as sensitivity and specificity(48).

When we develop a predictive model or classify cases we need to be concerned about how close we get to a correct identification of cases and non-cases, comparing our classification to a gold standard by a test based on proven capacity to discriminate positive from negative conditions.

Sensitivity refers to the ability of the test to correctly identify those patients with the disease while specificity refers to the ability of the test to correctly identify those patients without the disease. Diagnostic tests show an inverse proportion of these two characteristics; when a test, case definition or model increases its sensitivity, the specificity is reduced(48).

Dengue fever and severe dengue are a good example of the importance of measuring the sensitivity and specificity of classifications provided by a classification model or by diagnostics provided by a laboratory test or an expert team. Identifying true cases of dengue in an opportune way allows clinicians to treat them without delay avoiding complications and unnecessary tests for other febrile illness. Having a good sensitivity (closer to 100%) allows for efficient management, identifies population groups and geographic regions with intense transmission of dengue. However, the need to identify other febrile illness can be better solved if negative dengue results are identified in a high proportion with models or test of high specificity, contributing to better epidemiological surveillance and avoiding misdiagnosis and waste of therapeutic resources as well as redirecting the study of negative patients to other diagnostic alternatives (other febrile diseases)(45).

Agreement studies

An important concept when we classify cases is reliability or reproducibility defined as the extent to which results of a measurement can be replicated. There are circumstances in which we have to classify between two options: positive or negative, abnormal or normal, case or non-case. When two or more subjects observe the same event they opt for either one of them according to knowledge, experience, preference or other criteria. We assume that observers will have imperfect classifications based on the characteristics we just mentioned.

It is necessary to define the agreement among observers;. the easiest way is to calculate simple agreement, which is the proportion of observation among the observers agreed; in this case we have to consider that this agreement measure would be influenced by the distribution of the given values; if one of them predominates over the other we can have a high agreement value just by chance alone(49).

Ignoring the possibility of agreement by chance may lead us to conclude incorrectly about the quality of our measurement giving a misleading trust of the obtained agreement(51).

Considering the role of chance it is important to introduce the use of kappa to correct it in analyzing unrelated classifications. Agreement by chance will have a value of zero and a perfect agreement a value of 1, other results usually vary between these 2 values according to the level of agreement. The formula for kappa is

$$k = \frac{Observed\ agreement - Expected\ agreement}{1 - Expected\ agreement}$$

A value < 0.00 is a poor agreement, while from 0.00 to 0.20 is slight agreement, 0.21 to 0.40 is fair agreement , 0.41-0.60 as moderate agreement , 0.61 to 0.80 is substantial agreement , 0.81 to 0.99 was considered almost perfect agreement (51, 52).

Kappa is an important correlation coefficient of reproducibility when it reflects something about the accuracy of measurement beyond mere chance. Recent papers describe the possibility of having a kappa with negative values when the agreement level is lower than expected by chance, which introduces an issue for analysis of the criteria for classification . Negative agreement may represent the use of different criteria for each classification and the need to look for unique criteria (51).

The papers presented as part of this thesis describe my use of predictive models having access to similar information as would general practitioners in emergency departments. That is, the variables are based on mostly clinical evaluation of patients.   This thesis, using data from Honduras, illustrates the difficulty in the detection of dengue in an area where multiple febrile syndromes co-exist, as well as the challenge of predicting disease severity in order to initiate early supportive treatment. The thesis also explores the difficulty in increasing accuracy of national classifications of severe dengue cases measured through their sensitivity and specificity, but also assuming that local classifiers are observers of the process of severe dengue classification at similar level than WHO and needing to measure their level of agreement.

Chapter 2 of the thesis describes the development of a predictive model for dengue fever, to help differentiate it from other febrile syndromes. Here, logistic regression method was used to develop the model and internal validation was carried out using the bootstrap technique. The resulting model was tested for accuracy using ROC curves. A model equation was obtained and a score for the purpose of using it in the clinical setting was developed.

In chapter 3, patients with confirmed dengue were studied to develop a clinical predictive model for severe dengue. Logistic regression was used to develop the model. Internal validation was accomplished and accuracy of the model was also determined. A model equation and a score were developed.

In chapter 4, I studied the pattern of classification of severe dengue currently followed by the Ministry of Health in Honduras, comparing their classification with the criteria developed in 2009 by a consultative committee of the World Health Organization (WHO). The procedure was implemented by identifying the criteria established by WHO and tabulating the information of

the official database of the Ministry of Health. Accuracy measures of the national classification were obtained using as standard the WHO approved classification, and also simple agreement and kappa were obtained. I have included in this introduction a table with the samples for each one of the studies included in chapters 2 thru 4.

My personal role in the development of these papers included the formulating of the research questions, collecting the data, establishing databases, analyzing the data, and preparing the first draft of manuscripts and subsequent drafts based on the feedback of the other authors

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Flow Chart of Patients in Dengue Cohort

Patients admitted to tertiary hospitals as suspected dengue (n=718)

Patients included in the study

Model for dengue fever

Patients with confirmatory laboratory results

(n=548)

Patients included in the study **Classification of Severe dengue**

Dengue cases (Confirmatory positive test) (n=390)

Non dengue cases (Negative test) (n=158)

Dengue cases with two laboratory results for hematocrit in the first 24 hours of hospitalization (n=320)

Patients included in the study **Model for Severe dengue**

CHAPTER 2: A PREDICTIVE MODEL FOR DIAGNOSIS OF DENGUE FEVER IN HONDURAS

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Abstract

Introduction: Dengue is a major public health problem in tropical and subtropical countries but its clinical presentation may be similar to many febrile illnesses. Since in endemic countries laboratory confirmation is frequently delayed, the majority of dengue cases are diagnosed based on patient's symptomatology. This can often lead to misdiagnosis and potential serious health complications. The objective of this study was to identify clinical, hematological and demographical parameters that could be used as predictors of dengue fever among patients with febrile illness.

Methods: We conducted a retrospective cohort study of 548 patients presenting with febrile syndrome to the largest public hospitals in Honduras. Patients' clinical, laboratory, and demographical data as well as dengue laboratory confirmation by either serology or viral isolation were used to build a predictive statistical model to identify dengue cases.

Results: Of 548 patients, 390 were confirmed with dengue infection while 158 had negative results. Univariable analysis revealed seven variables associated with dengue: male sex, petechiae, skin rash, myalgia, retro-ocular pain, positive tourniquet test, and bleeding gums. In multivariable logistic regression analysis, retro-ocular pain petechiae and bleeding gums were associated with increased risk, while epistaxis and paleness of skin were associated with reduced risk of dengue. Using a value of 0.6 (i.e., 60% probability for a case to be positive based on the equation values), our model had a sensitivity of 86.2%, a specificity of 27.2%, and an overall accuracy of 69.2%; allowing for the diagnosis of dengue to be ruled out and for other febrile conditions to be investigated.

Conclusions: The application of predictive models can be valuable when laboratory confirmation is delayed. Among Honduran patients presenting with febrile illness, our data reveal key symptoms associated with dengue fever, however the overall accuracy of our model is

still low and specificity remains a concern. Our model requires validation in other populations

with similar pattern of dengue transmission.

Key Words: Dengue, fever, Predictive model, symptoms, Honduras

Introduction

Dengue is a vector-borne flavivirus infection transmitted by *Aedes* mosquitoes that poses a major public health threat in many tropical and subtropical regions (2-4). Worldwide, dengue infects 50 million people annually and causes 20,000 to 25,000 deaths (2). In Honduras, 66,814 cases were reported in 2010, with a case fatality rate of 3% (5) .

Clinical manifestations of dengue range from febrile illness to severe complications resulting in hospitalization and death. In its early stages, dengue resembles other febrile illness such as malaria, influenza, and leptospirosis, constituting a diagnostic challenge for clinicians in endemic countries (6). Further complications to dengue diagnosis arise from the presence of co-morbidities with overlapping manifestations such as the presence of hematuria, which can occur in cases of either falciparum malaria or dengue(7, 8). The ability to differentiate between dengue and other conditions is of prime importance to clinicians in dengue endemic areas.

A confirmatory diagnosis of dengue is made through laboratory diagnosis using serology (e.g., IgM titres, IgG seroconversion), antigen detection of non-structural protein (NS) in sera, or polymerase chain reaction (PCR) for dengue virus nucleic acids in blood. Of these laboratory methods, PCR is not available in many middle and low income countries. Similarly, antigen detection of NS is frequently unavailable or is not part of routine dengue confirmation. Although serology is available in many settings, there may be delays in obtaining results, and, in many areas, no laboratory testing is available at all.

Although there is no specific therapy for the disease identifying dengue can help clinicians make better decisions; for example they could triage patients by knowing which patients need to be monitored for possible dengue complications.

On the other hand, dengue diagnosis based solely on clinical presentation is challenging and can lead to misdiagnosis with the ensuing consequences for patients. In Honduras, for

instance, studies show that less than 50% of cases clinically diagnosed as dengue are confirmed by laboratory testing as positive (9, 10).

Various publications report predictive models for dengue, using symptoms, signs, and laboratory data to differentiate dengue from other febrile syndromes (11-17). Demographic factors such as age and sex(13, 14);clinical symptoms such as joint pain, vomiting, and myalgia (16); and laboratory findings such leucopenia and thrombocytopenia(17), have all been reported as predictors for dengue. Limitations of these studies include a small sample size (12-15), lack of validation of the models(18), limited geographic representation (15, 16), and limited predictive ability of the results (18-20).

In the present investigation, we studied a retrospective cohort of febrile patients seen at different health care facilities in Honduras. Patients presented a wide range of clinical signs and symptoms and had laboratory analysis for dengue infection. Based on this information, we built a logistic regression model to identify clinical factors that could predict dengue infection.

Methods

The study took place in Honduras, between 2009 and 2010. Data was collected from febrile patients receiving health care at hospitals and outpatient clinics located in Tegucigalpa and San Pedro Sula, the two largest cities in the country. Health care facilities attending patients can be broadly organized into: (I) hospital and clinics part of the Social Security system (Instituto Hondureño de Seguridad Social, IHSS); and (ii) hospital and clinics part of the Public Health system. The IHSS serves the insured employed population whereas the latter serves the general population with no restrictions (although patients with financial means utilize the private health case system).

Patients' information was organized into a database of clinical and laboratory variables. This information had been systematically collected by general practitioners, nurses, and microbiologists in a data collection form, either at the primary health care centres or at the tertiary hospitals. Patients were hospitalized when, according to treating physicians, further diagnosis and management were necessary.

Other conditions characterized by febrile presentation such as malaria, kidney infections, chronic hepatitis, leptospirosis, and respiratory infections were evaluated. In some cases confirmation for these differential diagnoses was possible. In addition, patients filled out a survey questionnaire specific for dengue.

Blood samples were collected from patients for dengue laboratory confirmation. If a patient presented within five days of the first symptom onset, samples were processed for viral culture following guidelines from the Centers for Disease Control and Prevention (CDC) of the United States (21). If the blood sample was taken on the sixth day or later of onset of symptoms, a test for antibody detection was done using IgM antibody-capture enzyme-linked immunosorbent (IgM Mac ELISA) from Standard Diagnostic of Bio Venture Company (Gyeonggi-do , South Korea) (21, 22). This commercial kit reports a sensitivity of 96.4% and specificity of 98.9%(22). Serology and viral isolation were performed at the Honduras National Laboratory of Virology according to CDC guidelines and standardized techniques.

For hospitalized patients, our analysis was based on serial blood tests taken over the three days following admission, and using additional radiological or laboratory testing if available. The initial clinical diagnosis of the treating physician (prior to laboratory confirmation) was

recorded and submitted to the Honduran National Classification Committee for classification as

uncomplicated dengue or severe dengue (dengue haemorrhagic fever or shock syndrome)(9, 23).

Among all patients, those who received a conclusive laboratory result for dengue (either

positive or negative) were enrolled in the study. Complete information was obtained for 548

patients, representing 13 of the 18 departments of the country.

Outcome variables:

Based on laboratory confirmation (by serology or virus isolation), the dependant variable

was either dengue positive or dengue negative result. Of 548 patients studied, 390 were

confirmed with dengue infection while 158 had negative results (see annex 3).

Predictor variables

Clinical information was grouped into three categories: 1) general symptoms, including

fever, headache, retro-ocular pain, myalgia, rash, anorexia and vomiting; 2) bleeding

manifestations, including petechiae, ecchymosis, hematemesis, melena, positive tourniquet,

epistaxis (nose bleeding), gingival bleeding, hematuria, and heavy menstrual bleeding

(metrorrhagia); and 3) symptoms and signs suggestive of capillary leakage, including abdominal

pain, cold extremities, sweating, paleness of skin, serous membranes' effusion (pericardial,

pleural effusion and ascites), and reduction of medial arterial pressure. Hematological analysis

(leucocytes count, and hemoglobin and hematocrit values) were recorded if they were available

for at least two consecutive days.

Power calculation

A sample size was not determined *a priori* since the study enrolled all patients admitted during the study period who had febrile illness and a completed laboratory testing for dengue. Since petechiae are both readily identifiable and are commonly seen in dengue infection we based the study's power calculation on the predictive value of petechiae to differentiate dengue from other febrile illness (24).

Based on the 548 records (390 dengue-positive and 158 dengue-negative), assuming a prevalence of petechiae of 25% in non-dengue febrile illness and an alpha level of 0.05, the study had 80% power to detect a 1.5-fold increase risk of dengue given the presence of petechiae. Calculation is shown in Annex # 3

Data analysis

The chi-square test was used to detect significant differences between presenting signs and symptoms (recorded during admission and on the first day of the patient's stay, prior to being tested for dengue) and the laboratory tests results.

A logistic regression model was built using a forward step-wise selection. Variables with $p < 0.2$ were entered into the multivariable models. We decided *a priori* to include both age and sex in the analysis**.** Correlation among pairs of variables was assessed calculating values for tolerance and the variance inflation factor (VIF). Values for tolerance >0.2 and for VIF< 5 were considered as compatibles with a low collinearity. Variables that were statistically significant (p$<$ 0.05) were kept in the final model.

Confidence intervals for the odds ratios, were calculated for each predictor. As part of the models' internal validation, the bootstrap method (resampling data) was used to estimate the accuracy of the estimators (standard error, confidence intervals and bias)(25-27). The model was also validated using subsamples, each corresponding to one tenth of the sample and with nine

tenths of the remaining sample used for a cross-validation. The subsamples were drawn using

systematic sampling (including every tenth case for each subsample) without leaving any patient

out of the procedure.

Additionally, as a sensitivity analysis, we created a dichotomous variable for the type of

health care facility where patients were captured: either at the Social Security system (accessible

only to the insured employed population) or at clinics of the Public Health system (open to the

general population). This was done account for potential differences between the two patient

populations (centre effect).

The analysis then was performed using logistic regression models; the sensitivity of the

method was obtained alternatively using the receiver operating characteristic (ROC) or ROC

curve, which is a graphic plot illustrating the performance of a binary classifier system like the

one used during this study.

