THE PREDICTIVE AND DIAGNOSTIC ACCURACY OF PENTRAXIN-3 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SEVERE DENGUE

GARY LOW KIM KUAN

DOCTOR OF PHILOSOPHY IN MEDICAL SCIENCES

FACULTY OF MEDICINE AND HEALTH SCIENCES

UNIVERSITI TUNKU ABDUL RAHMAN

March 2019

THE PREDICTIVE AND DIAGNOSTIC ACCURACY OF PENTRAXIN-3 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SEVERE DENGUE

By

GARY LOW KIM KUAN

A thesis submitted to the Faculty of Medicine and Health Sciences,

Universiti Tunku Abdul Rahman,

In partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Medical Sciences

March 2019

ABSTRACT

THE PREDICTIVE AND DIAGNOSTIC ACCURACY OF PENTRAXIN-3 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SEVERE DENGUE.

Gary Low Kim Kuan

Identifying severe dengue virus infection is essential for prompt management of patients. There is a need to evaluate biomarkers and clinical parameters that can predict the complication. Hence, the biomarkers' level must be able to predict at least 24 hours prior to the development of complication. Unfortunately, none of the biomarkers were evaluated in a 'day-to-day' sequence when the disease progresses. This study aimed to evaluate vascular endothelial growth factor (VEGF) and pentraxin 3 (PTX-3) as predictive and diagnostic markers in differentiating severe dengue from non-severe dengue. The study was conducted in Ampang Health Clinic, Ampang Hospital and Serdang Hospital. The dengue patients were followed up from the day of presentation until discharged. The plasma levels of VEGF and PTX-3 were compared between severe dengue and non-severe dengue by ELISA. The inclusion criteria were participants age 15 or more, presented within the first 72 hours of illness and with positive NS1 Ag test. Multiple logistic regression was used to develop predictive and diagnostic models by incorporating other clinical parameters. The Receiver Operating

Characteristics (ROC) analysis was used to assess the accuracy of the biomarkers. A total of 82 patients were recruited, 29 with severe dengue and four died. The Area Under the Curve (AUC) was statistically significant in VEGF as diagnostic marker at Day 2 and 3 of illness with sensitivity of 80.00%-100.00% and specificity of 76.47%-80.00%. The predictive model with AUC of 0.84 (95% CI: 0.73, 0.94, $p < 0.01$) has a sensitivity of 100.00% and specificity of 79.25% for predicting severe dengue. The diagnostic model with AUC of 0.71 (95% CI: 0.60, 0.85, $p < 0.01$) has a sensitivity of 76.19% and specificity of 73.58% for diagnosing severe dengue. The AUC for PTX-3 was not statistically significant. VEGF may be used in combination with other clinical parameters to predict the severity of the disease. As a single biomarker, it may be used as an adjunct investigation to support the diagnosis of severe dengue. PTX-3 was not able to differentiate severe dengue from non-severe dengue.

ACKNOWLEDGMENTS

I thank my supervisors Dr. Seng Chiew Gan and Dr. Thaw Zin who have been supportive throughout the process of my PhD candidature. Sue-Lynn Kong and Zhen Yu Tok for their help during the data collection process, and to all the doctors in Ampang Hospital for their kind assistance.

This work was supported by Universiti Tunku Abdul Rahman (UTAR) through UTAR Strategic Research Fund (UTARSRF) under project number 6274/009.

APPROVAL SHEET

This thesis entitled "**THE PREDICTIVE AND DIAGNOSTIC ACCURACY OF PENTRAXIN-3 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SEVERE DENGUE.**" was prepared by GARY LOW KIM KUAN and submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Sciences at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Assoc. Prof. Dr. Seng Chiew Gan) Date:
Supervisor
Department of Pre-Clinical Sciences
Faculty of Medicine and Health Sciences
Universiti Tunku Abdul Rahman

_____

(Assoc. Prof. Dr. Thaw Zin) Date:
Co-supervisor
Department of Pre-Clinical Sciences
Faculty of Medicine and Health Sciences
Universiti Tunku Abdul Rahman

FACULTY MEDICINE AND HEALTH SCIENCES

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 20 December 2019

SUBMISSION OF THESIS

It is hereby certified that **Gary Low Kim Kuan** (ID No: **14UMD07965**) has completed this thesis entitled "THE PREDICTIVE AND DIAGNOSTIC ACCURACY OF PENTRAXIN-3 AND VASCULAR ENDOTHELIAL GROWTH FACTOR IN SEVERE DENGUE." Under the supervision of Dr. Seng Chiew Gan (Supervisor) from the Department of Pre-Clinical Sciences, Faculty Medicine and Health Sciences, and Dr. Thaw Zin (Co-supervisor) from the Department of Pre-Clinical Sciences, Faculty Medicine and Health Sciences.

I understand that the University will upload softcopy of my thesis in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

Gary Low Kim Kuan

DECLARATION

I Gary Low Kim Kuan hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Gary Low Kim Kuan

Date: 20 December 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

WHO	World Health Organization
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
VEGF	Vascular endothelial growth factor
VCAM-1	Vascular cell adhesion molecule 1
PTX-3	Pentraxin 3
RNA	Ribonucleic acid
WBC	White blood cell
PLT	Platelet
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Ag	Antigen
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DSS	Dengue shock syndrome
TNF- α	Tumour necrosis factor- α
IFN-α	Interferon-α

IL	Interleukins
EDTA	Ethylenediaminetetraacetic acid
OD	Optical density
CV	Coefficient variation
CI	Confidence interval
ROC	Receiver operating characteristics
SE	Standard error
AUC	Area under the curve
LR	Likelihood ratio
SPSS	Statistical Package for Social Science
MREC	Medical Research & Ethics Committee
SD	Standard deviation
IQR	Interquartile range
NA	not applicable
SD	severe dengue

CHAPTER 1.0

INTRODUCTION

Only nine countries had experienced severe dengue epidemics before the year of 1970, but the disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific (World Health Organization, 2016). Not only is there a 30-fold increase in geographical expansion to new countries in the last 50 years, the spread of the disease is noted to be from urban to rural settings (World Health Organization, 2009).

Dengue virus infection is estimated to have affected 390 million individuals per year globally (Bhatt et al., 2013). World Health Organization (WHO) estimated 500 000 people with severe dengue require hospitalization each year and about 2.5% of those affected die (World Health Organization, 2016). Malaysia is also a dengue endemic country with 101,357 cases of dengue virus infection reported in 2016, of which, 1% was diagnosed with severe dengue. Selangor, a state in Malaysia, has the highest number of reported cases with the highest number of dengue death. This report has been consistent throughout the months in every year since 2011 (Ministry of Health, 2017). The incidence rate in Malaysia is approximately four times higher in individuals of

15 years of age or more than in individuals younger than 15 (Ministry of Health, 2010).

This has an impact on the country's economy. The mean health care cost per reported case in Malaysia between 2001 and 2005 was US$ 1234 per annum (Suaya et al., 2009). The cost is definitely higher in recent years.

Dengue clinical manifestation is divided into three phases: febrile, critical and recovery phase. Critical phase is the most important phase where the patient can either recover or die from the disease (Gubler, 1998; Rigau-Pérez et al., 1998; Nishiura & Halstead, 2007). Unfortunately, the critical phase is difficult to predict even with the latest classification recommended by the WHO 2009 Clinical Practice Guideline (World Health Organization, 2009). The warning signs listed in the WHO 2009 classification are predictors for severe dengue. Hence, patients presenting with these warning signs will be admitted to the hospital for close monitoring. However, the specificity and the positive predictive value of these signs are poor which could lead to high false positive rates (Leo et al., 2013; Thein, et al. 2013). In addition, the clinicians are unable to predict the severity accurately, leading to unnecessary hospital admissions for intensive monitoring. Hence, this will have an impact on the healthcare cost and workforce (Horstick et al., 2014).

In order to reduce the burden of the disease, an optimally accurate predictive or screening method is essential. This method should be easily performed and cheap enough to be applied in a dengue-endemic population. This will allow safe triaging of the patients to reduce unnecessary hospital admissions.