Finally the model equation was included and a score was obtained from the equation

transforming the values assigned to each predictor to integers by multiplying by ten (avoiding

decimal values). All statistical analysis were done using SPSS version 19 (IBM)((28))

Results

The final study sample consisted of 548 patients: 227 were female (41%); mean age 21

years of age (SD=15.5 years). Of these patients, 295 (53.8%) were admitted in tertiary-level

hospitals, 240 (43.8%) in hospitals providing care for insured workers, and 13 (2.4%) in regional

and local health clinics.

Complete clinical and demographic data was available for all 548 patients. However,

information on the date of onset of symptoms was missing in 10% of the sample.

Viral isolation by cell culture was done in 114 patients who presented within the first five days of symptoms, resulting in 9 (7.9%) positive isolates. Serology was done in the remaining 434 patients, 381 (87.8%) of whom were seropositive. Altogether, dengue laboratory tests revealed that 390 patients (71%) were positive (381 by serology and 9 by viral isolation).

Altogether, laboratory requests by health care facility were as follows. The Social Security system (IHSS) submitted 239 blood samples and 203 (84.9%) tested positive. The Public Health (PH) system submitted 309 samples and 187 (60.5%) tested positive. In terms of type of test requested, viral isolation was requested in 14 patients by the IHSS and in 100 patients by the PH system, for a positivity of 28.6% (5 cases) and 5% (5 cases) respectively. Antibody determination was requested for 225 patients by the IHSS and for 209 patients by the PH system, for a positivity of 88.4% (199 patients) and 87.1% (182 patients), respectively.

For 495 patients for whom the date of onset of symptoms was available, the mean time for taking the dengue sample was 6 days (range 0-65 days).

Nine variables (petechiae, skin rash, myalgias, retro-ocular pain, tourniquet test, cold limbs, gingival bleeding, epistaxis, and skin paleness) had a p value < 0.2 and were thus considered for multivariable analysis (Table 1).

The results of multivariable analysis are shown in Table 2. The following variables were independently associated with dengue fever: petechiae (OR=2.0, 95% CI: 1.3, 3.3), retro-ocular pain (OR=1.7, 95% CI: 1.1, 2.5), gingival bleeding (OR=3.7, 95% CI: 1.1, 13), epistaxis (OR=0.6, 95% CI: 0.4, 1.0), and skin paleness (OR=0.6, 95% CI: 0.4, 0.9). Statistics for collinearity were also applied and the values for tolerance were >0.7 and the VIF (variance inflation factor) was ≤1.3, which represent no significant collinearity among variables tested in the model.

The model had a sensitivity of 86.2% and a specificity of 27%, with a positive predictive value of 74.5% and a negative predictive value of 44.3%. The positive and negative likelihood ratios were 1.2 and 0.5, respectively, for an overall model accuracy of 69.2%. For the classification of cases (positive cases), the software's default threshold or cut-point was 0.5; however the specificity was extremely low (4.4%). Therefore, we increased the cut-point to 0.6 which reduced the overall model prediction (from 71.9% to 69.2%) but increased the specificity. In this cases the cut-point define the level of acceptance of the cases as positive dengue, ROC was used to identify the best cut-point for the classification that provided a higher specificity while keeping a level of sensitivity higher than 85%.

Applying the bootstrap method, we found that p values from the subsamples were similar to those obtained in the entire sample, and the bias values were < 25% of the standard error values of the predictors of the subsamples (Table 3). Including the health care facility as a variable in the multivariable analysis gave us an alternative model that included two clinical predictors with a positive association to confirmed dengue: petechiae (OR=1.80; 95% CI: 1.11 ,2.92) and gingival bleeding (OR=3.24,95% CI: 0.93,11.26). It also revealed paleness of skin as a negative association (OR=0.61; 95% CI: 0.41, 0.90). The health care facility was also a significant predictor for dengue (OR=3.42; 95%CI: 2.23, 5.26). Retroocular pain and epistaxis did not appear in this model and the ORs for the clinical predictors in this model have smaller ORs. At a cut point of 0.6, this alternative model correctly classified 69.9% of the patients, with a sensitivity of 84.1% and specificity of 34.8%, representing a slight improvement in overall classification and specificity but with a reduction in sensitivity over the previous model based only on clinical signs and symptoms. The model, which included health care facility, was also validated using the bootstrap test (tables 4 and 5). In the analysis of ROC we found that the area

under the curve was 0.663 (asymptotic 95% CI: 0.616, 0.710). The equation for the model was

y= 0.694 +0.718(petechiae) + 0.516 (retro-ocular pain)+ .316(bleeding gums) – 0.474

(epistaxis)- 0.535 (skin paleness)

The results given by the ROC curve showed a logistic regression model with an area

under the curve (AUC) of 0.65 (95% CI=0.60, 0.70) and a standard error of 0.025, being

statistically significant. The logistic regression model had a good overall prediction and this was

confirmed through the ROC analysis.

Finally we developed a score from the equation of our model (table #2), the equation is as

follows: 0.694 +0.718(petechiae) + 0.516 (retro-ocular pain)+ 1.316(bleeding gums) – 0.474

(epistaxis)- (0.535 (Pale skin).

A score was obtained rounding up those values of the equation to the close integer,with

the next values: Petechiae= 7 when the sign is present; Retro-ocular pain=5 when this symptom

is present; Bleeding gums=13 when this sign is present; Epistaxis= -5 when it is present

(negative value); and skin paleness =-5 (negative value). When only positive predictors are

present, the maximum score value would be 25 whereas when only negative predictors are

present such value would be -10. Intermediate values can be obtained with different combination

of predictors.

The score was simplified using the values of the equation and reducing it to values 0 and

1 assigning 1 to those considered cases and 0 to non-cases. That is, signs and symptoms scores

were added and a threshold value of 3 was selected to differentiate cases from non cases, (any

value $\leq$ 3 was coded as 0 and values >3 were coded as 1). This resulted in 336 of the 390 positive

correctly predicted, and 43 of the 158 negative patients predicted as no dengue cases. The

sensitivity and specificity for this score were 86.2% and of 27%, respectively. The area under the

curve was 0.567(asymptotic 95% CI were 0.512, 0.622) with a standard error of 0.028. The

accuracy is still low if is intended to operationalize the equation for clinical personnel providing

a rapid method to identify cases of dengue.

Discussion

Our logistic regression model found that five symptoms or signs helped differentiate

dengue from other febrile illness: (1) petechiae, (2) retro-ocular pain, (3) gingival bleeding, (4)

epistaxis and (5) skin paleness. Of these, the association was positive for the first three (likely to

be present in dengue cases), and negative for the last two (epistaxis and pale skin), consistent

with being less likely to be present in dengue cases.

The logistic regression model is based in the identification of the variables with the

lowest *p* value and the highest effect size, which is an approach different to other possible

methods that were considered for the analysis such as the Classification and Regression Tree

(CART) that emphasizes the identification of variables as splitters of the sample in consecutive

subsamples (29). For the purposes of developing our model the probabilistic approach of logistic

regression was an important advantage.

Including clinical signs in the model make intuitive sense. Petechiae and gingival

bleeding, for example, represent subcutaneous or mucosal bleeding and are therefore readily

noticeable by the patient and reported as such. Epistaxis can be frequently associated with

respiratory conditions rather than with dengue, and in our sample most patients with epistaxis

had a negative dengue test.

With respect to effect size, gingival bleeding had a higher OR than other predictors but

also had a wider confidence interval (OR 3.7, CI: 1.1, 13.0.) followed by petechiae, retro-ocular

pain. Compared to other studies, our ORs were smaller for petechiae, retro-orbital pain and gingival bleeding but clearly represented a positive association with dengue positivity ((11, 24, 30-36).

Unlike other studies that have found epistaxis and skin paleness positively associated with dengue (11, 36) (33, 36), our study identified an inverse association with both. Most of our patients with epistaxis had a negative dengue result. Mittal et al reported that skin paleness or pallor was associated with advanced stages of dengue. In fact, 13.3% of their patients with dengue were described as having pallor(36) (36).

In the present study, patients were investigated for other febrile conditions that can cause pallor, such as malaria, anemia, concomitant malnutrition and anemia but most of the dengue-positivity patients did not exhibit paleness of skin. No other studies have incorporated skin pallor as part of a statistical model and it is not possible to contrast with other results. Further studies taking into account the nutritional status of patients are needed to confirm our findings

Paleness of skin has been found associated to dengue presumably because of vascular events (bleeding, vasoconstriction) occurring when dengue evolves into severe forms. In Central America, as in other developing regions, paleness can be associated with chronic anemia and malnutrition but frequently the clinician is faced with limited laboratory resources and must resort to basic clinical evaluation to make a diagnosis.

Notably, proportionally more patients from the Social Security system hospitals were diagnosed correctly (84.9%) than those from the Public Health System (60.5%) as described in the results. The model that included health care facility reduced the number of symptoms to three: petechiae, gingival bleeding and paleness of skin, keeping the same direction of association and similar effect size as the model without health system. The sensitivity of the

34

model increased slightly to 84.1%, and the specificity to 34.8%, for an overall accuracy of 69.9% (Table 4). Including the health care facility as a predictor increased the accuracy of the model but resulted in a reduction in the number of clinical predictors. Although this finding may suggest differences in the populations receiving care at different levels of the Honduran health system, it is most probably due to differences in the clinical judgment of treating physicians at those levels.

We considered it appropriate to keep the model based on signs and symptoms as our main finding, and broadening it with the understanding that the health care facility has a role increasing the accuracy of the model.

Identification of groups at higher risk for dengue infections is a priority when developing a model. Prior to considering a model, we need to analyze the sensitivity and specificity of each of them. Our logistic regression model gave a sensitivity of 86.2% which allows the clinician a moderate level of identification of cases needing a management as dengue fever. However its low specificity (27%) can result in an increase of admissions. Although in theory, rather than missing true cases it would be best to manage an excess of patients who turn out not to be dengue cases, in practice this would result not only in excessive financial costs to the health system but also in unnecessary admissions and ensuing risks for the patients. This situation underscores the need for laboratory testing, in particular the need for rapid tests that could be use at a point of care in order to facilitate clinical decisions.

The score system we developed demonstrated a sensitivity exceeding the specificity which could be important if the priority is to detect a higher number of cases for confirmation.

A strength of this study is that it was conducted during a period of active dengue transmission allowing for a sufficient number of patients to be assessed. One limitation is that our sample may have been characterized by an overrepresentation of symptoms deemed

important to a subgroup of patients in the early stages of the syndrome, particularly with respect

to pain (e.g. headache, retro-ocular pain). Also, the difference in the clinical diagnostic accuracy

between health care facilities providing information is a factor to consider as this may represent

different levels of experience with the disease.

Prior laboratory experience in the Honduras shows that around 40% of suspected cases

with confirmed to be positive by laboratory testing (9, 10). We were unable to differentiate

between primary and secondary dengue, however it is unclear how this would have affected the

results. It is possible that our sample may have been biased by spectrum, with a representation of

patients with more severe illness including symptoms of more severe disease which is observed

in patients consulting the medical service but not in the community where milder disease is more

frequent (external validity could be limited in that population); it is important to remember that

the sample was taken from patients requiring some kind of hospital evaluation and not only

ambulatory care. Patients with ambulatory care had milder symptoms and were not considered

for observation and hospitalization receiving symptomatic management (pain relievers and

medication for fever). It should be noted that this model needs to be periodically validated and

compared with those clinical findings that characterize each epidemic cycle of transmission

In conclusion, we demonstrate that a simple model may identify useful predictors to

differentiate between dengue and other causes of febrile illness. The model should optimally be

used with a method for rapid confirmatory testing. The use of such a model can define those

cases more likely to be dengue and needing primarily a dengue test while other patients can be

directed to a different management. The use of a score table as the one presented in the results

can facilitate the effort of those working in the screening of febrile illness (dengue-like)

especially those requiring hospitalization. Predictive models can hasten treatment decisions and

improve the capacity to report cases, but the need for confirmation is a priority that requires

reliable and rapid diagnostic tests. We can rely on rapid tests with higher specificity to confirm

our findings with the model, with the current limitation of its limited accessibility in areas with

intense transmission, once they are confirmed with the model.

Tables

Table 1. Variables associated with dengue fever among febrile patients in univariable analysis.. Honduras 2009-2010

Variable	Positive dengue test		P value	Unadjusted Odds Ratio	95% CI
	Yes Number (%)	No Number (%)			
Demographic Characteristics					
Gender (n=543):					
Male	168 (43.5)	218 (56.5)		1.28	(0.9,1.8)
Female	59 (37.6)	98 (62.4)	0.204	1.0	
Signs and Symptoms					
Had Petechiae (n=548):					
Yes	120 (81.1)	38 (18.9)		1.52	(1.0.2.3)
No	270 (69.2)	130 (32.5)	**0.002**	1.0	
Had skin Rash(n=548)					
Yes	138 (76.7)	42 (23.3)		1.51	(1.0,2.3)
No	252(68.5)	116 (31.5)	**0.046**	1.0	
Had myalgias (muscle Pain) (n=548)					
Yes	336(72.6)	127 (27.4)		1.52	(0.93,2.47)
No	54(63.5)	31 (36.5)	0.091	1.0	

Had retroocular pain					
Yes	296(74.6))	101 (25.4)		1.51	(1.0,2.3)
				1.0	
No	94 (62.3)	57 (36.1)	**0. 040**		
A (+) tourniquet test identified					
Yes	26 (86.7)	4 (13.3)		2.75	(0.94,8.0)
No	364 (70.3)	154 (29.7)	0.054	1.0	
Cold limbs at evaluation (n=548):					
Yes	139 (66.8)	69 (33.2)		0.71	(0.5,1.0)
No	251 (73.8)	89 (26.2)	0.079	1.0	
Complains of bleeding gums (n=548):					
Yes	24 (88.9)	3 (11.1)		3.4	(1.0,11.4)
No	366 (70.2)	155 (29.8)	**0.037**	1.0	
Bleeding Nose (n=548):					
Yes	68 (63.6)	39 (36.4)		0.64	(0.41,1.01)
No	322 (73.0)	119 (27.0)	0.057	1.0	
Paleness (n=548)					
Yes	174 (65.9)	90 (34.1)		0.61	(0.4.0.9)
No	216 (76.1)	68 (23.9)	**0.009**	1.0	

*Pearson Chi square test
was performed for these
variables.

Table # 2

Final Logistic Model for Predictors of Dengue Fever (n=548)

Honduras 2009-2010

Predictor	P-value	Odds Ratio	95% Lower Confidence Interval	95% Upper Confidence Interval
Petechiae	0.003	2.0	1.3	3.3
Retro-ocular pain	0.014	1.7	1.1	2.5
Bleeding gums	0.038	3.7	1.1	13.0
Bleeding nose	0.045	0.6	0.4	1.0
Paleness	0.006	0.6	0.4	0.9
Constant	0.001	2.0		

Logistic Regression Model Statistics:

Rho square is 0. 33 Values between 0.20 to 0.40 suggest a very good fit (39)

Homer and Lemeshow p=0.788 . There is a good fit and the model prediction is not significantly different to the observed values.

Cox & Snell R-square = 0.059; Nagelkerke R-square= 0.084

69.2 % correctly classified.

Model equation:

 0.694 +0.718(petechiae) + 0.516 (retro-ocular pain)+ 1.316(bleeding gums) – 0.474 (epistaxis)- (0.535 (Pale skin).

Table 3

Bootstrap for the Logistic Regression Model (Final Model) after 1000 samples

Predictor	B	Bootstrap		
		Bias	Standard error	Significance (2 tails)
Petechiae	0.718	0.015	0.239	0.004
Retro-ocular pain	0.516	0.006	0.223	0.013
Bleeding gums	1.316	0.871	3.899	0.024
Bleeding nose	-0.474	-0.009	0.236	0.035
Paleness	-0.535	0.009	0.202	0.011
Constant	0.694	0.006	0.213	0.003

Graph # 1

ROC curve for the logistic regression model

Predictors of dengue fever (n=548)

Honduras 2009-1010

ROC Curve
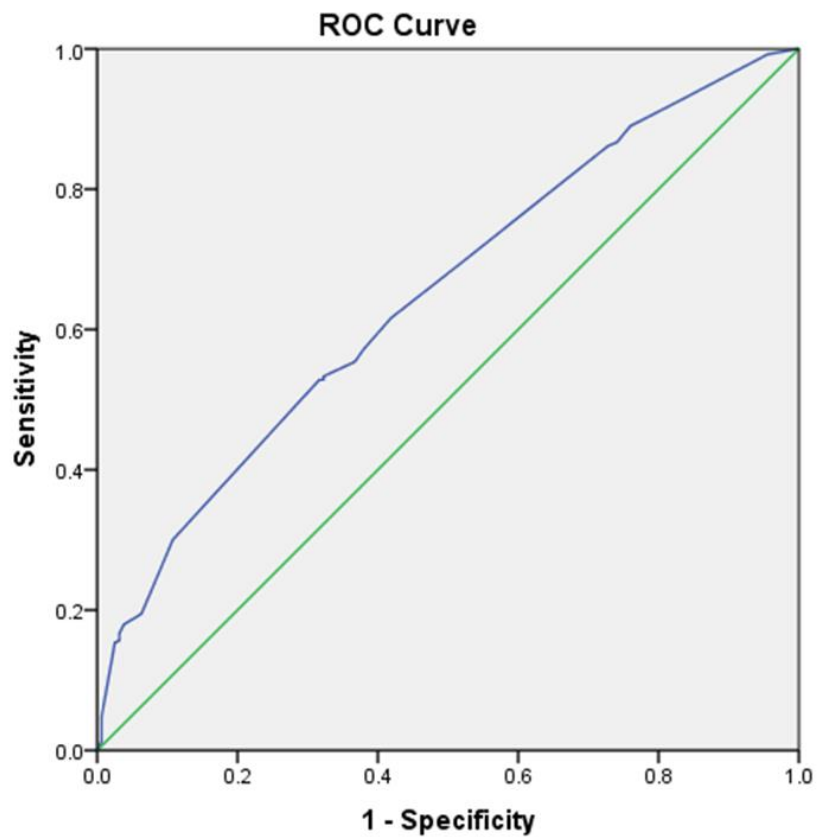
Diagonal segments are produced by ties.

Area Under the Curve

Logistic Regression Model

Test Result Variable(s): Predicted probability

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.649	.025	.000	.600	.698

Coordinates of the Curve

Test Result Variable(s):Predicted probability

Positive if Greater Than or Equal Toa	Sensitivity	1 – Specificity
.0000000	1.000	1.000
.4808102	.992	.956
.5449569	.926	.829
.5525384	.890	.759
.5771172	.867	.741
.6310601	.862	.728
.6648315	.700	.525
.6716019	.615	.418
.6912262	.572	.380
.7105347	.554	.367
.7168033	.533	.323
.7250380	.528	.323
.7508979	.528	.316
.7857709	.300	.108
.8026009	.195	.063
.8073870	.179	.038
.8154665	.167	.032
.8216195	.162	.032
.8355177	.156	.032
.8605604	.154	.025
.8765163	.049	.006
.8809368	.036	.006
.8840950	.028	.006
.8948422	.023	.006
.9147196	.021	.006
.9318116	.015	.006
.9500441	.013	.006
1.0000000	.000	.000

TABLE # 4

Logistic Regression Model including Health System (Centre)

Predictive Model for Dengue Fever

N=548

Variable	P value	Adjusted Odd Ratio	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Petechiae	0.017	1.8	1.11	2.92
Gingival Bleeding	0.064	3.2	0.93	11.26
Health system (centre)	0.000	3.4	2.23	5.26
Paleness	0.014	0.6	0.41	0.90
Constant	0.026	0.5		

Logistic Regression Model Statistics

Rho square=58.03

Cox-Schnell R-square=0.10 ; Nagelkerke R-Square=0.14

69.9% cases were correctly classified

No missing cases in variables included in the model

Equation:-0.712+0.589(Petechiae) +1.17 (gingival bleeding)+1.2 (health system) -0.494 (pale skin)

Table # 5

Bootstrap for Variables in the Equation with Centre included

Predictive Model for Dengue Fever. Honduras 2009-2008

N=548

		Bootstrap[a]				95% Confidence Interval	
		B	*Bias*	*Std. Error*	*Sig. (2-tailed)*	Lower	Upper
Step 1	*Petechiae*	.589	.011	.255	.020	.118	1.132
	Gingival bleeding	1.177	.909	3.893	.029	.193	20.173
	Health System (center)	1.232	.003	.227	.001	.808	1.701
	Paleness	-.494	-.012	.204	.016	-.903	-.142
	Constant	-.712	.006	.340	.030	-1.354	-.059

Graph # 2

Area Under the Curve
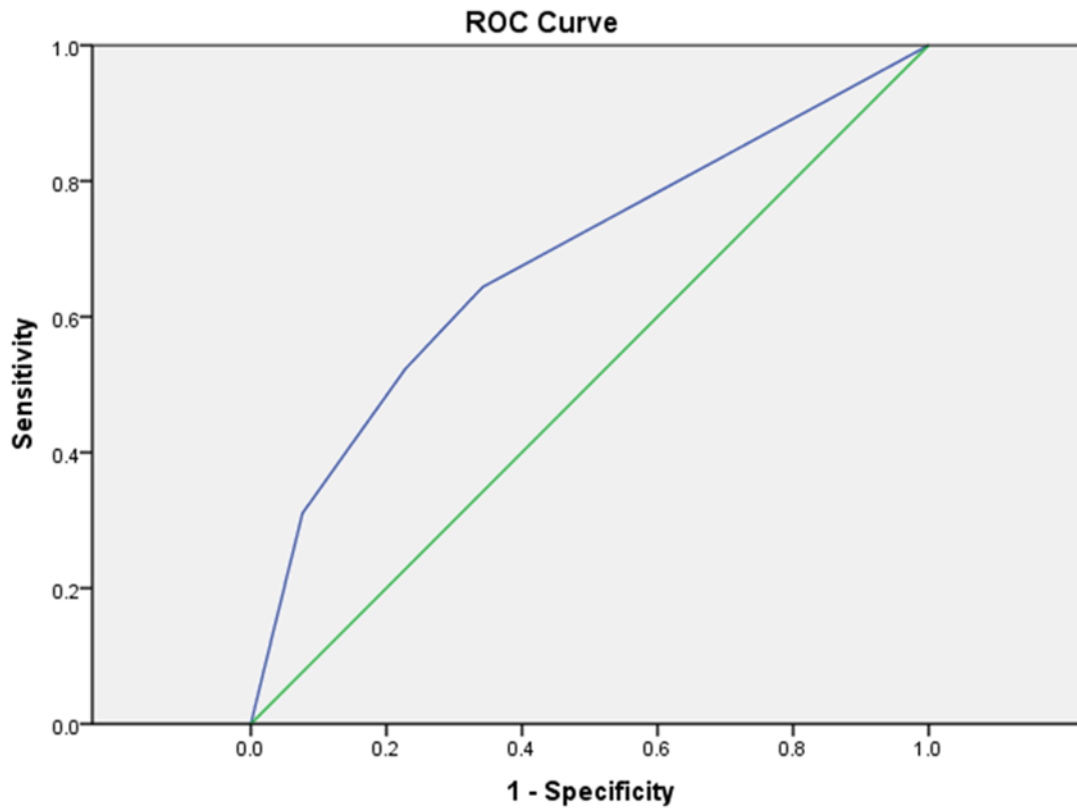
Logistic Regression Model Including Health System

N=548

ROC Curve

Diagonal segments are produced by ties.

Area Under the Curve

Logistic Regression Model Including Health System

N=548

Test Result Variable(s):Predicted Probability for

positividadtotal=1

| Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
			Lower Bound	Upper Bound
.682	.024	.000	.636	.729

Coordinates of the Curve

Test Result Variable(s):Predicted probability

Positive if Greater Than or Equal To[a]	Sensitivity	1 – Specificity
.0000000	1.000	1.000
.5673803	.841	.652
.6398478	.664	.348
.7032224	.600	.291
.7615875	.549	.247
.7733014	.541	.241
.8111549	.428	.146
.8478931	.415	.146
.8547469	.213	.076
.8613705	.208	.070
.8863127	.123	.013
.9103453	.121	.006
.9155020	.033	.000
.9336609	.023	.000
.9511472	.013	.000
.9623982	.010	.000
1.0000000	.000	.000

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CHAPTER 3: PREDICTIVE MODEL FOR SEVERE DENGUE

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ABSTRACT

Background: One of the challenges in dengue is to predict which patients will go on to experience severe illness, which is typically characterized by fever, thrombocytopenia, haemorrhagic manifestation, and plasma leakage. Accurate prediction could result in the appropriate hospital triage of high risk patients. The objective for this study was to identify clinical factors that predict severe dengue during the first 24 hours of hospital admission.

Methods: We conducted a retrospective cohort study of 320 patients with febrile illness who had microbiologic confirmation of dengue within one week of admission using data from the 2009-2010 Honduras Epidemiological Survey for Dengue. The outcome measure was plasma leakage defined using hemoconcentration $\geq 15\%$ determined by serial hematocrit testing. We conducted univariable analysis and multivariable logistic regression analysis to construct a predictive model for severe dengue.

Results. Sixty-four (20%) of patients in the 320 patient cohort had hemoconcentration $\geq 15\%$. We found that in the final multivariable logistic regression model the presence of ascites and the platelet count during admission to the hospital was associated with plasma leakage while the presence of petechiae and headache were negatively associated with leakage. Using regression and an ROC analysis we initially found that a cut-off point of 0.5 (50% chance of detecting a case of severe dengue) provided a low sensitivity while a moderate specificity. We subsequently found that using a minimum estimated probability of 7% that a person is a case led to a 70.3% specificity for an overall accuracy of 70.9%.

Conclusion: Using logistic regression, we identified signs and symptoms that can correctly

identify a majority of patients that eventually develop severe dengue. It will be important to

further refine the models and validate it in different populations.

Keywords: severe dengue, predictors, models.

Introduction

Dengue fever is a mosquito-borne disease, with a high incidence in tropical and

subtropical regions; most cases are reported in South East Asia, the Americas Region and the

Pacific Basin (1). Treatment for the disease is not yet available, and vaccines are in the process

of evaluation.

Identifying cases of dengue that are likely to eventually develop severe dengue is an

important challenge in endemic regions. Previous studies have had varied success in identifying

patients that go on to develop severe illness (2-6). Although a microbiologic diagnosis of dengue

can be made within days of clinical presentation, identifying those patients who will go on to

develop severe dengue could serve as an important form of triage for hospitals. That is, patients

at low risk would not require the intensive follow up of high risk patients and this could help

reduce the burden on the healthcare system. Conversely, high risk patients would be monitored

more closely and given early supportive care as they develop evidence of severe disease.

Symptoms and signs are typically recorded for patients with suspected dengue and widely

available testing, such as blood platelets and hematocrit is usually done. Thus, a combination of

early symptoms and laboratory tests could help quantify the likelihood of severe dengue disease.

Using data from Honduras, the objective of this study was to identify clinical and

laboratory factors that predict severe dengue, previously known as dengue hemorrhagic fever

(DHF) and dengue shock syndrome (DSS), during the time of admission of the patients that subsequently had a confirmatory test for the dengue fever diagnosis.

Methods

We conducted a retrospective cohort study using data involving patients with dengue from the Honduras Ministry of Health. This data was collected in accordance to guidelines put forth by the U.S. Centers for Disease Control and Prevention Dengue Branch (CDC) (7). The objective of the study was to determine clinical and laboratory predictor variables for plasma leakage, which is the key component of severe dengue (1, 8-10). The database included demographic, clinical, and laboratory variables of patients with suspected severe dengue that were systematically collected by general practitioners, nurses, and microbiologists. Original records were reviewed and the information was organized in a database in the statistical package Epiinfo version 3.5.1. The procedure was performed by two data entry technicians under the supervision of the investigator prior to the analysis with the statistical package SPSS version 19.

This cohort was a subset of patients that had initially presented to clinics in 13 of the 18 provinces of Honduras with suspected dengue and who were ultimately evaluated for severe dengue in tertiary care hospitals in the two major cities of Tegucigalpa and San Pedro Sula between 2009 and 2010 either through self-referral or through their primary care provider.

Only patients with documented dengue, as confirmed from blood specimens drawn within 48 hours of presentation and diagnosed as dengue serologically using Ig M or by viral culture were analysed for this study. Patients had to be on the sixth day or later with respect to symptom onset for the serology to be obtained, otherwise diagnosis was made using viral culture

(7, 11). The majority of the 320 cases were diagnosed with dengue by IgM (315) and five were diagnosed by viral isolation. Serology and viral isolation was performed by the Honduras National Laboratory of Virology according to CDC guidelines and standardized techniques, using commercial kits. The patients in this dataset had at least two consecutive readings of hematocrit and platelets in the first 24 hours after their admission to the hospital.

Predictor Variables

The independent variables consisted of : 1) demographic information (age, sex), symptoms reported within the first 24 hours of admission including general symptoms, such as fever, headache, retroocular pain, arthralgia, myalgia, rash, anorexia and vomiting; 2) bleeding manifestations, including petechiae, ecchymosis, hematemesis, melena, positive tourniquet, epistaxis, gingival bleeding, tourniquet test at admission, hematuria and heavy menstrual bleeding (metrorrhagia); and 3) symptoms and signs suggestive of capillary leakage, including abdominal pain, cold extremities, sweating, pale skin and serous membranes' effusion (pericardial, pleural effusion and ascites) and reduction of medial arterial pressure (based on blood pressure recorded within the first 24 hours of admission). These signs and symptoms of capillary leakage may also appear in other systemic or localized medical conditions.