Numerous biomarkers for dengue infection were identified and studied to differentiate the severity of dengue virus infection (Srikiatkhachorn & Green, 2010; Pawitan, 2011; John, et al. 2015). Biomarker tests for dengue severity are generally divided into three techniques: enzyme-linked immunosorbent assay (ELISA) (Bethell et al., 1998; Young et al., 2000; Murgue et al., 2001; van Gorp et al., 2002; Libraty et al., 2002; Gil et al., 2004; Koraka et al., 2004; Mairuhu et al., 2005; Srikiatkhachorn et al., 2007; Honsawek et al., 2007; Soundravally et al., 2008; Widjaja & Mantik, 2009; Michels et al., 2011; van de Weg et al., 2012), flow cytometry (Fadilah et al., 1999; Azeredo et al., 2006) and reverse transcription polymerase chain reaction (RT-PCR) (Calzavara-Silva et al., 2009; Nascimento et al., 2009; Ha et al., 2011). Among the different biomarker techniques, ELISA became relatively cheaper in recent years and easier to perform. Globally, most laboratories have the capability and expertise to conduct ELISA. More importantly, it has potential to be further developed into a ‘bedside’ rapid kit. Preferably, non-laboratory technicians such as short-course-trained nurses, medical assistants or doctors will be able to use this rapid kit.

A pilot study on ELISA-based biomarkers was conducted to evaluate the five most potential biomarkers: neopterin, vascular endothelial growth factor (VEGF), thrombomodulin, vascular cell adhesion molecule 1 (VCAM-1) and pentraxin 3 (PTX-3). VEGF and PTX-3 were the two best potential biomarkers in differentiating severe dengue from non-severe dengue cases (Low et al., 2015). VEGF and PTX-3 are involved in the pathogenesis of dengue virus infection. VEGF is specifically involved in plasma leakage when the endothelial cells in the blood vessels are damaged, releasing VEGF as a response to repair the damaged cells (Rathakrishnan et al., 2012). VEGF and cytokines were released into the circulation from dengue-infected mast cells, vascular endothelium, platelet and macrophages. VEGF along with other cytokines could stimulate other biochemical such as platelet-activating factor (PAF) to increase the vascular permeability through the activation of receptors on the endothelial cells (Malavige & Ogg, 2017).

Thus far, there is no in vitro study on PTX-3 in the pathogenesis of dengue virus infection. Based on other studies, PTX-3 is related but distinct from the C-reactive protein which is produced by a variety of cells such as macrophages and endothelial cells. These cells are activated during infection thereby releasing PTX-3 into the blood stream (Mantovani et al., 2003).

Detectable PTX-3 and VEGF levels in plasma before, during and after the identification of severe dengue, could offer various clinical usage. These proteins can serve as predictive markers to determine patient's admission, as

diagnostic markers or as prognostic markers to determine the likelihood of death. However, many studies conducted could not substantiate their use as predictive, diagnostic and prognostic markers because of the lack of generalizability and inappropriate study design (Bethell et al., 1998; Mairuhu et al., 2005; Tseng et al., 2005; Srikiatkhachorn et al., 2007; Furuta et al., 2012).

Level of biomarkers must be able to predict at least 24 hours prior to the development of complication. Hence, typical cross-sectional and case-control study designs could not address the utilisation of biomarkers as predictors due to the lack of 'day-to-day' follow-up of patients until the occurrence of severe dengue, i.e. temporality (Bethell et al., 1998; Mairuhu et al., 2005; Tseng et al., 2005; Srikiatkhachorn et al., 2007; Furuta et al., 2012). Furthermore, the studies conducted by using an older version of WHO 1997 case classification which is not contemporary to the current clinical practice. Thus, this poses difficulty in translating research results into clinical practice.

There is a need to conduct a study in which the patients were follow-up daily for the biomarkers and compare against the latest WHO 2009 case classification. Therefore, the primary objective of this study was to evaluate VEGF and PTX-3 as predictive and diagnostic markers in differentiating severe dengue from non-severe dengue. The secondary objective was to evaluate the biomarkers as prognostic markers to predict death among the severe dengue cases.

The null hypothesis for the primary objective was no difference in VEGF and PTX-3 levels between severe dengue and non-severe dengue. The alternative hypothesis for the primary objective was that there is difference in VEGF and PTX-3 levels between severe dengue and non-severe dengue.

The null hypothesis for the secondary objective was no difference in VEGF and PTX-3 levels between severe dengue patients who died and who did not. The alternative hypothesis for the secondary objective was that there is difference in VEGF and PTX-3 levels between severe dengue patients who died and who did not.

CHAPTER 2.0

LITERATURE REVIEW

2.1 The dengue virus

Dengue virus is a mosquito-borne flavivirus with four distinct serotypes namely, DEN-1, 2, 3 and 4. There is another distinct dengue serotype found. This new fifth serotype so far has been linked to only one human outbreak (Vasilakis et al., 2013). The four serotypes will give rise to the different severity of clinical presentation (Vaughn et al., 2000).

Dengue virus is a single-stranded ribonucleic acid (RNA) virus (Henchal & Putnak, 1990). Viral replication involves:

1. Attachment, penetration and uncoating
2. Primary translation and early RNA replication
3. Synthesis of viral proteins
4. RNA replication
5. Virus assembly and release

The virus binds to the host cell via the Fc receptor on the host cell and the trypsin-sensitive virus receptor. This is mediated by the viral envelope E glycoprotein. After binding with receptors of the host cell, the virus may fuse

with the membrane of the host cell or gain entry by disruption of the host cell membrane. The virus releases the nucleocapsid into the cytoplasm of the host cell after penetrating the membrane. A negative strand RNA is converted to a positive strand RNA for translation and synthesis of a negative strand template for the synthesis of viral proteins and RNA replication (Rothman, 1999). Following assembly, progeny virions are released by way of either 'budding' through the host membrane or by exocytic vesicles.

Dengue is transmitted by *Aedes aegypti*, *Aedes albopictus* and other less common types of *Aedes* which can be found mainly in tropical countries (Chen et al., 2006; World Health Organization, 2009; Ministry of Health, 2010). They breed in water-filled containers in the house, rain-filled discarded containers and blocked gutters. The mosquito is able to fly within 100 meters and feed during daylight (dawn and dusk) on a human (World Health Organization, 2009). Thus, vector control and preventive programmes are targeting on these key behavioural features of the mosquito.

2.2 The clinical manifestation

Dengue virus has an incubation period of four to seven days (ranging from three to fourteen days). The clinical manifestation begins with three phases: febrile, critical and recovery phase (Rigau-Pérez et al., 1998; Gubler, 1998; Nishiura & Halstead, 2007). During the febrile phase, the clinical features are generally non-specific such as facial flushing, skin erythema, generalized

body ache, myalgia, arthralgia, headache, anorexia, nausea, sore throat, injected pharynx and conjunctiva (Kalayanarooj et al., 1997; Gubler, 1998; Rigau-Pérez et al., 1998). Recently, diarrhoea was also found to be more pronounced in dengue virus infection (Seet et al., 2005; Kuan et al., 2015; Low, 2016). Thus, diarrhoea was also included in the Malaysian clinical practice guideline as one of the warning signs (Ministry of Health, 2015).

The critical phase is characterised by the warning signs of dengue virus infection which last about 24-48 hours (World Health Organization, 2009). The warning signs of dengue virus infection such as persistent vomiting, abdominal pain and mucosal bleed may sometimes occur early in the febrile phase. These warning signs are used as a guide to admit patients for close observation. During the critical phase, the patient may also develop a complication which is called severe dengue. Severe dengue is diagnosed when any one or more of the three criteria is fulfilled. These criteria are severe plasma leakage, severe haemorrhage and severe organ impairment (World Health Organization, 2009).

After 24-48 hours, the patient will enter the recovery phase if the patient does not succumb to the complication of severe dengue. The recovery phase is characterised mainly by the halt of plasma leakage followed by reabsorption of extravascular fluid (Gubler, 1998). Paradoxically, the presence of antibody against dengue virus will not reduce the severity of the disease (Vaughn et al., 2000). Hence, the disease will be more severe in secondary dengue cases.

The paradoxical severity of secondary dengue could be explained by the antibody-dependent enhancement hypothesis. It consisted of three functional states: neutralization, enhancing virus growth and antibody degradation. In neutralization phase, the dengue virus antibody is too low to block receptors that present in the cell surface. With the enhancement effect of antibodies such as anti prM monoclonal antibody and the interaction with DC-SIGN and FcγRIIA, Vitamin D receptors, the virus could easily enter the cells to replicated further (Guzman & Vazquez, 2010).