Laboratory data on leukocytes, platelets, hemoglobin and hematocrit counts was also collected during the admission and 24 hours later. Thrombocytopenia was defined as a platelet count of 100,000 platelets/mm^3 or less (8).

Outcome

The outcome or dependent variable was plasma leakage. Although death is the most serious outcome for severe dengue, since it is uncommon in the Americas, plasma leakage was selected as the outcome (8, 12). Plasma leakage is a central event in the pathogenesis of severe dengue, leading to hypovolemia, hypotension, edema, shock, and potentially death (1, 8).

Key elements in the classification of severe dengue include the presence of plasma leakage manifestations and hemorrhagic symptoms. In our study, plasma leakage was measured by hemoconcentration using two consecutive readings of hematocrit, the first hematocrit taken at admission and the second 24 hours later. Hemoconcentration gradually increases over the course of severe dengue. Although a threshold of 20% above baseline is frequently used, we selected a threshold of 15% to increase the sensitivity of the analysis (8, 13). Previous studies in Colombia and Puerto Rico have considered thresholds as low as 10% of hemoconcentration having found that sensitivity is increased and specificity is not significantly reduced (14, 15). We included an intermediate value between the traditionally accepted value and such a lower threshold as a way to include a larger number of cases while maintaining specificity.

Analysis

We conducted a univariate analysis assessing the relationship between the predictor variables and plasma leakage as defined by a 15% increase in hematocrit. Variables with a p value <0.2 were considered for multivariable analysis using logistic regression built using forward step-wise selection. The variables included in the final model were statistically significant (p< 0.05),. Variables were included if no collinearity was observed among them. Collinearity was assessed using the tolerance statistic (values >0.2 were acceptable) and the variance inflation factor (VIF) which was acceptable if values were < 5 Using a cut-off of 12

years, we defined children as 12 years and under, and those older than 12 years as adults.

Goodness of fit was determined using Hosmer Lemeshow statistics (p>0.05 for the model).

The final model was validated internally using the bootstrap technique (with the sample of 320 individuals) where 1,000 subsamples were drawn randomly such that estimators from the subsamples could be compared (16).

Clinics and hospitals came from two different health systems: the social security system, which provides medical services to workers of the public and private sector, and the public health system which is open to all the population but mainly to rural population, unemployed or under-employed population. In what we refer to as a "centre effect", we assessed the effect of the type of health system so that we could compare estimator values based on each system to rule out differences on this basis. Bootstrap was done by dividing the sample by health systems (Public Health System and Social Security) to assess if this would lead to differences in estimator values.

The rationale for selecting logistic regression was based on the outcome being a binary variable and the fact that the model allows predictor variables to be categorical or continuous. Advantages are that logistic regression can control for confounders, has a good capacity to detect effects that are small, can identify interactions, is better than other methods at identifying predictors that contribute additively, and makes no assumptions about the distribution of predictor variables. A potential disadvantage is the need of larger samples than those required by other methods in order to guarantee enough observations for each of the independent variable categories (minimum of 10 observations per variable) and sensitivity to outliers (17).

RESULTS

Of the 320 patients in the cohort, 139 (43.4%) were female and the mean age was 22.4

years. 244 (49.2%) of cases were hospitalized six or more days after the first symptom. There

were 34 (10.6%) of cases that met our definition of plasma leakage, based on a

hemoconcentration of 15% or more. Descriptive statistics are shown in Table 1. The mean

hematocrit during admission was 40.6 while at 24 hours it was 40.1. At baseline, the mean

number of platelets was 73, 684 platelets/mm^3 and 24 hours later 58,124platelets/mm$^{3.}$

In univariable analysis, cases were less likely to have petechiae (p<0.05, O.R.=0.3, with

95%CI=0.1,0.8) but were more likely to have ecchymosis (p<0.05, O.R. =1.1 with 95%

CI=1.0;1.2). The following variables were considered for multivariable analysis: ascites,

petechiae, ecchymosis, headache, skin rash, retro-ocular pain, platelet count on admission.

Platelets were included as a binary variable with a threshold of 50,000 platelets/mm$^{3.}$ Obtaining

two categories (<50,000 and≤ 50,000) provided for a statistical significant x^2 test and ORs >The

recoding procedure had the purpose to identify a threshold associated to statistical significance,

The values for platelets were included as potential predictors. As specified, a priori, age and sex

were included in the model.

The collinearity test gave a tolerance ≤ 1.0 and a VIF <5 (i.e. the higher VIF value was for

platelets and headache: 1.007).

The final model included the variables headache, petechiae and ascites and low platelets

at baseline. The model was tested for fit using the Hosmer Lemeshow test (p=0.1) which

showed that the observed events match the expected events (given by the model); the Nagelkerke

R-square showed an improvement of our model of 15.1% over a null model with no predictors

(Cox Schnell R-square was only 7.4%); and reviewing the final classification table the sensitivity

reached 76.5% and the specificity was 70.3% for an overall 70.9% correct classified

observations. The model was also tested for fit using Rho square, it was compared with model

zero (with no predictors) and the result was 0.11524. A value between 0.20 and 0.30 suggests a

good fit (18). The odds ratio for headache was 0.37 (95%CI=0.15, 0.94), for petechiae it was

0.24 (95%CI=0.080, 0.73), for baseline platelets 2.9 (95% CI=1.4,6.30) and for ascites

7.3(95%CI=1.86, 28.7) (Table 3).

The equation for the model was: y= - 1.631-0.978(Headache)-1.417(Petechiae) +1.99

(Ascites) +1.087(Platelets on admission). Values obtained from running the equation were

grouped according to frequency and the values were assessed as threshold to establish the values

0 and 1 in a new variable score; we obtained a ROC graph to verify the sensitivity and specificity

for the selected threshold, the value we tested was -2.6, predicting correctly 26 of the 34 cases

(sensitivity 76.4%) and 201 of the 286 non-cases (specificity of 70.3%). Our score then was set

in -2.6, any value lower was included as 0 (non- case), and those $\geq$-2.6 was included as 1(case).

Any patient with presence of the predictors adding $\leq$-2.6 was classified as non-case (0),

and those with value >-2.6 was classified as case. Using the threshold of -2.6 provided better

specificity and classified correctly a higher number of patients.

The validation of the model with bootstrap showed estimates (standard error and bias)

with values close to the predictors (B) and relatively narrow confidence intervals; when samples

with 50% observations were validated the estimators became larger in value and confidence

intervals became broader. The results of model validation are shown in Tables 4 and 5.

Since one group of patients belonged to the public system (N=120) and another to the

Social Security system that provides services to public and private company workers (N=200)

we split the sample in two subsamples corresponding to each one of these systems (Tables 6 and 7) to do bootstrapping; the estimators were larger than those obtained for the original sample and their confidence intervals were broader than those in the complete sample.

When the centre (health system) effect was analyzed by including it in the model its role was not significant in the logistic regression model, its p=0121 and the odd ratio was 1.8 (95% CI=0.85, 3.9) with a positive direction to the Social Security system. The role of the centre did not affect the logistic regression model (it did not alter the predictors).

In the model (logistic regression) ascites was the key predictor followed by petechiae and headache. The platelets at hospital admission had a cut-off of 50 000 platelets/mm3 was present in the logistic regression model and was also statistically significant.

A ROC analysis was done for the model, obtaining an area under the curve of 0.746 (95% CI: 0.6630, 0.8290) with a cut off of 0.07, these results are in moderate values but at the same time show that a specificity over 90% will have a very low sensitivity (around 10%) and vice versa. Our selection at cut off 0.07 gives us lower specificity but higher sensitivity (Charts 3 and 4).

DISCUSSION

Our final logistic regression model included the following predictors: ascites, headache, petechiae and having platelets < 50,000 at admission. The logistic regression model improved its accuracy (specifically for sensitivity and specificity) with a change in the cut-off to 0.07 as suggested by the ROC curve with a decrease in the sensitivity and no changes in the predictors; maintaining the overall correct prediction of the status of patients (case or no case), which was 70.9%.

Ascites has been reported to be present in the very early stages of dengue fever and has frequently been associated with evolution to severe stages of dengue; in previous studies ascites has also been noted to be an early predictor for severe dengue, and for admission to intensive care as well(19-21). The reduction of platelets to values under 100,000/mm3 has also been shown to be to be a warning sign for severe dengue (1, 22). Although platelets of less than 100,000platelets/mm^3 were observed in our study we found that only values under 50,000/mm^3 were significantly associated with severe dengue in our regression model. Headache has been identified in severe and non-severe dengue cases. Previous studies described headache as a symptom more likely to be associated with non-severe dengue and occurring with other symptoms like ocular pain. (23, 24); this close relationship to non-severe dengue is consistent with the negative association found in our study. Unlike previous studies that have identified it as a predictor of severe dengue (25-28) petechiae were negatively associated with severe dengue in our model which is related to the high prevalence in non-severe cases in our study sample.

Prior models that have been developed for severe dengue differed from ours in that they derived different combinations of predictors based on the inclusion of more laboratory variables (e.g.white blood cells count, aspartame aminotransferase) or other clinical symptoms like

myalgia, arthralgia and abdominal pain that do not appear in our model. Thrombocytopenia has

been included in these models with higher thresholds, and petechiae with different direction of

association (4, 13, 27). Our study sample consisted of patients with dengue fever admitted to a

health facility. Our methodology and sampling were different from previous studies and notably

our study had fewer laboratory variables. On the other hand our study had access to information

available in the first few hours of admission, in a situation resembling that of medical personnel

doing a triage with just basic information. In our logistic regression model the predictors that

consistently were identified with a positive association were ascites and first platelets reading,

but the rest of those provided by the logistic regression model were positively associated in the

other studies, and we found a negative association with headache and petechiae, which could be

influenced by the fact that our sample was formed by patients with dengue positivity and who

constituted the majority of individuals with those symptoms.

A strength of our study is that the model equation was transformed into a score that could

be applied to patients to classify them as cases or non-cases. Validation of the model, preferably

in a different study sample, is an integral part of developing a model. Although lack of external

validation is a limitation of our model, our use of bootstrapping sampling led to the development

of accurate estimators. We acknowledge the fact that death could not be used as an outcome

because of the low death rate and so we selected difference of hematocrits in the first two

readings after the admission of patients. We acknowledge that using changes in platelet levels

would be an alternative approach (3).

A key limitation of our study is that our model presented a high level of specificity but

low sensitivity, in a population where the cases represented close to 20% of the sample; the

alternative is reducing the cut-off to values lower than 0.2 to increase sensitivity but a decrease

of the specificity. Since clinical practice is centered on the identification of cases with high risk, it is important to increase that capacity to detect true cases (high sensitivity) to allow the ruling out of severe dengue, which means a reduction of the cutoff point may detect a larger number of true cases (around 80 %) but also increasing the report of false positives (reduction of specificity).

Under ideal conditions the type of model we derived should be used in conjunction with other diagnostic methods that add their own accuracy to the model. The use of the model equation and ROC can provide a better sense of the best cut-point, or verification of the adequate selection of this threshold. The development of a score point system provide a way to determine what patients are under higher risk of having severe dengue depending the presence of the predictors identified by the model; this can contribute to a better patients triage in hospitals and their emergency rooms.

The findings in our study are relevant to regions with reduced access to diagnostic tests such as imaging to detect fluid extravasation. From a public health perspective, our model can be a valuable tool to screen populations during epidemic periods and in areas with high transmission can assist in the classification of cases. It is important to include statistical tools to the interpretation of the cases classification, otherwise we are just tabulating for descriptive statistics, and to keep a record of warning symptoms.

Table 1

<div align="center">

Descriptive Statistics

Severe Dengue in Honduras 2009-2010

Variable means

</div>

Variable	N	Mean
Systolic Pressure (mm Hg)	100	106
Diastolic Pressure (mm Hg)	100	68.75
Age (years)	304	22.6
Time between symptoms onset and hospital admission (days)	244	4.4 days
Hematocrit at hospital admission	320	40.6
Hematocrit after 24 hours	320	40.1
Platelet 1(per/mm^3)	320	73684
Platelet 2(per/mm^3)	320	58124
Leucocytes at admission	313	5246
Leucocytes after 24 hours	311	5674

Table 1

Table 2 .Variables associated with severe dengue in patients with dengue fever confirmed by laboratory. Severe dengue in Honduras 2009-2010 (n=320)

VARIABLES	Hemoconcentration > than 15%		O.R.	95% C.I.	P
	No	Yes			
	Number (%)	Number(%)			
Ascites			5.3	[1.5,19.2]	0.021
N=320					
No	279(90.3%)	30(9.7%)			
Yes	7 (63.6%)	4(36.4%)			
					**
Headache			2.3	[1.0, 5.4]	0.101
N=320					
No	34(81%)	8(19.0%)			
Yes	252(90.6%)	26(9.4%)			
					**
Skin Rash			1.8	[0.8,4.0]	0.139
N=320					
No	173(87.4%)	25(12.6%)			
Yes	113(92.6%)	9(7.4%)			
					*

Ocular pain			1.6	[0.7, 3.5]	0.2
N=320					
No	65(85.5%)	11(14.5%)			
Yes	221(90.6%)	23(9.4%)			*

Petechiae			0.3	[0.1, 0.8]	0.011
N=320					
No	191(86.4%)	30(13.6%)			
Yes	95(96.0%)	4(4.0%)			
					*

Ecchymosis			1.1	[1.0 ,1.2]	0.036
N=320					
No	251(88.1%)	34(11.9%)			
Yes	35(100%)	0(0%)			*

Sex			0.8	[0.4,1.6]	0.47
N=316					
Female	126(90.6%)	13(90.4%)			
Male	156(88.1%)	21(11.9%)			*

Age			1.1	[0.5, 2.3]	0.74
N=314					
≤ 12yrs	107(88.4%)	14(11.6%)			
>12yrs	173(89.6%)	20(10.4%)			*
Time between first symptom and hospital admission			0.5	[0.2,1.3]	0.16
N=244					
≤3 days	64(92.8%)	5(7.2%)			
>3 days	151(86.3%)	24(13.7%)			
					*
Platelets (first count)			0.9	0.8,1.2	0.8
N=320					*
≤ 100K	65(90.3%)	7(9.7%)			
>100K	221(89.1%)	27(10.9%)			

			0.9	[0.8,1.0]	1.0
Platelets2 difference (second count) N=320					
≤ 100K	31(96.9%)	1(3.1)			
>100K	255(88.5%)	33(11.5%)			*
Platelets 1 (second count) N=320			0.63	[0.49,0.82]	0.04
					*
50k/mm3	128(84.2%)	24(15.8%)			
>50k/mm3	158(94.8%)	10(15.8%)			

* Pearson chi-square   ** Fisher exact test

Table 3

Logistic Regression Model

Severe dengue Honduras 2009-2010 (N=320)

VARIABLE	Odd Ratio	95% Confidence Interval	p-value
Ascites	7.3	[1.86, 28.7]	0.004
Petechiae	0.24	[0.08,0.73]	0.012
Platelet 1(Admission)	3.00	[1.4,6.3]	0.005
Headache	0.37	[0.15,0.94]	0.037
Constant	0.16		0.000

Statistics for the Predictive Model

Rho square is 0.11

Cox-Schnell R-square =0.074

Nagelkerke R-square=0.151

Hosmer and Lemeshaw p=0.1

Overall accuracy : 70.9% (For a cutoff in 0.07)

Sensitivity=76.5% ; Specificity=70.3%

No missing cases

Equation: - 1.631-0.978(Headache)-1.417(Petechiae)+1.99 (Ascites) +1.087(Platelet1 admission)

Bootstrap for variables in the equation

Severe dengue Model

Honduras 2009-2010 (n=320)

Predictors	B	Bootstrap			95% Confidence Interval	
		Bias	Std. Error	Sig. (2-tailed)	Lower	Upper
Ascites	1.988	-.182	-2.488	.001	.371	3.454
Petechiae	-1.417	-.419	2.596	.006	-3.221	-.573
Platelets1 (Admission)	1.087	.007	.426	.008	.265	1.984
Headache	.978	.038	.546	.041	-1.945	.260
Constant	-1.631	-.078	.577	.002	-3.125	-.720

Table 4

Logistic Regression Model

Severe Dengue Honduras 2009-2010 (N=200)

SOCIAL INSURANCE SYSTEM

VARIABLE	Odd Ratio	95% Confidence Interval	p-value
Ascites	34.00	[1.66, 697.18]	0.022
Petechiae	0.07	[0.01,0.80]	0.033
Platelet 1(Admission)	8.30	[2.31,29.71]	0.001
Headache	0.49	[0.11,2.18]	0.347
Constant	0.07		0.001

Equation: -2.667-.719(Headache) -2.691(Petechiae)+ 3.526 (Ascites) + 2.114 (Platelet1 admission)

Logistic Regression Model

Severe dengue Honduras 2009-2010 (n=200)

SOCIAL INSURANCE SYSTEM

Bootstrap for Variables in the Equation

					Bootstrap	
					95% Confidence Interval	
	B	Bias	Std. Error	Sig. (2-tailed)	Lower	Upper
Ascites	3.526	.899	17.133	.001	-18.278	41.642
Petechiae	-2.691	-9.841	8.054	.001	-19.530	-1.732
Headache	-.719	.859	4.236	.358	-2.513	18.474
Platelets1 (admission)	2.114	.718	4.852	.002	.880	4.588
Constant	-2.667	-1.619	7.127	.009	-22.767	-1.124

Table 5

Logistic Regression Model

Severe dengue Honduras 2009-2010 (n=120)

PUBLIC HEALTH SYSTEM

VARIABLE	Odd Ratio	95% Confidence Interval	p-value
Ascites	3.96	[0.83,18.93]	0.085
Petechiae	0.54	[0.14,2.10]	0.377
Platelet 1(Admission)	1.22	[0.45,3.34]	0.698
Headache	0.39	[0.12, 1.31]	0.130
Constant	0.38		0.092

Equation: -.959-.931(Headache) -.609(Petechiae)+ 1.376 (Ascites) + .199 (Platelet1 admission)

CHART # 1

ROC   FOR LOGISTIC REGRESSION MODEL

SEVERE DENGUE HONDURAS 2009-2010
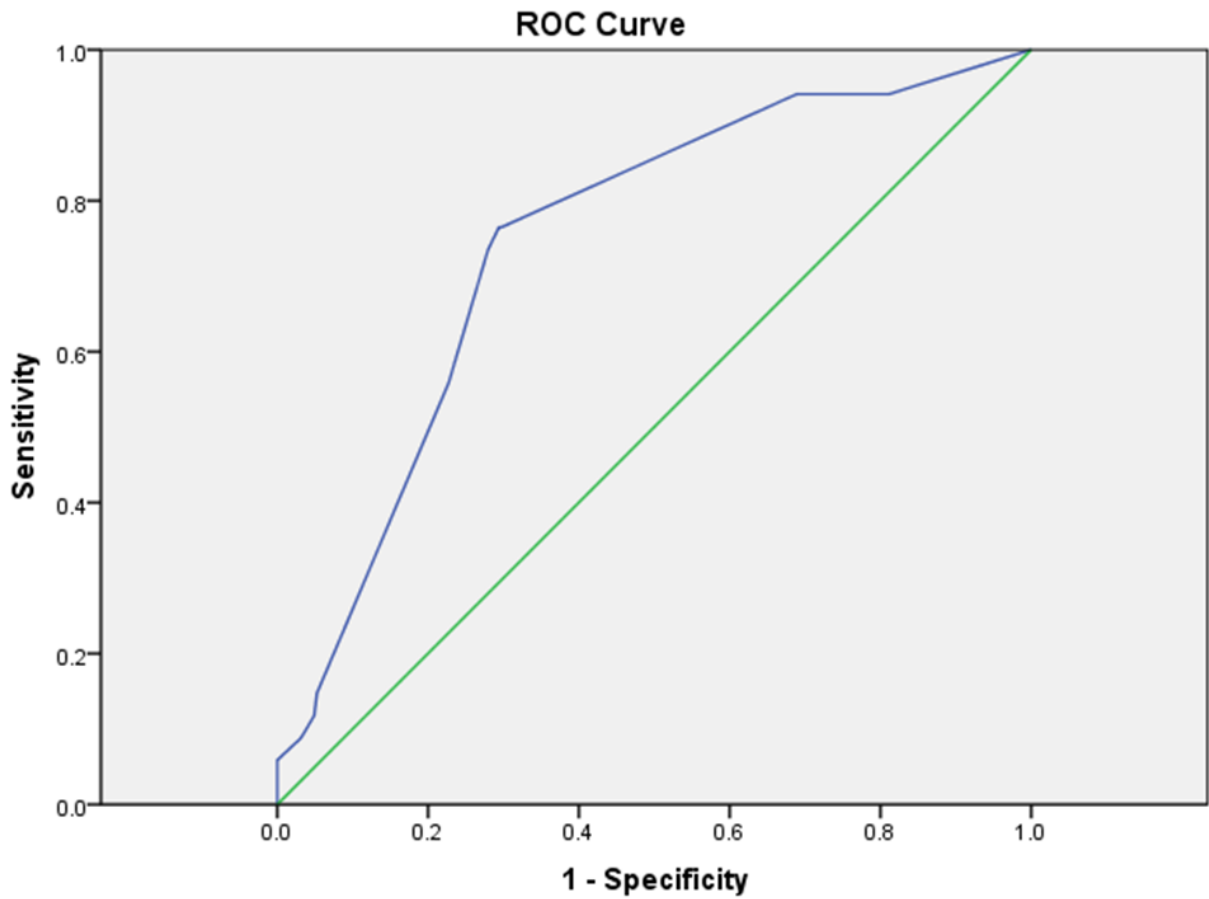
Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s):Predicted probability

Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.746	.042	.000	.663	.829

Coordinates of the Curve

Test Result Variable(s):Predicted probability

Positive if Greater Than or Equal Toa	Sensitivity	1 - Specificity
.0000000	1.000	1.000
.0314198	.941	.811
.0477742	.941	.790
.0594169	.941	.689
.0919476	.765	.297
.1193121	.765	.294
.1435380	.735	.280
.1714661	.559	.227
.2289226	.147	.052
.3141950	.118	.049
.3584954	.088	.031
.4908923	.059	.000
1.0000000	.000	.000

Appendix # 1

Dengue Model for Severe Dengue

Previous Studies for classification of Severe dengue

Researcher and year	Place	Dependent Variable	Type of Study	Sample size # participants	Results	Strengths	Limitations
Diaz Quijano F (2005)	Colombia	Dengue stage (of severity)	Cross-sectional study	891	Model	Includes clinical and laboratory predictors	No validation is described Accuracy is not mentioned.
Thomas L (2008)	Martinique	Dengue classification	Prospective observational study	715	Model	Information was reviewed by two experts before confirmation of severe dengue	Patients coming from one emergency department Some clinical categories can be biased Confounding with variables not considered in the analysis
Potts JA (2010)	Thailand	Severe dengue (WHO criteria)	Retrospective cohort study	1384	Identification of predictors	Validation with an alternative dataset	Only in children Includes only laboratory predictors.

Cavalcanti (2010)	Brazil	Dengue Hemorrhagic Fever (WHO 1997)	Retrospective Cohort study	450	Description of prevalent clinical manifestations	Information related to circulation of specific serotype (Den3) General population	Selection and Information bias No model was developed No validation
Biswas HH(2012)	Nicaragua	Dengue hemorrhagic fever (WHO 1997)	Prospective cohort	3800	Identification of severe dengue cases Identification of predictors	Use of different statistical models Prospective studies Community - based study	Pediatric population Low statistical power Older classification was used Cases from one city
Mena Lara (2013)	Dominican Republic	Severe dengue based in WHO definition	Retrospective study	796	Identification of predictors	Sample size with Few missing values	Retrospective design Data from one clinical centre

Huy NT (2013)	Vietnam	Dengue Shock Syndrome	Prospective cohort analysis	444	Model equation Model with 7 predictores	10 fold Validation of model Equation Development of score	Information from 2 hospitals only Some markers were not included No treatment variables were included Patients with shock risk only

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CHAPTER 4: Agreement between a national definition and World Health Organization for classification of severe dengue

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Article summary line:

Given reports of differences between the definition of severe dengue by the World Health Organization and the classification used locally in developing countries, we determined the level of agreement of these in Honduras when classifying severe dengue fever.

Running title: Agreement on severe dengue classification.

Keywords: Dengue, national classification, agreement, plasma leakage, haemorrhagic manifestations.

ABSTRACT

Introduction: In 2009, a technical committee of the World Health Organization (WHO) issued new criteria for the classification and clinical management of severe dengue. There are reports that these criteria may over-diagnose severe dengue in comparison to prior WHO criteria. The purpose of this study was to determine the level of agreement between classification using national Honduras classification results and WHO criteria.

Methods: The level of agreement between the national classification of dengue cases in Honduras and the classification according to WHO was determined using a sample of 390 patients with confirmed dengue suspected of having severe dengue who were treated in tertiary hospitals in Honduras during 2009 and 2010. This sample was drawn from a total of 718 individuals presenting with a febrile syndrome and included a secondary analysis to assess the classification agreement in the absence of complete confirmatory testing. The 1997 WHO classification was also assessed for agreement with Honduras national classification.

Results: The observed agreement in 390 cases with confirmatory testing was 51.5%, the expected agreement was 45.8% and kappa was 10.5% which is a slight agreement. A total of 236 patients (60.5%) had evidence of plasma leakage, and 56 patients (14.4%) had severe bleeding. The odds ratio for having severe dengue with the Honduras classification was 0.17 (95% CI 0.11, 0.25) compared to the 2009 WHO classification. There were statistically significant differences in the frequency of plasma leakage and severe bleeding when the two classifications were compared. The 2009 WHO criteria classified more cases as severe dengue while the national classification tended to classify more patients as non-severe. In the secondary analysis of 718 patients with incomplete confirmatory testing, the observed agreement between

both classifications was 42.9%, expected agreement 42.0% and kappa was 2.4%. Plasma

leakage was found in 458 patients (63.8%) and severe bleeding in 95 patients (13.2 %). The

1997 WHO criteria classified fewer cases as severe dengue, 65 compared to 164 classified by the

national committee; the expected agreement was 55.0%, the observed agreement was 57.4% and

a kappa of 5.1%. The odds ratio for having severe dengue with the Honduras definition was 4.0

(95% CI 2.7, 6.0) compared to the 1997 WHO definition.

Conclusions: The level of agreement between the Honduras national classification and the

WHO classification for severe dengue is suboptimal. To increase the use of the most recent

definition guidelines issued by the WHO, we suggest further training for personnel involved in

the process of severe dengue case classification.

Introduction

Dengue fever is the most important arboviral infection worldwide, and is endemic in many tropical and subtropical regions. There are 50 to 100 million cases reported annually worldwide (1, 2). Dengue is characterized by a broad spectrum of clinical manifestations, with approximately 85% of cases manifesting only vague or even absent symptoms. Fewer than 5% actually progress to severe clinical disease (3).

Formally classifying severe dengue cases can allow uniform case management on a local level, and reporting cases will improve surveillance efforts of the disease nationally and internationally. In 2009 the World Health Organization (WHO) published classification criteria for "severe dengue", previously referred to as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (1-3). Prior to 2009, the WHO definition of DHF was characterized by fever, hemorrhagic tendency, thrombocytopenia and plasma leakage as evidenced by a hematocrit increase of 20% (in comparison to normal values), and clinical manifestations such as pleural effusion or ascites. In contrast, the 2009 definition of severe dengue was met if one or more of the following criteria were present: (i) plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress, and/or (ii) severe bleeding, and/or (iii) severe organ impairment (1, 2).

Countries typically classify cases according to expert judgment and guidelines given by WHO; various publications have highlighted discrepancies between the WHO criteria and the classification system used locally in each country (4-7). In this paper we assess the level of agreement between the classification done in Honduras by the national classification committee

and by one of the authors (EF) using the 2009 WHO classification. We sought specifically to assess whether the level of agreement was in an acceptable range, i.e. moderate or high.

Methodology

Design

We performed a cross-sectional study in Honduras with data collected in 2009 and 2010. Data was collected from patients with clinical symptoms compatible with dengue who were admitted for treatment and evaluation as suspected severe dengue cases. Admission was based on clinical criteria requiring dengue confirmatory testing and clinical observation. A total of 718 patients were included from four different tertiary level hospitals. The hospitals were located in the two major cities of Honduras (Tegucigalpa and San Pedro Sula). We included patients who presented directly to the emergency departments, were from outpatient clinics, or were referred to hospital from other primary care clinics. Patients' records were reviewed by a committee of the Honduras Ministry of Health, comprised of clinical personnel, laboratory personnel and epidemiologists, who classified cases as being severe or non-severe dengue (8).

For the purpose of classification the national committee included all 718 records received even when the dengue confirmatory test was not available or was negative. These records included 390 confirmed cases of dengue, 158 that tested negative, and 170 with indeterminate results. Only those with confirmed dengue were included in our primary analysis. The national committee based its classification on the information provided by completion of a survey following the WHO guidelines, but also considered the experience of its members, their previous knowledge and technical discussions held during their meetings. Classification by the committee was ultimately done by consensus. The committee decided to classify cases where there was no

consensus or cases that had multiple comorbidities where attribution of complication to dengue

was uncertain as not severe.

Analysis Plan

One of the authors (EF) classified all cases using 2009 WHO guidelines for severe

dengue and then compared this classification of cases with those classified by the national

classification committee. Signs and symptoms specified in the 2009 WHO guidelines were

abstracted by the researcher from data collected by the survey (which was reviewed by the

Honduras national committee) that consisted of close ended questions, for example, the presence

or absence of symptoms and signs. Variables were created for each sign and symptom in the

survey. A code was generated electronically to classify cases according to 2009 WHO

classification as outlined below. This was compared to the classification of the national

committee which was included as a variable in the database.