2.3 Biochemical and haematological changes

During the febrile phase, the first change to occur is the white blood cell (WBC) count which will decrease from about 11,000 cells/μL to about 6,000 cells/μL (Kalayanarooj et al., 1997). However, WBC count is a non-specific blood index because other medical conditions can also cause leukopenia.

Following leukopenia, a rapid decrease in platelet (PLT) count will occur in the critical phase and it precedes plasma leakage. Platelet count will fall to less than 100,000 cells/mm^3 during this phase (Phuong et al., 2002). Haematocrit will rise from the baseline and it precedes blood pressure and pulse volume changes. This indicates haemoconcentration and it is clinically significant if there is a rise of more than 20% from the baseline (Srikiatkhachorn, Krautrachue, et al. 2007). When dengue infection involves the liver, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) will be elevated (Souza et al., 2004).

Viremia will occur in the first three to five days of fever. Hence, test to isolate the virus, detect the viral genome or virus particle NS1 antigen (Ag) for diagnosis of dengue is best conducted during this period. Antibodies (IgM) against the dengue virus are usually detectable after day five of illness (Vaughn et al., 1997).

In the recovery phase, the WBC count will rise back to normal range followed by the rise of platelet count. The liver function test will also show a normalisation of AST and ALT levels (World Health Organization, 2012). These biochemical and haematological changes are used to guide clinicians in their management of dengue patient.

2.4 The challenges of dengue virus infection

In the early febrile phase, it is difficult to distinguish dengue virus infection clinically from non-dengue febrile diseases (Rigau-Pérez et al., 1998). The presentation of the disease in the febrile phase is similar to that of upper respiratory tract infection and gastrointestinal disease. Symptoms such as vomiting, diarrhoea, abdominal pain, headache, cough, sore throat, runny nose, rashes are common in dengue virus infection. Therefore, the NS1 Ag rapid diagnostic kit has been employed to diagnose dengue virus infection in the first three days of illness.

In other viral infections, the patient's condition improves as the temperature subsides. In dengue virus infection however, the condition will deteriorate further when the fever subsides. This is the critical phase of dengue which lasts for 24 to 48 hours (Rigau-Pérez & Laufer, 2006). This confusing pattern of fever often leads to a false sense of illness recovery whereby patients would seek medical attention late.

2.5 WHO classification

The WHO 1997 case definition for dengue has been used for more than 10 years. Dengue cases are classified into dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Alternatively, dengue can be graded from I to IV with grade I and II representing DHF and grade III, and IV representing DSS (World Health Organization, 1997).

DHF and DSS require four criteria to be met. Without any one of the criteria, the dengue patient will be classified as dengue fever even though the patient might already have developed shock (World Health Organization, 1997). Thus, the use of the WHO 1997 case definition was not sensitive enough to include some of the severe dengue cases (Balmaseda et al., 2005; Bandyopadhyay et al., 2006; Deen et al., 2006). The tourniquet test was evaluated to have low sensitivity and positive predictive value (Kalayanarooj et al., 1997; Phuong et al., 2002). Having a positive tourniquet test is one of the four criteria of DHF and DSS which indicates that there is a haemorrhagic tendency (capillary fragility and thrombocytopenia). A positive tourniquet test

is one of the earliest signs to indicate a haemorrhagic tendency. Due to the low sensitivity of tourniquet test, undiagnosed severe dengue cases can only be classified as dengue fever. This has prevented early treatment of the disease which can be fatal.

Due to the limitation of the WHO 1997 classification, the classification has been revised to form the WHO 2009 clinical practice guideline. The revised classification of severe dengue was based on the requirement of major intervention such as fluid resuscitation, blood products transfusion and other additional supports (Alexander et al., 2011). The WHO 2009 classification for severe dengue consisted of three subcategories: severe plasma leakage, severe bleeding and severe organ involvement. These subcategories were diagnosed clinically by the physician using the WHO 2009 clinical practice guideline. Severe plasma leakage was determined by shock (also known as dengue shock syndrome) or fluid accumulation with respiratory distress. Physical examination and investigations such as blood pressure, chest X-ray, haematocrit levels, serum albumin and ultrasound were used to determine the plasma leakage. Severe bleeding was determined by clinical judgement. Lastly, severe organ involvement was determined by the AST or ALT of more than 1000 U/L if liver was affected, heart or central nervous system involvement. The new classification has a sensitivity of 96% and specificity of 97%. The old WHO 1997 classification of DHF/DSS has a sensitivity of 76% and specificity of 54% (Alexander et al., 2011). Further study on comparing the old and revised versions of WHO classification showed that there is a reduction of unclassified dengue cases and misclassification of dengue cases with the use of the WHO

2009 classification (Barniol et al., 2011). However, the criteria of severe dengue in the revised classification indicate that pathological changes have already occurred and thus there is a short window period for treatment and intervention. The list of warning signs is based on symptoms and signs one day prior to the development of severe dengue (Alexander et al., 2011). This also does not allow the clinician to timely institute treatment within the short time frame.

2.6 Vascular endothelial growth factor (VEGF)

Cytokines such as tumour necrosis factor- α (TNF- α), interferon-α (IFN-α), interleukins (IL) and VEGF, are involved in the immunopathogenesis of dengue virus infection particularly in increasing the vascular permeability leading to plasma leakage (Bethell et al., 1998; Juffrie et al., 2001; Srikiatkhachorn & Green, 2010; Pawitan, 2011; Kumar et al., 2012). Some of the cytokines have been evaluated as possible predictive or screening markers (Bethell et al., 1998; Tseng et al., 2005; Srikiatkhachorn et al., 2007; Furuta et al., 2012; Kumar et al., 2012).

Most studies have shown the potential of cytokines in predicting the disease outcome (Bethell et al., 1998; Mairuhu et al., 2005). VEGF and the associated forms such as VEGF receptors and total VEGF-A have been evaluated and found to be particularly useful in differentiating dengue haemorrhagic fever (DHF) from dengue fever (DF) (Tseng et al., 2005; Srikiatkhachorn et al., 2007; Furuta et al., 2012). One study demonstrated that the VEGF level two days before fever defervescence was statistically

significantly different between DF and DHF groups of patients (Srikiatkhachorn et al., 2007).

Studies have demonstrated the predictability of cytokines on the days around the defervescence period (Bethell et al., 1998; Kumar et al., 2012). The cytokines measurement of the cytokines might pose difficulty in less resourceful laboratory centres. However, VEGF is one of the cytokines that could easily be measured by ELISA test kit which relatively cheaper and easier to performed compared with other techniques such as flowcytometry.

Nevertheless, some limitations found in the existing literature hampered the use of VEGF in a clinical setting such as the type of study designs i.e. case-control and cross-sectional study (Tseng et al., 2005; Thakur et al., 2016).These study designs only require an examination of the biomarkers at a single time point. This could not allow the development of prediction model because of the lack of temporality or a 'day-to-day' follow up sequence. Therefore, a cohort study is the preferred study design to evaluate the biomarker as a predictor. Cohort study requires a follow-up of patients from the development of disease to recovery from the disease.

A cohort study conducted blood samples collection since the day of admission (Furuta et al., 2012). Ideally, the blood samples should have been collected on the day when patients presented to the emergency department of

the hospital where they might or might not be admitted. Blood samples obtained upon admission could have been biased towards patients who has severe infection. The results of two other studies on VEGF were also not applicable to the clinical practice presently because an older version of WHO classification was employed (Srikiatkhachorn et al., 2007; del Moral-Hernández et al., 2014).

Lastly, some studies were conducted in a cohort of children (Srikiatkhachorn et al., 2007; Furuta et al., 2012). Dengue studies were previously focused on children population might be generalisable to the specific population where the study was conducted. Such studies may not be representative of dengue in Malaysian where the incidence of dengue virus infection is higher in the adult population (Ministry of Health, 2010).

2.7 Pentraxin 3 (PTX-3)

A study demonstrated that pentraxin 3 levels were significantly different between DF/DHF and DSS. The levels of pentraxin 3 were higher in DSS compared to DF/DHF in the first five days of admission (Mairuhu et al., 2005). The study population was children and the sample size was small. This was a retrospective study on frozen blood stored for several years, thus the stability of this biochemical compound is uncertain (Mairuhu et al., 2005). To our knowledge, this is the only study that evaluated PTX-3 in dengue virus infection.