Using the 2009 WHO classification for severe dengue, patients from Honduras had to

present with fever and have at least one of the following clinical manifestations: plasma leakage,

severe bleeding, or severe organ impairment. Evidence for plasma leakage was met if one or

more of the following were present: a 15% increase in hematocrit, pleural effusions, pericardial

effusion, or ascites. Pleural and pericardial effusions, as well as ascites, needed to be

documented by radiographic imaging. The criterion for plasma leakage was also met if there

was circulatory compromise or shock. This was based on the presence of one or more of the

following clinical manifestations: low blood pressure, pallor, cold and clammy extremities. The

criterion for severe bleeding was met if any of the following clinical manifestations were

reported: epistaxis, ecchymosis, melena, menorrhagia, gingival bleeding. The criterion for

severe organ involvement was met if there was severe abdominal pain, persistent vomiting,

changes in level of consciousness, or elevated liver function tests.

We also assessed how frequently cases met the 1997 WHO criteria for severe dengue (i.e.

dengue hemorrhagic fever) where all four of the following criteria needed to be met: 1) fever, 2)

hemorrhagic tendencies defined by at least one of the following: a positive tourniquet test;

petechiae, echimosis or purpura; bleeding of mucosa, gastrointestinal tract, injection sites;

hematemesis or melena; 3) thrombocitopenia: platelets count $< 100000/mm^3$), 4) evidence of

plasma leakage (defined by at least one of the following: rise of hematocrit $\geq 20\%$; a drop $\geq 20\%$

following volume replacement compared to baseline; pleural effusion or ascites). We created a

variable for each component of these clinical manifestations and required the presence of all four

for a patient to meet the definition.

We calculated the observed agreement and kappa (agreement beyond chance) between

the classifications given by the WHO definitions and the national classification committee.

National classification of severe dengue and the classification following the WHO criteria

were considered binary variables with values of either severe or non-severe dengue. The national

classification was made by consensus by the committee. The information used by the national

committee for classifying cases included consideration of data from the national dengue survey

and as specified above our analysis collected information from the same source.

We also examined the effect of using only one criterion for classification (only plasma

leakage or only severe bleeding) and by combining plasma leakage and severe bleeding to assess

the effect on agreement with the national classification.

Calculation of Agreement

Observed agreement was determined by considering those observations that were confirmed as cases of severe dengue by both classifications (i.e. WHO 2009 or 1997 and national) and those observations that were not considered as severe dengue (by both classifications), and dividing by the total number of observations (i.e. 390 for the primary analysis and 718 for the secondary analysis). Since a level of agreement between unrelated classifications can occur by chance it was necessary to correct for this using the coefficient kappa. Chance expected agreement was calculated as $p_1 p_2 + (1 - p_1)(1 - p_2)$ where p_1 is the proportion of observation classified as cases by the first classification and p_2 the proportion of observation classified as cases by the second classification (9). The coefficient kappa is expressed through the formula:

$$\frac{\text{Observed agreement- Expected Agreement}}{1 - \text{Expected Agreement}}$$

By convention, a kappa value < 0.00 is considered poor agreement, while from 0.00 to 0.20 slight agreement, 0.21 to 0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 0.99 almost perfect agreement (10, 11).

In order to determine whether the odds ratio derived from discordant pairs (b/c in the table below) differed significantly from an odds ratio of 1, McNemar's chi-square statistic was calculated, given by the formula: $X^2 = (b-c)^2/(b+c)$ for one degree of freedom.

WHO dengue	Honduras dengue classification		
classification[a]	Non-severe dengue	Severe dengue	Total
Non- severe dengue	a	b	
Severe dengue	c	d	

Results

2009 WHO Versus National Classification for Cases of Confirmed Dengue

When considering the 2009 WHO definition for severe dengue, 300 of 390 patients

(77%) were classified as severe dengue compared to 165 of 390 using the Honduras

classification system; the observed agreement was 51.5%, and kappa was 0.105 or 10.5%, which

is a slight agreement beyond chance. The odds ratio for having severe dengue with the Honduras

classification was 0.167 (95% CI 0.107,0.252) compared to the 2009 WHO classification.

There was concordance in classification of 201 of the 390 patients, that is, 138 patients were

classified as severe dengue by both classifications and 63 as non-cases (Table 1). There was no

agreement for 189 patients, as 27 were classified as severe by the Honduran classification and as

non-severe by the classification based in the 2009 classification; and 162 were classified as

severe according the 2009 WHO criteria but were considered as non-severe by the Honduran

classification.. The classification based on the 2009 WHO criteria classified a higher number of

patients as severe compared to the Honduran classification.

Using severe bleeding as the only criterion for classification of severe dengue led to an expected agreement of 55.5%, an observed agreement of 56.1% and a kappa of only 1.5%. There was concordance in classification of 219 patients, that is, 25 patients were classified as severe using the Honduran national classification. We found an expected agreement of 45.8% for dengue by both classifications, and 194 as non-cases. There was no agreement for 171 patients.

If the only criterion for severe dengue was plasma leakage, the expected agreement was 48.4%, observed agreement 51% and kappa of 5.1%. There was concordance in classification of 199 patients, that is, 105 patients were classified as severe dengue by both classifications, and 94 as non-cases. There was no agreement for 191 patients.

With the inclusion of both criteria (bleeding and plasma leakage), the expected agreement was 56%, observed agreement 55.6%, and a negative kappa of -0.9%. There was concordance in classification of 217 of the 390 patients, that is, 17 patients were classified by both classifications, and 200 as non-cases. There was no agreement for 173 patients.

1997 WHO Versus National Classification for Confirmed Dengue

When we compared the national classification with the 1997 criteria for severe dengue (DHF) in a sample of 387 patients, we found that 65 of the 387 patients or 17%, were classified as severe dengue with the 1997 definition, for an observed agreement of 57.36%, an expected agreement of 55.06%, and the kappa value was 5.1% (Table 2). The odds ratio for having severe dengue with the Honduras definition was 4.0 (95% CI 2.7, 6.0) compared to the 1997 WHO definition. There was agreement classifying 32 patients as severe dengue, and 190 patients as non-severe. There was no agreement for 165 patients; the national classification included 164

patients as severe dengue, while using the 1997 WHO criteria only 65 cases were classified as severe.   There were 132 patients classified as severe dengue by the national classification and as severe by the classification based in the 1997 WHO guidelines; while 33 patients were classified as severe dengue based on the 1997 WHO guidelines and as non-severe by the national classification.

2009 WHO Versus National Classification for Cases of Suspected Dengue

In the secondary analysis of 718 cases, all the records presented to the committee were analyzed regardless of the laboratory result (Table 3). Symptoms of plasma leakage were present in 450 patients (62.7 %), and significant bleeding in 88 (12.3%). Both types of manifestations were present in 68 patients (9.5%). According to the 2009 WHO definition for severe dengue, 477 patients had at least one sign/symptom of the two categories and were classified as severe dengue. When compared with the national classification, we found that 146 patients were classified as severe dengue in both classifications, and 111 as non-severe dengue, there was not agreement for 461 patients; for an observed agreement of 35.8 %, an expected agreement of 34.0% and a kappa of 2.6%. The 2009 WHO definition for severe dengue classified 580 patients as severe dengue while the national classification classified 173.

Examining disagreement we found 27 patients classified as severe dengue by the national classification and as non-severe by the classification based on the 2009 WHO classification; while 434 patients were classified as non-severe by the national classification and as severe by the classification based on the 2009 WHO classification.

1997 WHO Versus National Classification for Cases of Suspected Dengue

The guidelines for the 1997 classification were also used for the sample of 718 patients, and the observed agreement was 70% (there was agreement classifying 35 cases and 466 non-cases; and non-agreement for 217 patients); the expected agreement was 67.7%, and the value for kappa was 6.5% (Table 4). The agreement between the national classification and the 1997 WHO classification was greater for non-cases; which is related to a more restrictive definition (requiring to fulfill all the stated criteria). The national classification included 173 cases while the one based in the 1997 WHO guidelines only classified as cases 114 patients indicating that the Honduran classification is more likely to classify cases.

A disagreement between both classification was identified in the classification of 138 patients who were classified as severe dengue by the national classification and as non-severe dengue by the 1997 WHO-based classification; and and 79 were classified as non-severe dengue by the national classification and as severe dengue cases by the 1997 WHO- based classification..

Descriptive Analysis Based on Individual Factors

Symptoms of plasma leakage were reported in 225 of the 390 cases (57.7 %), and severe bleeding in 50 cases (12.8%). Hemorrhagic manifestations were present in the absence of plasma leakage in 13 cases (3.5%) and plasma leakage manifestations in the absence of hemorrhagic manifestations in 188 cases (49.7%). Both types of manifestations were present in 37 cases (10.7%). In terms of severe organ impairment, abdominal pain was present in 188 cases (48.0%), sweating in 189 cases (48.5%), cold limbs in 139 cases (35.6%), hematemesis in 35 cases (9.0%) and pale skin in 174 cases (44.6%).

A breakdown of the 225 individuals with plasma leakage shows that all of them were included in the severe dengue classification based on 2009 WHO criteria, while in the national classification only 111 cases with plasma leakage were classified as severe dengue (41.9 %). All 50 patients with severe bleeding manifestations were included as severe dengue in the classification based on the 2009 WHO criteria, but only 23 of them were classified as severe dengue by the national committee (46%).

DISCUSSION

We assessed agreement between the 2009 WHO criteria for the classification of severe dengue and the national classification using the kappa coefficient. We found what would be considered "slight agreement", indicating that the national classification diverges substantially from that established by the 2009 WHO guidelines. Use of the 1997 WHO classification did not result in a substantial improvement in agreement. Even though the 2009 WHO classification is more inclusive, as it allowed cases to be defined as severe dengue based on one criterion, agreement was minimal. The Honduras classification followed an intermediate pattern, classifying more cases than the 1997 WHO guidelines, but less than the current 2009 WHO guidelines, showing little agreement with either WHO classification. The timing of this study, during a transition period between the two WHO criteria might explain the difference in the number of severe dengue cases classified by the national committee, higher than that of WHO 1997 classification, but still lower than the 2009 WHO guideline. Our findings are in contrast to a similar study in Nicaragua, where there was 62% kappa agreement between a local Nicaraguan classification and the 2009 WHO criteria (6). We suspect the difference in kappa agreement (Honduran kappa agreement 10.5% and Nicaraguan 62%) is due to more extensive training in Nicaragua using the WHO criteria.

An important finding in our study population was the frequency of plasma leakage which occurred in 60.5% of the patients, compared to the severe bleeding reported in 14.4%. The most frequent finding related to plasma leakage was abdominal pain, which is consistent with findings in other studies (4, 6, 7, 12). We also discovered that plasma leakage provided a substantial level of agreement by itself, which was slightly increased by the presence of severe bleeding. Considering cases with any of these clinical features as severe increased the level of agreement of the national classification with the 2009 WHO criteria. Moreover, when we introduced the criterion of hemoconcentration greater than 15%, we observed a further increase in agreement. Similar findings occurred when a lower threshold for thrombocytopenia was introduced and kappa was above 10%. However, all of these values were demonstrative of just slight agreement.

We found that the number of patients classified as severe dengue with the Honduran classification was greater than that given by the 1997 WHO classification, but less than that given using the 2009 WHO classification; this is reflected in the direction of the odds ratios for discordant pairs comparing the national classification with both sets of WHO guidelines. Interestingly, patients with evidence of bleeding were classified by the Honduras classification in a manner not significantly different to the one given by the 1997 WHO classification.

The finding of such a low level of agreement in classification of severe dengue between the Honduran classification and the WHO guidelines raises the question of what weight the national classification committee assigned to various factors to classify patients. One possibility is that the committee gave more weight to hemoconcentration. However, when we took this under consideration, agreement improved but not enough to increase kappa beyond slight agreement. Another possibility is that the national committee used geography, in regions where

they felt there was a need for more public health resources, thereby assigning more severe

dengue status to patients in that area (no reports were found about this, but we cannot discard it

as a possibility). Such a reason for classification, of course, would invalidate estimates of severe

dengue. In our study, the classification based on WHO guidelines included hemorrhagic

manifestations and plasma leakage, with the latter constituting the majority of classified cases. In

contrast, Gupta et al found that hemorrhagic symptoms were present at a higher frequency in

their study, but cases did not completely fulfill criteria for severe dengue (2). Srikiatkhachorn

and colleagues found that manifestations of plasma leakage increased specificity of severe

dengue, while hemorrhagic manifestations discriminated less between severe and non-severe

dengue (4).

Studies from Thailand, Indonesia, Nicaragua and Brazil show a pattern of plasma leakage

predominance in patients with severe dengue, similar to our results (4, 6, 13-15). In studies done

prior to 2006, a broader range of plasma leakage and hemorrhagic manifestations were present,

with hemorrhagic manifestations ranging in frequency from 22% to 93% of severe cases, and

plasma leakage from 6% to 95% of severe cases (16). Such variations may be explained by

differences in study design, sample size, timing of measurement, and therapeutic interventions

(16).

We acknowledge that the lack of access to more detailed laboratory data was a limitation

of our study. Also, only a subset of cases had confirmed dengue. We encourage other regions to

conduct similar evaluations to determine if similar discrepancies exist between national and

WHO classifications. Improved physician education and auditing of decision making may help

reduce the discrepancies we have observed. It would be of value to conduct further research over

a longer time frame to assess patterns of dengue.

Tables:

TABLE 1

Agreement between the Honduran national classification and the 2009 World Health Organization (WHO) classification for severe dengue cases based on 390 febrile patients evaluated in Honduras in 2009-2010.

WHO dengue classification[a]	Honduras dengue classification		Total
	Non-severe dengue	Severe dengue	
Non- severe dengue	63	27	90
Severe dengue	162	138	300
Total	225	165	390

Agreement	
Expected agreement (chance)	0.458 (45.8%)
Observed agreement	0.515 (51.5%)
Kappa coefficient[b]	0.105 (10.5 %)

[a]Severe dengue defined as the presence of plasma leakage and/or severe bleeding and/or organ failure (2).

[b]A kappa coefficient between 0-20% is considered a slight agreement (10).

TABLE 2

Agreement in severe dengue case classification between the Honduran national definition and the 1997 World Health Organization (WHO) classification (n= 390 patients evaluated in Honduras in 2009-2010).

WHO 1997 dengue classification(2)	Honduras dengue classification		
	Non-severe dengue	Severe dengue	Total
Non- severe dengue	190	132	322
Severe dengue	33	32	65
Total	223	164	387

Agreement	
Expected agreement (chance)	0.551 (55.1%)
Observed agreement	0.574 (57.4%)
Kappa coefficient	0.051 (5.1%)

[a]Severe dengue defined as the presence of fever, hemorrhagic tendencies, thrombocytopenia and/or severe bleeding and/or organ failure (2).

[b]A kappa coefficient between 0-20% is considered a slight agreement (10).