CHAPTER 3.0

MATERIAL AND METHODS

3.1 Study design

This was a prospective study in which dengue patients were monitored from the day of presentation until hospital discharge. Blood samples were obtained daily after the patient was recruited. Hence, the trend of biomarkers for each day of the illness can be assessed. The plasma levels of VEGF and PTX-3 were compared between severe dengue and non-severe dengue (Definition of non-severe dengue: combined dengue without warning signs and dengue with warning signs).

The clinical classification of dengue virus infection was based on clinical practice guidelines from WHO (World Health Organization, 2012). Demographic data and clinical diagnosis were obtained using a standard data extraction form. The treating physicians were blinded from the biomarker test results. Fever in dengue virus infection was defined as more than 37.5 Celsius (>37.5˚C) (World Health Organization, 2009), or a complaint of fever from the patients. Patients might not be having fever when the temperature was measured. Hence, complaint of fever by the patient was acceptable as having a fever because patients often would have been prescribed with antipyretics (Ministry of Health, 2010). All patients with fever were included in the study if they were NS1Ag positive. NS1 Ag detected in the blood of the patient is

indicative of acute infection. The diagnosis of acute dengue virus infection was also determined by virus isolation and serotyping via RT-PCR. The presence of virus in the plasma is indicative of acute infection because viraemia only occurs during the first three days of illness.

3.2 Settings

Patients who visited Ampang Health clinic and Ampang Hospital from January 2016 until October 2017 were recruited prospectively from the outpatient and emergency departments. In Serdang Hospital, blood was collected via the Department of Pathology where blood samples were stored. Ampang Health Clinic is a primary care facility where the majority of patients will seek their first consultation with the general practitioner. Ampang and Serdang Hospitals are the referral centres for the primary health clinics in Malaysia if patients require specialist care. The hospitals also cater for emergency care for dengue patients.

3.3 Participants

3.3.1 Inclusion criteria:

The included participants were: aged 15 or older; presented within the first three days (72 hours) of illness; and had tested NS1 Ag positive.

3.3.2 Exclusion criteria:

Patients were excluded if the participants were pregnant, had an autoimmune disorder, haematological disorder, cancer, cardiovascular disease or were on long term warfarin and aspirin. (These patients were already in a high risk of severe dengue virus infection and thus they did not necessarily need to be tested for biomarkers. Furthermore, the blood disorders or medication that change the blood parameters could possibly confound the level of biomarkers)

3.4 Specimen transport, storage and analysis

3.4.1 Blood specimen

The daily blood samples were obtained either separately by the investigator or concurrently with the managing health practitioner/s. The blood samples were collected using ethylenediaminetetraacetic acid (EDTA) tube. The EDTA tube was used because it is compatible with all biomarkers and other antibodies testing. A plain tube was not recommended for use with PTX-3 ELISA test kit as specified by the manufacturer. The blood samples were centrifuged and the plasma was obtained. The plasma was transported in an ice box until it could be stored at -20 degree Celsius (°C). The blood samples were stored at 4 °C if the test was performed within 24-48 hours. The handling of the specimen from collection, centrifuge of specimen, transportation using ice box until the storage of plasma samples in UTAR laboratory is the same from the hospitals and health clinic.

3.4.2 Specimen analysis

A commercial ELISA kit was purchased to compare with the 'in-house' optimised ELISA test kit. The reason for optimising is to reduce the cost and duration of the test performed. The duration needed to produce result for the commercial test kit of PTX-3 (Human Pentraxin 3/TSG-14 Immunoassay, DPTX30, R&D Systems, Inc, Minneapolis, USA) and VEGF (Human VEGF-A, Platinum ELISA, BMS277/2 / BMS277/2TEN, eBioscience, Bender MedSystems GmbH, Vienna, Austria) was approximately six hours.

The levels of VEGF and PTX-3 were measured separately using an optimised sandwich ELISA. The ELISA procedure was optimised to be similar to the commercial test kit with the exception of a shorter duration (around one to two hours in total during samples, biotinylated detection antibody, Streptavidin-HRP and TMB solution incubation period). The ELISA plates of 96 wells were coated with 100 µl capture antibody. The capture antibody is the respective PTX-3 and VEGF monoclonal antibody purchased commercially. It was in powder form which had to be reconstituted. The capture antibody was diluted in 50 mM carbonate buffer with pH 9.6. The plate was then sealed with a plastic film and incubated overnight for about 16-17 hours at 4°C. The wells were washed with 350 µl washing buffer on a shaker for 30 seconds. The process of washing was repeated for at least three times. After the plate was washed, it was blotted dry. Three hundred microlitres of blocking buffer (1% bovine serum albumin) were then pipetted into each well. Blocking buffer was used to block non-specific binding sites. The plate was incubated for an hour at room temperature. The plate was then washed with 350 µl washing buffer on a shaker

for 30 seconds. The process of washing was repeated for three times and blotted dry after the final wash. The plate was added with 300 µl of 2% sucrose solution into each well and incubated for seven minutes at room temperature. The sucrose solution was removed and air dried at room temperature for two hours. The plate was stored at 4°C with desiccant in a sealed plastic bag until plasma samples were tested. The plate can be stored up to a week before using it. When the ELISA plate was ready for testing, 100 µl of each standard (recombinant antibody), plasma samples, control was pipetted into the wells in duplicates. The plate was sealed and incubated for 30 minutes on a shaker at room temperature. Samples of inadequate volume were diluted up to 1:1 ratio. After 30 minutes, the plate was washed with 350 µl, washing buffer for 30 seconds on a shaker for three times. After the final wash, the plate was blotted dry. 100 µl biotinylated detection antibody was subsequently added to each well. The plate was incubated for 15 minutes on a shaker at room temperature. A 100 µl of Streptavidin-HRP was added for another 15 minutes' incubation on a shaker at room temperature. The plate was washed after 15 minutes with 350 µl of washing buffer for 30 seconds on a shaker for three times. After the final wash, the plate was blotted dry. TMB solution is the substrate solution which was added to produce a blue colour. A 100 µl of the substrate solution was added to each well to incubate for 4-5 minutes, at room temperature and in the dark. (PTX3 was incubated for four minutes and 30 seconds and VEGF was incubated for five minutes. The substrate solution is sensitive to light. If the solution is exposed to light, the colour will change rapidly to blue and thus, it has to be incubated in the dark. Stop solution (50 µl sulfuric acid) was then added which turned the blue colour into yellow colour. The plate was then analysed

immediately (within 30 minutes). The setting of the optical density (OD) reader for reference wavelength was 540 nm and the measurement wavelength was 450 nm. For samples that were diluted with 1:1 ratio, must multiply the OD reading was multiplied by a factor of two. All of the antibodies need (capture, detection and standard antibodies) were purchased from R&D Systems (Minneapolis, MN). The commercial ELISA was performed according to the manufacturer's instructions.

3.4.3 Validation of optimised ELISA procedure

The Spearman's correlation coefficient which is also known as validity coefficient was calculated to compare between the commercial ELISA results and the optimised ELISA results. The validity coefficient ranges from zero to one with the highest being perfectly correlated. Coefficient of more than 0.7 is considered acceptable validity (Baumgartner & Hensley, 2006). The precision of optimized ELISA protocols was determined by coefficient variation (CV) expressed in percentage. CV is defined as standard deviation divided by the mean of the biomarker level. CV is calculated in intra-assay and inter-assay precision. The validity of the optimised ELISA kit was based on 20 samples. These 20 samples were randomly selected by generating random numbers that matched to the specimen number to minimise bias. However, potential bias could occur as these samples were derived from the remaining samples at the end of the pilot study (Low et al., 2015). Therefore, the not all samples had a n equal chance of being selected for testing because of inadequate samples.

3.4.4 NS1 Ag test and RT-PCR

NS1 Ag rapid test kit was used to diagnose dengue patients for the inclusion into the study. The test kit from Panbio has a sensitivity of 71.9% (95% confidence interval (CI) 64.1–78.9) and specificity of 99% (95% CI 83.1–99.4) (Pal et al. 2014). Virus isolation and RT-PCR was performed to determine the virus serotypes. The virus isolation and RT-PCR method were performed in the Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The procedure is described elsewhere (Suppiah, et al., 2018). Failure to serotype the virus can occur due to inadequate plasma or serum samples, virus no longer present in the plasma after day three of illness or repeated freeze-thaw cycle that could degrade the viral RNA.

3.4.5 Dengue IgM and IgG

Dengue IgM assay was performed to confirm the dengue status of the patients. Secondary dengue virus infection was determined by a positive dengue IgG assay on the blood samples collected within the first four days of illness. Dengue IgM and IgG ELISA tests were performed using commercial test kits from SD Bioline. The dengue IgM test has a sensitivity and specificity of 96.4% and 98.9%, respectively. Dengue IgG test has a sensitivity and specificity of 98.8% and 99.2%, respectively. Both test kits of dengue IgM and IgG were performed according to the manufacturer's instructions. Briefly, the samples and diluent were dropped in the wells of the precoated plate for incubation. The plate was then analysed in the OD reader after another solution was dropped into each well.

All blood tests were performed in the same UTAR laboratory using the same equipment. This reduces the variability of test kit results. The laboratory technicians were blinded from the diagnosis to avoid bias. Other blood investigation results (full blood count and liver function test) were recorded from the laboratory of the healthcare centres. The biomarker analysis and the test performed by the healthcare centres were conducted on the tubes of blood collected at the same time from the patients.

3.5 Sample size

The sample size was calculated by using receiver operating characteristics (ROC) based on the previous study conducted (Bradley & Longstaff, 2004; Low et al., 2015). The mean and standard deviation of the biomarkers in severe dengue and non-severe dengue were obtained. In SPSS Version 20 (Statistical Package for Social Science), a simulated data was created to produce a normal distribution of biomarker values by using the mean difference (the difference in mean between severe dengue and non-severe dengue group). The mean difference was adjusted three fold lower to produce a higher number of sample size. In this simulated data, 200 samples were assigned (100 sample for DF and 100 for DHF). The number of samples decided for the simulated data is arbitrary, but should be large enough to ensure that the biomarker levels follow a normal distribution.

Using this simulated data, the standard error (SE) of the area under the curve (AUC) was generated. Therefore, this SE was labeled as alternative hypothesis (SE_A). Sample size (n) was computed in the following formula:

$$SE\ of\ AUC \times \sqrt{(100 \div n)}$$

The same method was used with the null hypothesis (SE_O) in which the mean difference is zero. This produced a 95% CI of AUC which includes 0.5. The standard errors of both null and alternative hypotheses were then used to calculate the sample size (n) and power of study (Z) with the following formulas:

$$Z = \frac{(AUC_A - AUC_O) - 1.96 \times SE_O}{SE_A}$$

$$Z = \frac{(AUC_A - AUC_O) - 1.96 \times (SEO \times \sqrt{100 \div n})}{(SEA \times \sqrt{100 \div n})}$$

The power of the sample size calculated could be obtained from the Z score. With a power of 80%, the sample size for VEGF and PTX-3 were 45 and 20 per group respectively. The highest calculated sample size was used in this study: a total of 90 patients. An interim analysis was performed when the total sample size had achieved more than 60%.

3.6 Statistical analysis

3.6.1 Univariate analysis of biomarker

A ROC analysis was used to analyse the biomarker levels for each day comparing severe dengue and non-severe dengue. The analysis included biomarker levels up to the day before they developed severe dengue. This was to ensure the results produced are valid for prediction.

3.6.2 Predictive model using multivariable analysis

The logistic regression model was employed to develop a predictive model by identifying other clinical parameters that can be incorporated with the biomarkers. The outcome variable for the multivariable logistic regression model was severe dengue and non-severe dengue. Other predictor variables were age, gender, race, IgG status, dengue serotype, PTX-3, VEGF, WBC, PLT, HCT, ALT, and AST levels. A backward likelihood ratio (LR) was used to build the predictive model. The equation of the model was used to calculate various cut-offs and its corresponding sensitivity and specificity in the ROC curve. The cut-offs from the equation was the log odds denoted by ‘ln(p/1-p)’.

3.6.3 Diagnostic model using multivariable analysis

For diagnostic model, the analysis included biomarker levels on the day the severe dengue diagnoses were made. Biomarker levels for severe dengue were compared with non-severe dengue by stratifying the analysis according to the day of illness and without any stratification. Similarly, logistic regression

was employed to develop a diagnostic model by incorporating the same variables as in the predictive model. However, the patient with encephalopathy was removed since it did not change the diagnostic model. The logistic regression model cut-off values were chosen after a ROC analysis was performed.

Both the predictive and diagnostic models were constructed using data with valid, non-missing data and subsequently validated in the full data set. The prediction model was developed based on 44 patients and the diagnostic model was developed based on 60 patients.

3.6.4 Cut-off values selection

The ROC produces the AUC and the 95% CI, the sensitivity and specificity. AUC value of 0.5 and 95% CI of AUC which includes 0.5 were deemed as not statistically significant. The cut-off value and its sensitivity and specificity were presented only if it was statistically significant. The best few cut-offs for all models were chosen based on Youden's index and clinical applicability. Youden's index is an index which provides a guide to select a well-balanced sensitivity and specificity:

$$Youden's\ Index = Sensitivity + Specificity - 1$$

Cox regression was planned to analyse the prognostic value of biomarkers. This model allowed the estimation of the time of death. The

outcome variable was ‘dead’ or ‘survived’. Unfortunately, due to inadequate number of patients that died (four patients), no conclusion could be drawn from the analysis. Thus, this planned analysis was not performed.

A p-value of less than 0.05 was considered statistically significant. Statistical Package for Social Science (SPSS) version 20 was used in the statistical analysis.

This study was approved by the Medical Research & Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-15-1045-25937) and the study was registered in ClinicalTrials.gov (NCT02606019).

CHAPTER 4.0

RESULTS

The validity coefficients for PTX-3 and VEGF were 0.778 and 0.830, respectively. The intra-assay CV for VEGF and PTX-3 was 13.52% and 5.04%, respectively. The inter-assay CV for VEGF and PTX-3 was 20.35% and 19.88%, respectively.

A total of 82 patients with positive NS1 Ag test were included into this study. Acute dengue virus infection status of nine patients cannot be confirmed by IgM test or serotyping. The characteristics of patients were tabulated in Table 4.1. Samples that were not tested for the biomarkers were deemed as missing data accounting for 7.83%. The untested samples were due to insufficient plasma obtained and inability to obtain blood samples from the patients. A total of nine patients were excluded: five patients were lost from follow-up, two patients' samples were lost, one patient was asymptomatic and one patient was less than 15 years old.

Among the 28 severe dengue patients, 78.60% had severe plasma leakage alone leading to circulatory shock and respiratory distress. All patients with severe bleeding had to upper gastrointestinal bleed. All patients with severe bleeding were due to upper gastrointestinal bleed. Four patients with severe organ involvement were due to severe hepatitis except one patient who was

diagnosed with encephalopathy. The complications that occurred in patients with severe dengue are described in Table 4.2. Seven severe dengue patients were transfused with blood product and four died. One non-severe dengue patient had anaemia, one severe dengue patient had hypertension and another severe dengue patient had both diabetes and hypertension.

Table 4.1: Characteristics of all included patients.

	Severe dengue (n=29)	Non-severe dengue (n=53)
Male, n (%)	12 (41.40)	35 (66.00)
Female, n (%)	17 (58.60)	18 (34.00)
Race and nationality, n (%)		
Malay	22 (75.90)	38 (71.70)
Chinese	2 (6.90)	10 (18.90)
Indian	1 (3.40)	4 (7.50)
Other Malaysian	1 (3.40)	1 (1.90)
Other non-Malaysian	3 (10.30)	0 (0.00)
Serotype (57 samples detected), n (%)		
1	7 (28.00)	11 (34.38)
2	13 (52.00)	13 (40.63)
3	3 (12.00)	5 (15.63)
4	0 (0.00)	1 (3.13)
1 & 2	0 (0.00)	2 (6.25)
2 & 3	2 (8.00)	0 (0.00)
Number of secondary dengue (positive IgG), n (%)	9 (31.00)	8 (15.10)*
Age (years), mean (SD)	34.38 (15.96)	30.17 (13.36)
Day of discharge from care #, median (IQR)	7 (2)	6 (2)
Day of defervescence #, median (IQR)	4 (2)	4 (2)

Abbreviation: n, number of patients; SD, standard deviation; IQR, interquartile range.
* Two patients were not able to test IgG serology due to insufficient plasma.
#According to the day of illness.

Table 4.2: The complications that occurred in patients who were diagnosed with severe dengue.