TABLE 3
Agreement between the Honduran national classification and the 2009 World Health Organization (WHO) classification for severe dengue cases based on 718 febrile patients evaluated in Honduras in 2009-2010.

WHO dengue classification[a]	Honduras dengue classification		Total
	Non-severe dengue	Severe dengue	
Non- severe dengue	111	27	138
Severe dengue	434	146	580
Total	545	173	718
Agreement			

Expected agreement (chance)	0.340 (34.0%)
Observed agreement	0.358 (35.8%)
Kappa coefficient[b]	0.026 (2.6%)

[a]Severe dengue defined as the presence of plasma leakage and/or severe bleeding and/or organ failure (2).

[b]A kappa coefficient between 0-20% is considered a slight agreement (10).

TABLE 4

Agreement between the Honduran national classification and the 1997 World Health Organization (WHO) classification for severe dengue cases based on 718 febrile patients evaluated in Honduras in 2009-2010.

WHO dengue classification[a]	Honduras dengue classification		Total
	Non-severe dengue	Severe dengue	
Non- severe dengue	466	138	604
Severe dengue	79	35	114
Total	545	173	718

Agreement	
Expected agreement (chance)	0.677 (67.7 %)
Observed agreement	0.700 (70.0%)
Kappa coefficient[b]	0.065 (6.5%)

[a]Severe dengue defined as the presence of fever, hemorrhagic tendencies, thrombocytopenia and/or severe bleeding and/or organ failure (2).

[b]A kappa coefficient between 0-20% is considered a slight agreement (10).

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CHAPTER 5: Conclusions

The following paragraphs summarize the key findings of my thesis:

In chapter 2 I developed a predictive model to discriminate cases of dengue fever from other febrile illnesses in a selected cohort of patients attending tertiary level hospitals. Analysis was conducted using logistic regression and a similar analysis was done using the classification and regression tree method (CART) which is included in Appendix1. For both models I conducted an internal validation. For the logistic regression model bootstrapping with one thousand subsamples was used for validation; the resulting validation showed robust estimators and confidence intervals.

I found higher accuracy in the logistic regression model with the predictors petechiae, retro-ocular pain, gingival bleeding, epistaxis and pale skin. The CART model included three of these predictors: petechiae, pale skin, retro-ocular pain, consistent with what has been reported elsewhere (1-3). CART identified skin rash as an additional predictor but failed to include epistaxis (1, 2).

The methodology of ROC was used to identify different cut off points for sensitivity and specificity and allowed for the selection of a cut-off point that allowed for optimal sensitivity and specificity. The logistic regression model had a larger area under the curve (AUC) which provides evidence for the higher accuracy for this model compared to CART.

In chapter 3 I described the development of a predictive model for severe dengue. There were four predictor variables identified in this logistic regression model, only two of which were positively associated with severe dengue (ascites and platelet count) while the remaining two (pale skin and headache) were negatively associated. I found a higher sensitivity with the logistic regression model compared to CART (Appendix 2). The AUC also was greater for the logistic regression model adding to the superiority of the model.

The number of predictors for severe dengue in both logistic regression and CART models was fewer than those in the models of prediction for dengue fever. Ascites was a key predictor variable that has been reported previously (4). The association of headache and retro-ocular pain with severe dengue as identified by CART has previously been described (4,5).

In the chapter 4 I describe a study about the agreement of a Honduran national classification for severe dengue with 2009 and 1997 WHO classifications. Low levels of agreement were noted indicating that guidelines for classification are not being followed which indicated the need for more training and education (6-8).

Information on CART was included in Appendixes 1 and 2. Of note is the fact that the CART model for dengue fever was validated using split sample validation where the model was derived using a training subsample of approximately 50% of individuals and then validated in the other subsample of 50 %; splitting and terminal nodes were similar as was the standard error and estimator. The CART model for severe dengue was developed and validated using a similar process.

The research approach that I used in this thesis is further describing below along with key specific issues, reflections on the work done, and final conclusions.

Selecting variables for the dengue models

When developing predictive models it is important to understand the purpose of the modeling, the audience that can benefit from their use, and the resources available for development. A key consideration for the models developed as part of this thesis is that the models were intended for use in dengue endemic areas where other febrile illness are common, and where there is therefore an important need to differentiate dengue fever from other similar syndromes. The users of such models include general practitioners (the first line of disease in dengue treatment and control) as well as specialists in internal medicine and infectious diseases. Resources to develop the models for Honduras rarely include laboratory facilities, and therefore model building relies primarily on patient symptoms and signs.

Some predictor studies select laboratory indicators of the clinical and pre-clinical stages of illness, in others the emphasis is in analysing the role of clinical presentation available to front line physicians (1). Our dengue models fall more in the latter category as mentioned previously.

With better resources and with immediate access to laboratory technology more information for prediction models can be obtained, such is the case in recent studies where biomarkers have been used to define very early disease and to differentiate it from other illness (9).

The types of variables to be selected need to take into account qualitative aspects of the study including timing. Seasonality can make it more difficult to have real time data or requires extended studies to observe peaks and plateaus that guarantee the inclusion of a larger population and a variety of clinical manifestations (10). In the case of dengue, transmission can vary and the

reporting of cases is influenced by the presence of symptomatic cases with clinical manifestation

that lead to clinic and hospital visits.

In these studies I used variables included in the clinical history (symptoms reported by

the patients) findings of physical examinations and laboratory results for platelets and

hematocrit, as well as results of confirmatory testing for dengue. A limitation is that data

describing clinical evolution was not readily available in the survey form. Information about the

treatment and response to management could also be important and should be integrated where

available when studying classification of severe dengue. Most of the variables, in the Honduran

dengue study, were collected at the first medical evaluation but laboratory values and results of

serology or viral isolation that were included were collected subsequently.

Criteria for entry into the dengue model

In my thesis the main approach was to include variables available in the first encounter of

the physician with the patient, that is, symptoms and signs. Available basic laboratory

information was considered for the predictive models. We had access to variables such as

hemoglobin, hematocrit, and platelets which provided objectively measured values.

Demographic information was derived from patients and symptoms that were reported to be

present or absent were included, or was the tourniquet test which was performed by the physician

and reported as present or absent. In both models (i.e. dengue fever and severe dengue) the

dependent variable was binary, and potential predictors were defined as present or absent, in

some cases like age or laboratory values there was an opportunity to include continuous

variables.

The dependent variable for the dengue fever model was binary (positive or negative

result) based on whether dengue was confirmed or not. In the model for severe dengue our

preferred option was to identify mortality as the outcome but because of the extremely low case fatality rate we decided to use a measure of plasma leakage, hemoconcentration.

Multivariable analysis was performed using logistic regression, and as an alternative analysis we used CART.   For the logistic regression model, we assessed the statistical significance of variables through univariate analysis with chi square, and those with p values < 0.2 were considered for model entry. For CART we used a similar approach, including variables with p values less than 0.2. A review of the literature provides a broad range of variables that could be used including biomarkers and genetic variants (11). An integration of epidemiologic data and biomarkers is probably the optimal method for prediction models.

Use of different analytic approaches

A model provides an approximation to the real event that we wish to identify. Using more than one model can help validate findings and reduces reliance in one particular model that may have important weaknesses. We analyzed our data using both logistic regression and CART. Logistic regression was an obvious choice for a model because we were analyzing a dichotomous outcome for both prediction of dengue and for the prediction of dengue severity.

As an alternative approach I used the Classification and Regression Tree (CART) first described by Breiman as a method for recursive partitioning (12). The purpose of this was to see if I could identify the method which led to better predictive accuracy and to assess whether there would be differences in predictor variables. In doing so I learnt that different methods could lead to similar but also different results. I also learnt about methodology for comparing models.

Methods for comparison of models

Some methods are more applicable to data that are normally distributed but in our case both methods could handle nonparametric distributions. When comparing results, it was valuable to identify similarities and differences in the predictors and to verify their sensitivity and specificity. I used ROC analysis did derive the area under the curve (AUC) using alternative cut points leading to different sensitivities and specificities. For both logistic regression and CART models it was possible to identify the degree of correct prediction when different cut-off points were used. The ROC analysis allowed for sensitivity and specificity to be determined. Because of better accuracy, logistic regression was selected as the main model. Identifying the accuracy of the model using sensitivity and specificity allowed me to evaluate the utility of the model. This is particularly important in the context of Honduras where a model with the potential to rule out cases in the absence of immediate laboratory confirmation in an emergency setting as part of a triage for patients presenting with dengue-like syndromes is critical.

Presentation of results

The results of a logistic regression model yield a list of predictors with odd ratios indicating the direction and strength of association to the outcome (dependent variable), the statistics of the model including its classification of cases (sensitivity and specificity), the equation of the model, and values of the estimators for each predictor and their 95% confidence intervals.

The CART model presents branches of a tree with nodes at each level determined by a splitter or predictor; terminal nodes indicate subgroups of the population each with a different risk for the disease based on the absence or presence of the splitter. The model specifies the

importance given to each predictor(12). My interest in using CART was to determine the extent to which both models selected similar variables. The level of importance of predictors such as ascites in the severe dengue model or petechiae in the dengue model in CART could be determined based on their position in the tree, with predictors at the top of the tree generally affecting a broader part of the sample, while those in the lower levels affecting smaller subgroups. In the CART analysis for severe dengue it was possible to observe how the predictor platelet1 (first count of platelets) was a splitter at different levels of the tree but with different split values affecting sub-groups with decreasing sizes and previously affected by other splitters.

Validating the models

It is optimal to have a separate population in order to validate a model. When this is not feasible, it is possible to validate the model using random subsamples of the existing study population. If the sample is split and jackknife validation used the number of individuals is reduced and error might increase. A good alternative is the use of bootstrapping where multiple random subsamples are drawn from the original sample with replacement. This does not reduce the units of observations in the subsamples and can generate a large number of subsamples (we used 1000 subsamples). Through such validation we obtained estimators (bias and standard error; and 95% confidence intervals for the regression coefficient). This is internal validation, since it corresponds to subsamples with similar characteristics of the original sample. For CART we used cross-validation which is another type of internal validation technique, where the derivation of the model is done using half the sample and tested in the remaining half to verify its results.

External validation of our dengue models are needed to ensure their application beyond the sample from which they were derived. A limitation is that our study was done with patients

seeking medical attention in hospitals or being referred to them by the primary care person, which may have resulted in more severely ill populations compared to those in other populations those visiting ambulatory clinics. Our models should be validated among patients with milder clinical symptoms.

Level of prediction

Models can be compared based on accuracy (sensitivity and specificity) or using ROC (13, 14). Issues about reproducibility are also of importance. The data source is of relevance here, and for both logistic regression and CART models a standardized epidemiologic survey was used to collect variables for the models. External validation was not however carried out to identify the performance of the models using different data sets so as to verify applicability. We found CART provided a better visual presentation of results than logistic regression, however the regression model outperformed CART as judged by the AUC. Although the models differed, we felt it was important to evaluate both models. Variables from both models might need to be considered in new studies. Previous studies recommend verifying if adding new predictors to a model improve its accuracy and usefulness [14].

Agreement

Simple agreement can be a function of random coincidence and it is here that kappa provides a way to remove this factor. Usually a kappa coefficient greater than 60% is considered moderate agreement and over 80% is strong agreement while the unusual circumstance of negative kappa leads us to think that criteria for classification can be openly different (15). Our kappa calculation for the local classification of severe dengue compared to one WHO set of guidelines showed negative values that we interpreted as suggesting that the classification criteria were totally different to the ones provided by the WHO. This suggests the need to

thoroughly review the procedures for classification. It is entirely possible that the process for review included consideration of components of the previous WHO classification by the committee. The same situation may apply to countries other than Honduras, stressing the importance of review and evaluation when adapting new guidelines.

Sensitivity and Specificity

It is ideal to obtain optimal sensitivity and specificity for diagnostic tests, but it also important keep in mind that the balance of the two may depend on the purpose of the test. If the goal is to identify those at risk of death, as it is in severe dengue, then obtaining high sensitivity is important as long as specificity is not excessively low. On the other hand, when considering costs associated with admitting patients with mild illness, where missing a case will not be life threatening, then specificity becomes more important.

Related to this topic is selection of cut off points for the test, which in our case was for a predictive model. The cut-off point can create a tension between opting for higher sensitivity or for higher specificity, and in the process sacrificing the overall capacity to identify and classify cases (16). When developing the predictive model for severe dengue, we were faced with a small proportion of true cases and a larger proportion of non-cases (true negatives) that using a default 0.5 cut off point led to accurate classification of most of the non-cases, but misclassification of our true cases. To correct this misclassification, we modified the cut off point which allowed us to increase sensitivity (classifying more cases), even though it reduced the capacity to identify true negatives (reducing specificity). Finally our ROC analysis showed us that the optimum cut off point to increase sensitivity was close to 0.1 but it generated poor specificity (20%) because negative observations were also classified as cases.

The prevalence of cases can influence the predictive value in a model like ours, having a small number of cases in a sample and a larger number of no cases can lead to different values for positive and predictive value. In our studies we identified different levels of specificity and sensitivity according to the cut off points given that a small number of cases (severe dengue) required a lower threshold (cut off point) to be classified which was also verified by the ROC method and reviewing the list wise obtained after running the model equation.

Potential contributions to the field

Although there are an abundance of studies about agreement between physicians and about how patients self-report clinical symptoms (17-19), where agreement typically ranges from moderate to high, few studies exist about agreement when following guidelines for the classification of illness severity (20). We identified one study where there was clear disagreement in classifying patients with ventilator-associated pneumonia according to two different criteria, one using clinical criteria and the other using administrative criteria (21). As we have previously discussed, an important source of disagreement arises when comparing early clinical diagnosis to laboratory findings (22). Assessment of patient severity after direct observation can also generate low levels of agreement unless the assessment is probability of death (23). We anticipate that our findings of disagreement between the WHO classification of severe dengue and the classification reported by the Honduran committee will lead to a review of how criteria are applied and ultimately to better adherence to updated WHO guidelines.

Policy implications

The results of our models, particularly where limitations in the types of variables used are evident, can potentially affect the type of data that is collected for epidemiological surveillance.

Our predictive models can be incorporated into clinical practice guidelines, and in so doing will help familiarize users with the potential utility of such models in practise. Knowing the level of agreement between various classification methods for severe dengue can provide insight into the source for divergence and may offer ways to correct them. The models developed are pragmatic in that they are suited for countries with limited diagnostic and laboratory resources, relying mainly on clinical signs and symptoms.

The development of predictive models can be adversely affected by the limited availability of information and laboratory access, but as long as real time diagnostic tests and predictive biomarkers are not available to all clinicians, the models have utility. It is important to be aware that although decisions can be made based on technical knowledge in public health, such decisions are often influenced by physician experience, public pressure, and political influence. The discrepancy in the classification of severe dengue between the local classification and WHO standards shows how important these influences may be.

Dengue statistical models are based on knowledge of how clinical characteristics of disease are distributed in the population during transmission. Dengue fever and severe dengue are cyclic events that need continuous preparedness to reduce mortality, temporary disability and hospital costs. The use of predictive models needs to be implemented and externally validated by health organizations at national and international levels.