Complications	Number of patients(%)
Severe plasma leakage	22 (78.60)
Severe plasma leakage and severe bleeding	1 (3.60)
Severe plasma leakage, severe bleeding and severe organ involvement	2 (7.10)
Severe plasma leakage and severe organ involvement	2 (7.10)
Severe organ involvement*	1 (3.60)

* Dengue encephalopathy was diagnosed in one patient.

Note: one patient had severe dengue diagnosis but the complication was not documented.

The mean and 95% CI of PTX-3 and VEGF levels of each day are displayed in Figure 4.1 and 4.2, respectively. The removal of one patient with encephalopathy did not change the AUC significantly in all models for both biomarkers. Hence, the result of the analysis that was tabulated in Table 4.3, 4.4 and 4.5 included this patient with encephalopathy as part of the analysis. None of the AUC were statistically significant except for VEGF as a diagnostic marker at Day two and three of illness with AUC of 0.88 (95% CI: 0.73, 1.00) and 0.78 (95% CI: 0.60, 0.95), respectively. The best two cut-offs of VEGF level for the statistically significant AUC models were tabulated in Table 4.6.

The best predictive and diagnostic model with $p < 0.001$ has been tabulated in Table 4.7. The predictive model with AUC of 0.84 (95% CI: 0.73, 0.94, $p < 0.01$) has a sensitivity of 100.00% and specificity of 79.25% for predicting severe dengue with a cut-off of 0.34. The diagnostic model with AUC of 0.71 (95% CI: 0.60, 0.85, $p < 0.01$) has a sensitivity of 76.19% and specificity of 73.58% for diagnosing severe dengue with a cut-off of -1.07. However, with a cut-off of -3.22, the sensitivity and specificity was 100.00% and 3.77%, respectively. The equation for prognostic model was:

$\ln(p/1-p) = 217.03 + (-110.35) \times \text{Gender(Female=1)} + 0.33 \times \text{VEGF} + (-16.86) \times \text{WBC} + (-6.15) \times \text{HCT} + 0.46 \times \text{ALT}$

The equation for the diagnostic model was:

$\ln(p/1-p) = (-4.27) + 0.01 \times \text{VEGF} + 0.02 \times \text{PLT} + 0.01 \times \text{ALT}$

Figure 4.1: The mean PTX-3 level for each day of illness.

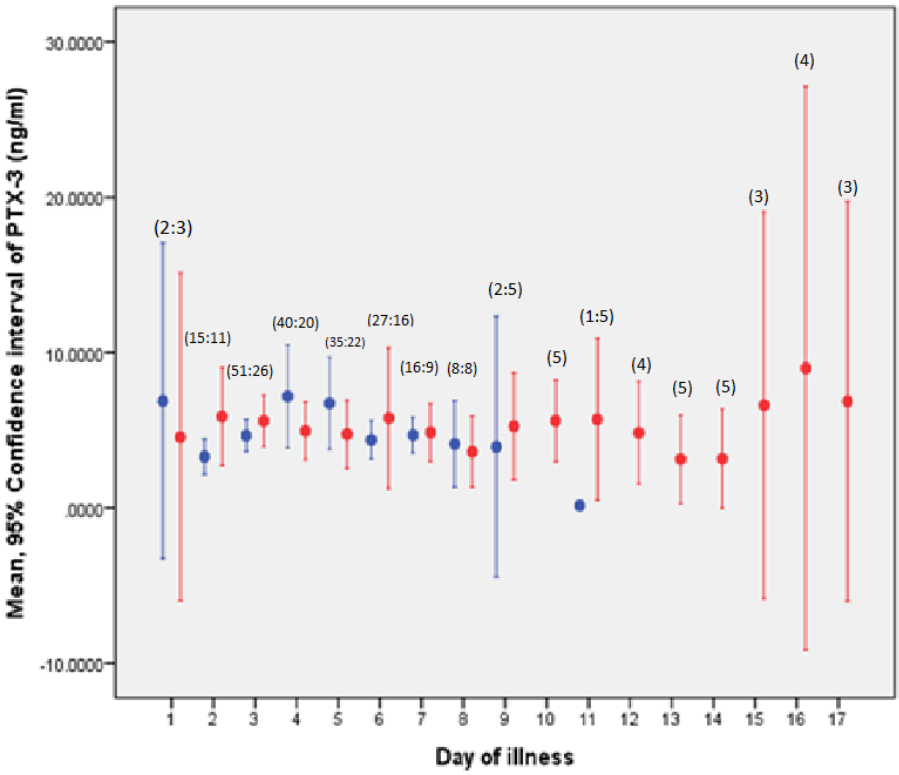

Figure legend:

Non-severe dengue is represented by the blue line: –

Severe dengue is represented by the red line: –

The brackets above each line represents the number of patients in each category: (non-severe dengue: severe dengue)

Note: Dot represents the mean and the line represents the confidence interval of the biomarker. None of the comparisons between non-severe dengue and severe dengue for each day were statistically significant ($p>0.05$).

Figure 4.2: The mean VEGF level for each day of illness.

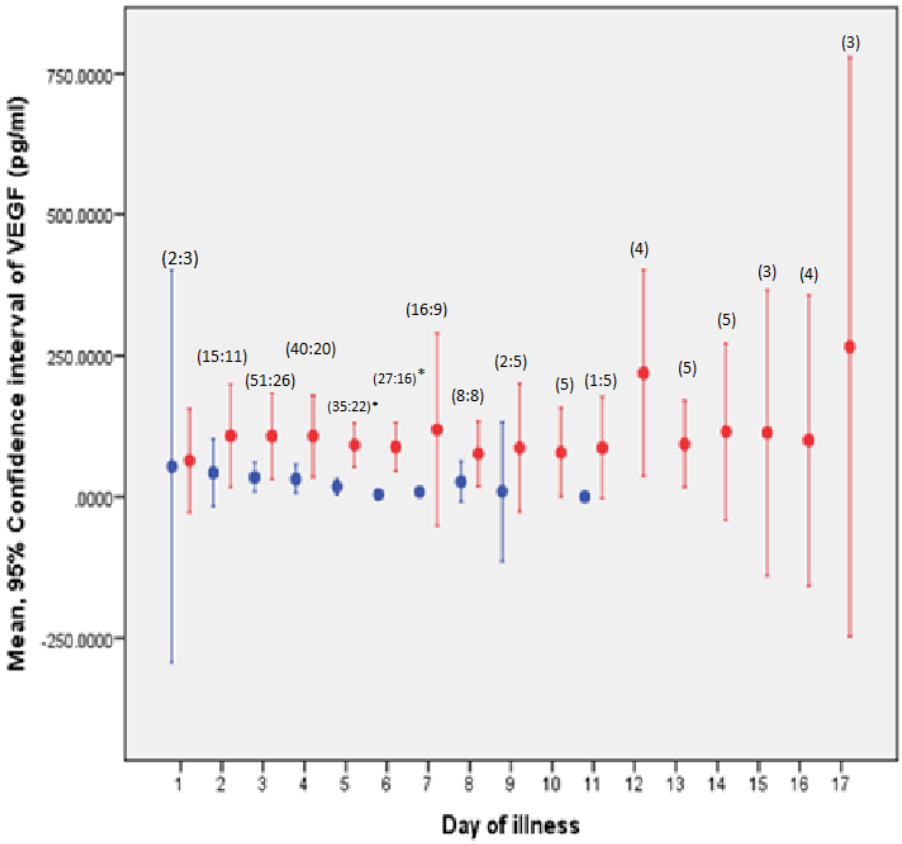

Figure legend:

Non-severe dengue is represented by the blue line: –

Severe dengue is represented by the red line: –

The brackets above each line represents the number of patients in each category: (non-severe dengue: severe dengue)

*p<0.05

Note: Dot represents the mean and the line represents the confidence interval of the biomarker.

Table 4.3: Receiver operating characteristics of PTX-3 and VEGF as predictive markers of severe dengue.

AUC (95% CI)	
PTX-3	VEGF
0.41 (0.15, 0.68)	0.54 (0.29, 0.78)

Abbreviation: AUC, area under the curve; CI, confidence interval.

Note: total number of patients is 44.

Table 4.4: Receiver operating characteristics of PTX-3 and VEGF as diagnostic markers stratified according to the day of illness.

Day of illness	Number of cases (SD: Non-SD)	AUC (95% CI)	
		PTX-3	VEGF
1	2:2	0.50 (0.00, 1.00)	0.50 (0.00, 1.00)
2	5:15	0.68 (0.34, 1.00)	0.88 (0.73, 1.00)*
3	10:51	0.45 (0.23, 0.66)	0.78 (0.60, 0.95)*
4	1:40	0.85 (0.74, 0.96)	0.33 (0.00, 0.72)
5	3: 35	0.68 (0.48, 0.88)	0.50 (0.11, 0.88)

Abbreviation: SD, severe dengue; Non-SD, non-severe dengue; AUC, area under the curve; CI, confidence interval.

* $p < 0.05$

Table 4.5: Receiver operating characteristics of PTX-3 and VEGF as diagnostic markers without stratification of the day of illness.

AUC (95% CI)	
PTX-3	VEGF
0.50 (0.34, 0.66)	0.71 (0.56, 0.86)*

Abbreviation: AUC, area under the curve; CI, confidence interval.

* $p < 0.05$

Note: total number of patients is 60.

Table 4.6: Cut-offs of VEGF levels as a diagnostic marker.

VEGF level (pg/ml)	Diagnostic accuracy (%)	
	Sensitivity	Specificity
Day 2 of illness		
37.50	100.00	80.00
50.53	80.00	80.00
Day 3 of illness		
19.03	80.00	76.47
48.65	70.00	88.24
Without stratification		
19.36	76.19	62.26
44.59	66.67	79.25

Table 4.7: Logistic regression model for prediction and diagnosis.

Predictor variables	Beta (SE)
Prediction model	
Constant	217.03 (25421.02)
Gender = Female	-110.35 (13928.20)
VEGF	0.33 (36.36)
WBC	-16.86 (2335.28)
HCT	-6.15 (633.79)
ALT	0.46 (52.53)
Diagnostic model	
Constant	-4.27 (1.19)
VEGF	0.01 (0,00)
PLT	0.02 (0,01)
ALT	0.01 (0,00)

Abbreviation: WBC, white blood cell count; HCT, haematocrit; ALT, alanine aminotransferase; PLT, platelet; SE, standard error.

CHAPTER 5.0

DISCUSSION

VEGF in combination with other variables was able to predict the complication of dengue infection i.e. severe dengue. VEGF as a single marker was also able to diagnose severe dengue on day two and day three of illness. However, PTX-3 was not able to predict and diagnose the severe dengue.

VEGF is involved in the pathogenesis of many diseases such as cancer and diabetic retinopathy particularly in the healing process of damaged blood vessel (Ferrara et al., 2003). However, VEGF is also involved in the pathogenesis of plasma leakage of dengue virus infection (Malavige & Ogg, 2017). Hence, a higher VEGF level is expected in a dengue patient who develops circulatory shock (Tseng et al., 2005; Srikiatkhachorn et al., 2007; Furuta et al., 2012). One patient with dengue encephalopathy had almost all zero VEGF level which is consistent with findings from existing literature (Misra et al., 2014). This is expected because VEGF is derived mainly from endothelial cells found in blood vessels. The dengue virus causes inflammation of the brain cells

The VEGF levels detected were consistent with the observations of Thakur et al., 2016 and Yong et al., 2017. However, the study designs of both studies did not examine the VEGF daily before and after the development of severe dengue in contrast to this study. Hence, severe dengue could have already

occurred when the analysis was performed (Yong et al., 2017). This does not allow the biomarker to be predictive before the complication occurs thereby reducing the chance of early treatment to prevent death. Another study was a cross-sectional study whereby the temporal sequence of the VEGF levels in the patient and the diagnosis of severe dengue was unclear (Thakur et al., 2016). As such, the findings from our study is difficult to be compare with although generally agrees to the increase VEGF level in dengue with complications.

PTX-3 is related to but distinct from the C-reactive protein produced by a variety of cells such as macrophages and endothelial cells (Mantovani et al., 2003). Thus, PTX-3 might be involved in the pathogenesis of dengue virus infection. Unfortunately, the difference in PTX-3 level between severe dengue and non-severe dengue was not statistically significant. The finding is not in agreement with the existing study whereby dengue shock syndrome has a higher PTX-3 level (Mairuhu et al., 2005). To date, no other study has evaluated PTX-3 in dengue virus infection after 2005 indicating that PTX-3 may not be useful in predicting or diagnosing severe dengue because negative results were not easily publishable (Easterbrook et al., 1991).

The specificity of the prediction model (VEGF and other clinical parameters such as female gender, WBC, HCT and ALT) was better than the warning signs employed by the WHO 2009 guideline. The WHO 2009 guideline has a sensitivity similar to our study but the specificity is less than 50% (Leo et al., 2013; Thein et al., 2013). Other studies also developed models to predict

severe dengue but both sensitivity and specificity obtained were lower than those of our prediction model (Tanner et al., 2008; Potts et al., 2010; Soundravally et al., 2015). However, our diagnostic model and VEGF levels as a single marker have comparatively lower accuracy than the WHO 2009 severe dengue diagnostic criteria (Horstick et al., 2014). Therefore, it could not replace the current diagnostic guideline but may serve as an adjunct investigation to support the diagnosis.

The logistic regression model was chosen as the predictive method after exploring few other prediction models such as decision tree and neural network. The selection was based on simplicity and accuracy. All models explored had similar accuracy but logistic regression was considered relatively simple to compute than the others. The simplicity of a model is important to facilitate easier development of its usage in the population. It is also easier to create a mobile application or online software with the simple mathematical equation. The function of the backward LR of multiple logistic regression models was used to eliminate the least statistically significant variable in each step. When the final step was reached, the model was again tested to evaluate its accuracy in predicting the severe dengue. Until the model is satisfied with the best accuracy, the elimination step is stopped. This model was again tested in larger sample size to evaluate its accuracy. This is because when the model was developed, only valid data was used to build the model. Other non-valid data which is due to missing values were excluded. Thus, the model was again being evaluated in the full data set which consisted of some missing data. This is the actual scenario as some patients would not undergo some of the blood testings.

This study has achieved up to 91% of the total calculated sample size. It is unlikely that the result will change significantly even when 100% of sample size is achieved. Therefore, the study halted when the interim analysis indicated statistical significant result. This reduced the need for more patients to be recruited and avoided research fund wastage. Instead, allocation of funding for future multicentre study with bigger sample size for generalisability could be optimised.

The ‘in-house’ ELISA test was optimised with an acceptable degree of variation in comparison with the commercial ELISA test. However, the limitation of this ‘in-house’ ELISA test is that it must first be validated by the respective laboratories before being used to test patients’. Each ELISA kit has some amount of variability but the commercial product may have more control over the variability. Even with a commercial ELISA kit, when it is used in a different laboratory setting, variation may occur. This is due to the different equipment used to perform and analyse the ELISA. ELISA is operator dependent and thus, variation can also occur. Therefore, a well-trained technician may minimise the variation. Researchers or laboratory users who wish to replicate and employ any ELISA test kit, will be required to undertake validation by using controls of known biomarker concentrations in the ELISA plate.

CV is a form of variability measurement and is expressed as percentages. It is calculated for each sample that is tested in duplicates. Less than 20% of CV is considered acceptable in this study and thus, when any variation exceeds this threshold; the ELISA was repeated until it achieved the threshold. However, "inaccuracy" according to CV estimation might not be significant in a clinical setting. For example, if the value falls within a wide range of standard error but still above the estimated ROC cut-off value, then the value generated is still useful to predict the disease. Hence, the CV threshold should be determined only after multiple testing in a clinical setting. In clinical practice, if the value cannot be correlated with clinical findings, then the test should be repeated as in any other test. This is in accordance with the concept of "regression towards mean" in statistics whereby the subsequent test value would move closer to the average value. Unfortunately, the cost of multiple testing is a burden to the healthcare system. Hence, the first step for the clinician is to ensure the accuracy of the dengue diagnosis before interpreting the biomarker results so that an accurate correlation between the clinical condition of dengue and the biomarkers can be made.

Although this study was designed as a cohort study, the incidence rate of severe dengue could not be reliably calculated because patients were only recruited in the first 72 hours of illness. Hence, a high proportion of severe dengue found in this study does not reflect the true incidence rate of severe dengue because most cases of non-severe dengue that presented after 72 hours of illness could not be captured. Thus, the denominator of the incidence rate is

smaller than expected which leads to a falsely higher incidence rate of severe dengue.