Our results may improve the evaluation of ambulatory cases of dengue and may lead to more efficient triage of cases needing close monitoring and hospitalization. Together, a model for dengue fever and one for severe dengue can improve the capacity to identify and diagnose true cases, differentiating them from other illnesses, and allow resources to be focused on those

requiring immediate management. Ultimately, we hope that quality of care may be improved

and health resources optimized both in Honduras and in countries with similar health resources

and dengue transmission.

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ANNEXES

ANNEX 1

Final Predictive Model for Dengue Fever using the CART Method .

Honduras 2009-2010. (n=547)
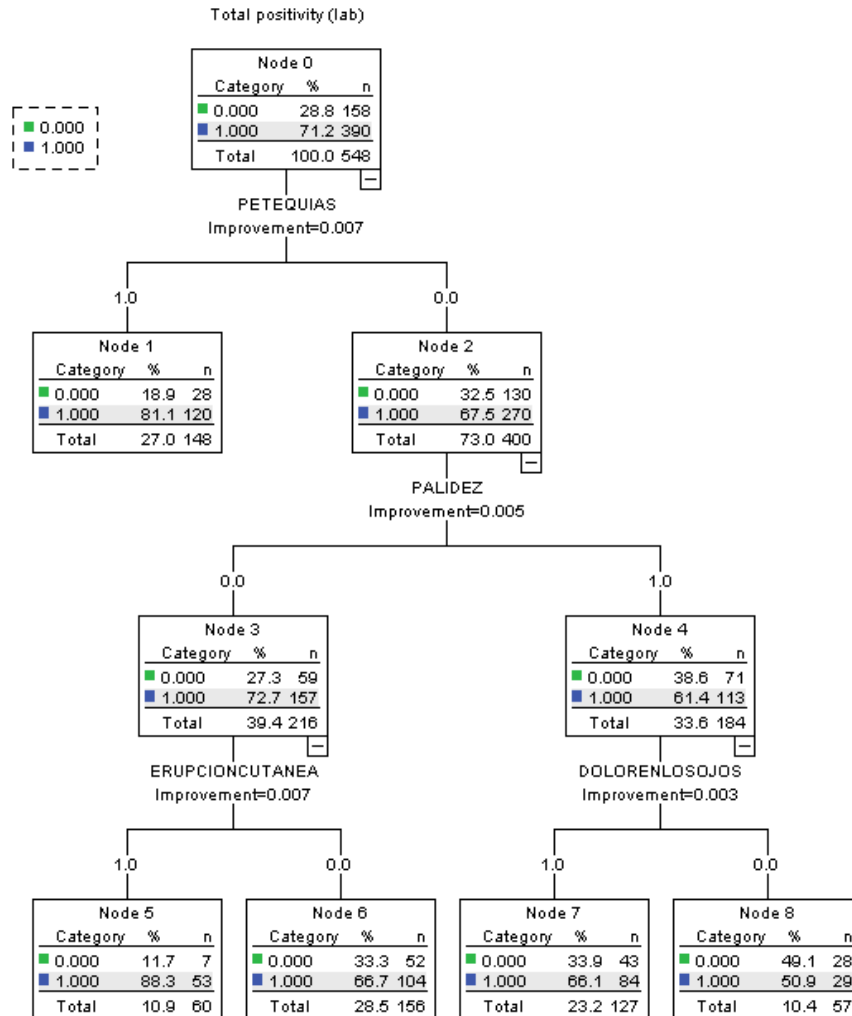
Legend : the splitters (predictors) included in the model are : Petechiae (Petequias);

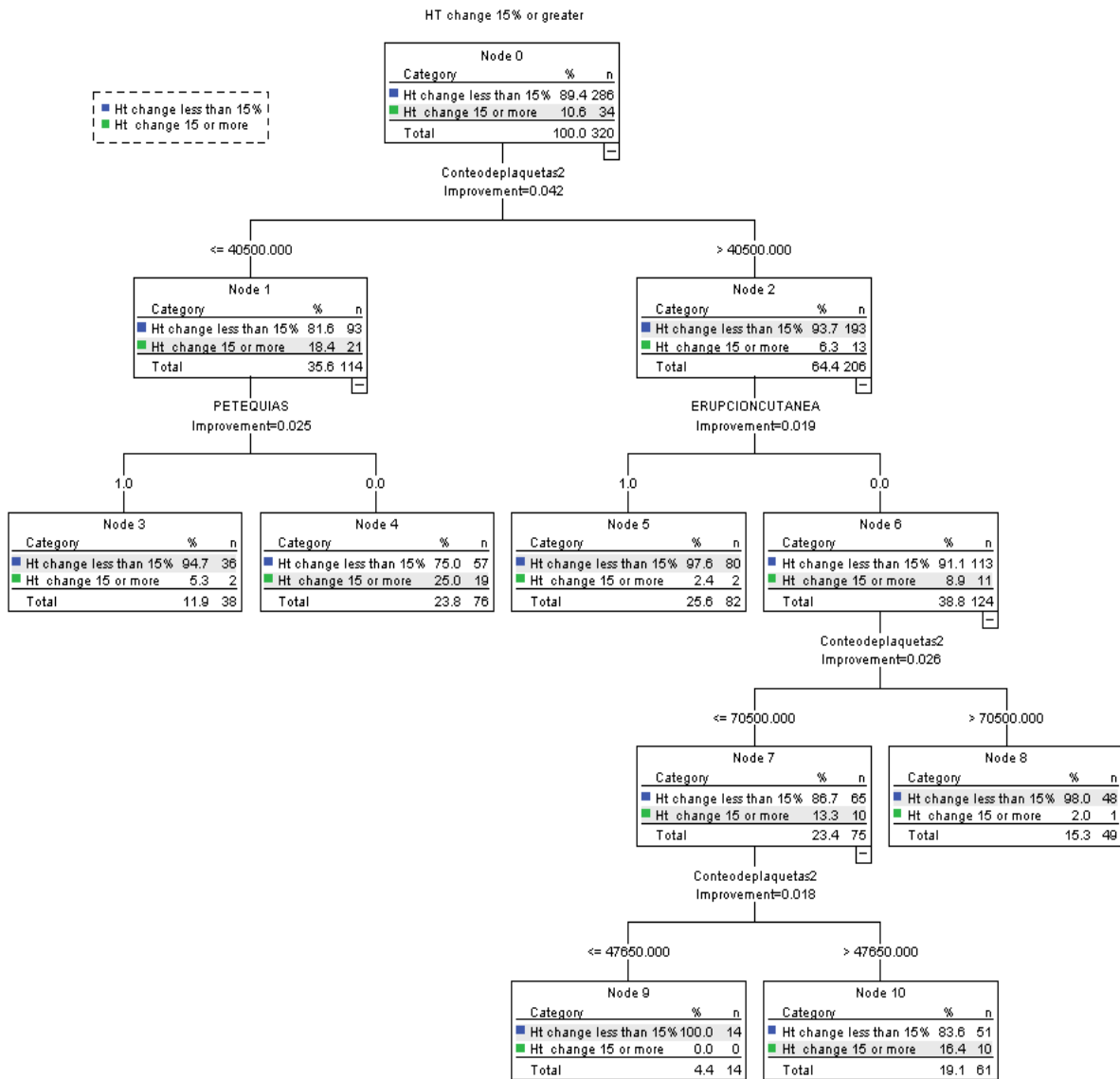Paleness(Palidez); Skin Rash (Erupcion cutanea); and Retroocular pain (Dolor en los ojos)

ANNEX # 2

Final Predictive Model for Severe Dengue Using the CART Method.

.Honduras 2009-2010. (n=320)

HT change 15% or greater

Node 0		
Category	%	n
■ Ht change less than 15%	89.4	286
■ Ht change 15 or more	10.6	34
Total	100.0	320

Legend:
■ Ht change less than 15%		
■ Ht change 15 or more		

Conteodeplaquetas2
Improvement=0.042

<= 40500.000

Node 1		
Category	%	n
■ Ht change less than 15%	81.6	93
■ Ht change 15 or more	18.4	21
Total	35.6	114

> 40500.000

Node 2		
Category	%	n
■ Ht change less than 15%	93.7	193
■ Ht change 15 or more	6.3	13
Total	64.4	206

PETEQUIAS
Improvement=0.025

ERUPCIONCUTANEA
Improvement=0.019

1.0

Node 3		
Category	%	n
■ Ht change less than 15%	94.7	36
■ Ht change 15 or more	5.3	2
Total	11.9	38

0.0

Node 4		
Category	%	n
■ Ht change less than 15%	75.0	57
■ Ht change 15 or more	25.0	19
Total	23.8	76

1.0

Node 5		
Category	%	n
■ Ht change less than 15%	97.6	80
■ Ht change 15 or more	2.4	2
Total	25.6	82

0.0

Node 6		
Category	%	n
■ Ht change less than 15%	91.1	113
■ Ht change 15 or more	8.9	11
Total	38.8	124

Conteodeplaquetas2
Improvement=0.026

<= 70500.000

Node 7		
Category	%	n
■ Ht change less than 15%	86.7	65
■ Ht change 15 or more	13.3	10
Total	23.4	75

> 70500.000

Node 8		
Category	%	n
■ Ht change less than 15%	98.0	48
■ Ht change 15 or more	2.0	1
Total	15.3	49

Conteodeplaquetas2
Improvement=0.018

<= 47650.000

Node 9		
Category	%	n
■ Ht change less than 15%	100.0	14
■ Ht change 15 or more	0.0	0
Total	4.4	14

> 47650.000

Node 10		
Category	%	n
■ Ht change less than 15%	83.6	51
■ Ht change 15 or more	16.4	10
Total	19.1	61

Legend: The splitters include platelets count (threshold of 40,600/mm3); petechiae, skin rash,, platelets count (threshold of 70600/mm3) and platelets (threshold of 47,650), having platelets splitting the sample in three different levels of the tree for subsets of it.

ANNEX 3

Power calculation

Value of power with different Sample sizes

Sample size	Z$_\beta$	Value of the Power	Significance level (α)
434	0.820985	79.4	0.05
440	**0.840143**	**80**	
500	1.024962	84.6	
550	1.170495	88	
600	1.309557	90.3	
650	1.443381	92.5	
719	1.619466	94.6	

(With Information from Kelsey, J. Methods in Observational Epidemiology,1996).[23]

Assuming the difference in proportion is 20% and the occurrence of the outcome is occurring in 0.35 of the interest population.

Annex 4

Epidemiological survey sample (used in the studies)

SECRETARIA DE SALUD DE HONDURAS
VIGILANCIA EPIDEMIOLOGICA DEL DENGUE, SOLICITUD DE EXAMENES

SOSPECHOSO DE DENGUE HEMORRAGICO   SI ☐   NO ☐

NOMBRE COMPLETO DEL PACIENTE_____

EDAD: ____Años___Meses___SEXO___ NOMBRE DEL JEFE DE FAMILIA_____

DIRECCION: DEPARTAMENTO_____ MUNICIPIO_____

BARRIO O COLONIA_____ CALLE Y/O AVENIDA_____ CASA No._____

DIRECCION EXACTA_____ TEL._____

HOSPITALIZADO EN _____ FECHA INGRESO_____ SALA_____ No. EXPED_____

LUGAR DE TRABAJO O ESTUDIO_____

DATOS CLINICOS: FECHA PRIMER SINTOMA_____ DIAS DE EVOLUCION_____

SEMANA EPIDEMIOLOGICA_____ FECHA DE TOMA DE MUESTRAS 1era_____ 2da_____

TIPO DE MUESTRA   SUERO ☐   SANGRE EN DISCO ☐

No.	SIGNOS Y SINTOMAS	SI	NO
1	FIEBRE		
2	DOLOR DE CABEZA		
3	DOLOR EN LOS OJOS		
4	MIALGIAS (DOLOR MUSCULAR)		
5	ARTRALGIAS (DOLOR ARTICULAR)		
6	ERUPCION		
7	FALTA DE APETITO		
8	PETEQUIAS		
9	EQUIMOSIS		
10	VOMITO CON SANGRE		
11	SANGRE EN LA EXCRETA		
12	HEMORRAGIA DE NARIZ		
13	HEMORRAGIA DE ENCIAS		
14	SANGRE EN LA ORINA		
15	HEMOR. VAG. / F.U.M: / /		
16	VOMITOS PERSISTENTES		
17	DOLOR ABDOMINAL INTENSO		
18	FRIALDAD DE MIEMBROS		
19	SUDORACION		
20	PALIDEZ		
21	PRUEBA TORNIQUETE	(+)	(-)
22	DERRAME PERICARDICO		
23	DERRAME PLEURAL		
24	ASCITIS		
25	PRESION ARTERIAL		

PRUEBAS DE LABORATORIO	FECHA 1			FECHA 2			FECHA 3		
	D /	M /	A	D /	M /	A	D /	M /	A
HB									
HTC									
PLAQUETAS									
LEUCOCITOS									
PAM*									
IMAGENES RX/USG									

*PAM= Presión Arterial Media (P/A S - P/A D)/ 3 +P/AD

DATOS ADICIONALES:

1. Durante los 10 días antes de enfermarse viajó a otro lugar?
   SI ☐   NO ☐
2. A donde viajó?_____

3. Tuvo dengue antes? SI ☐   NO ☐   Cuando_____
OTROS DIAGNOSTICOS: _____
_____

Embarazo: SI ☐ NO ☐

4. Otras pruebas de laboratorio:
   a. Hematozoario/ Gota Gruesa_____
   b. Leptodridot_____ HA_____ MAT_____
   c. Hemocultivo o coprocultivo tifoidea_____

Nombre de la persona que llenó la ficha_____

_____

Dirección Dptal._____No._____Área_____

Unidad de Salud: _____Tel._____

Enviar resultado a: _____

CLASIFICACION: A ☐   B ☐
                 C ☐   D ☐

RESULTADO DE LABORATORIO

ENGRAPE MUESTRA DE PAPEL FILTRO   REGISTRO No.

Annex 5

Some comments about the CART models

In the alternative predictive model for dengue fever   based on the classification and regression tree (CART) were included the symptom of retro-ocular pain (absent in the logistic regression model), and the clinical signs petechiae, pale skin and skin rash (but without the epistaxis and gingival bleedings) that were presents in the logistic regression model. Petechiae, skin rash and pale skin provided more improvement to the model than other symptoms. The specificity for this model was 44.3 and sensitivity 72.8% for an overall sensitivity of 67.9% assuming a cut-off of 0.543 (54.3% of probability for a case of dengue).

For the predictive model of severe dengue the final CART model identified 69.1% of cases, its sensitivity was 85.3% (29 of the 34 cases were correctly classified) and its specificity was 67.1% (192 of the 286 non cases were correctly classified). This model included as predictors petechiae, platelets and skin rash. Its cut-off point was 0.55 (55% probability to detect a severe dengue).Ascites was absent from the CART model.