VEGF may be useful in predicting severe dengue along with other available clinical parameters. The model was developed and validated in a full data set. However, to apply in another hospital or country, the equation must be revalidated because the incidence of severe dengue and clinical parameters may differ. If re-validation fails, a new prediction model must be developed. Therefore, replication of this study is essential before we can conclude its use internationally.

VEGF is a non-specific biomarker which can occur in many disease condition. The biomarker must be interpreted only if the dengue status of a patient is confirmed such as by a positive NS1 Ag or IgM. Other possible conditions that could have an effect on the biomarker levels were excluded in this study. Nevertheless, if dengue patients have these condition such as heart disease and pregnancy, they are already at higher risk of complication and death. Thus, the patient should be admitted for close monitoring with or without VEGF testing.

Another limitation of this study is that the fluid intake and other supportive treatments were not included in the development of the model. Treatment might affect the VEGF levels. For example, during blood transfusion

or dialysis, the VEGF levels might be reduced. Similarly, the fluid therapy administered parenterally could also have diluted the biomarker in the plasma. However, the analysis particularly for predicting severe dengue only included samples from patients who have not been treated parenterally because they were discharged from outpatient. This should not have impacted greatly on the prediction model.

Future studies should concentrate on the development of a biosensor for VEGF detection. A biosensor is a device that detects chemical reactions and converts then into digital data for interpretation. The detection via a transducer can be of various forms such as electrochemical, mass-based, thermometric, magnetic and optical. Relevant to dengue, the biosensor can be used as an immunosensor to detect the VEGF antigen-anti-VEGF reaction (Byrne et al., 2009). The development of an immunosensors could potentially reduce the time to produce results. Complications of dengue virus infection can occur rapidly and the patient might die within few hours or minutes. Hence, predictive or diagnostic results that can help the clinician to make a decision should be produced in a short period of time. More importantly, immunosensors could potentially reduce variations that are found in various steps of the ELISA. Immunosensors employ materials such as gold and electrical conducting material to detect signals from the antigen-antibody reaction. Materials used as a detecting probe must be able to bind to the capture antibody as in ELISA wells. The signal will be then transmitted via a transducer to form digital data. Hence, only samples were needed to produce a signal in contrast to ELISA which requires streptavidin-HRP and TMB solution to produce a colour of different

intensity (Byrne et al., 2009). The TMB solution is also light sensitive which can change the colour rapidly if it is exposed to light. Therefore, immunosensor has an advantage over ELISA by reducing the variation found in an ELISA.

An immunosensor can also be customised into a 'bedside' rapid kit. This will help clinician, especially in the rural areas where laboratory facility is not available. One widely used example is the glucometer. Glucometer requires only a drop of blood to create an enzymatic reaction which produces electrical activity. The signal of this electrical charge is then analysed within a palm-size machine to provide a measurement of the glucose level in the body (Dzyadevych et al., 2008). If the VEGF test can be customised into such a bedside kit for clinical use, there will be an enormous potential for it to be applied in many resource-poor dengue endemic countries. The variation of the measurement using such a 'bedside' test can be reduced to a minimum because most of the steps of the ELISA would have been eliminated.

One other way to make the test more user-friendly is to test on urine samples. Urinary VEGF may be detectable in dengue patients although no studies have been conducted on that. However, studies on cancers and other diseases particularly in the genitourinary system have offered insight that VEGF could be present in the urine (Matsumoto & Kanmatsuse, 2001; Bok et al., 2001; Chan et al., 2004). Studies can be conducted in dengue patients to determine whether VEGF is also excreted in the urine. If VEGF is present in the urine, then blood taking can be avoided. Furthermore, this can be used as a home-based test

kit that can be sold over the counter like the urine pregnancy test. This further reduces the cost of the healthcare system as the patient will buy the kit on their own. Detailed instruction should be provided to guide patients on how to use the test and calculate the probability of developing severe dengue using a standardised formula similar to what has been proposed in this study.

The prediction model developed in this study was based on a single blood sample from each patient one day prior to the development of severe dengue. Though high VEGF levels may indicate that plasma leakage has already occurred, it may still be useful as a predictor because clinical evidence of plasma leakage can only be detected a day later.

In conclusion, VEGF may be used in combination with other clinical parameters to predict the severity of dengue. As a single biomarker, it may be used as an adjunct investigation to support the diagnosis of severe dengue. PTX-3 was not able to differentiate severe dengue from non-severe dengue.

LIST OF REFERENCES

Alexander, N. et al., 2011. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. *Tropical Medicine & International Health*, 16(8), pp.936–948.

Azeredo, E.L. et al., 2006. Activated peripheral lymphocytes with increased expression of cell adhesion molecules and cytotoxic markers are associated with dengue fever disease. *Memórias do Instituto Oswaldo Cruz*, 101(4), pp.437–449.

Balmaseda, A. et al., 2005. Short report: Assessment of the World Health Organization scheme for classification of dengue severity in Nicaragua. *American Journal of Tropical Medicine and Hygiene*, 73(6), pp.1059–1062.

Bandyopadhyay, S., Lum, L.C.S. & Kroeger, A., 2006. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue haemorrhagic fever. *Tropical Medicine & International Health*, 11(8), pp.1238–1255.

Barniol, J. et al., 2011. Usefulness and applicability of the revised dengue case classification by disease: multi-centre study in 18 countries. *BMC infectious diseases*, 11(1), pp.106.

Baumgartner, T.A. & Hensley, L.D., 2006. *Conducting and reading research in health and human performance* 4th ed. New York: McGraw-Hill.

Bethell, D.B. et al., 1998. Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. *Journal of Infectious Diseases*, 177(3),

pp.778–782.

Bhatt, S. et al., 2013. The global distribution and burden of dengue. *Nature*, 496(7446), pp.504–507.

Bok, R.A. et al., 2001. Vascular endothelial growth factor and basic fibroblast growth factor urine levels as predictors of outcome in hormone-refractory prostate cancer patients. *Cancer Research*, 61(6), pp.2533–2536.

Bradley, A.P. & Longstaff, I.D., 2004. Sample size estimation using the receiver operating characteristic curve. *Proceedings of the 17th International Conference on Pattern Recognition*, 26 August 2004 Cambridge, UK. UK: IEEE, pp. 428–431.

Byrne, B. et al., 2009. Antibody-Based Sensors: Principles, Problems and Potential for Detection of Pathogens and Associated Toxins. *Sensors*, 9(6), pp.4407–4445.

Calzavara-Silva, C.E. et al., 2009. Early molecular markers predictive of dengue hemorrhagic fever. *Anais da Academia Brasileira de Ciências*, 81(4), pp.671–677.

Chan, L.W. et al., 2004. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: A longitudinal study of protein kinetics throughout tumor progression and therapy. *Journal of Clinical Oncology*, 22(3), pp.499–506.

Chen, C.D. et al., 2006. Dengue vectors surveillance in endemic areas in Kuala Lumpur city centre and Selangor State , Malaysia. *Dengue Bulletin*, 30, pp.197–203.

Deen, J.L. et al., 2006. The WHO dengue classification and case definitions: time for a reassessment. *The Lancet*, 368(9530), pp.170–173.

Dzyadevych, S. V et al., 2008. Amperometric enzyme biosensors: Past, present and future. *IRBM*, 29(2), pp.171–180.

Easterbrook, P.. et al., 1991. Publication bias in clinical research. *The Lancet*, 337(8746), pp.867–872.

Fadilah, S.A. et al., 1999. Quantitation of T lymphocyte subsets helps to distinguish dengue hemorrhagic fever from classic dengue fever during the acute febrile stage. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 30(4), pp.710–717.

Ferrara, N., Gerber, H.-P. & LeCouter, J., 2003. The biology of VEGF and its receptors. *Nature Medicine*, 9(6), pp.669–676.

Furuta, T. et al., 2012. Association of mast cell-derived VEGF and proteases in dengue shock syndrome. *PLoS Neglected Tropical Diseases*, 6(2), pp.e1505.

Gil, L. et al., 2004. Oxidative stress in adult dengue patients. *American Journal of Tropical Medicine and Hygiene*, 71(5), pp.652–657.

van Gorp, E.C.M. et al., 2002. Changes in the plasma lipid profile as a potential predictor of clinical outcome in dengue hemorrhagic fever. *Clinical Infectious Diseases*, 34(8), pp.1150–1153.

Gubler, D.J., 1998. Dengue and dengue hemorrhagic fever. *Clinical Microbiology Reviews*, 11(3), pp.480–496.

Guzman, M. G., & Vazquez, S., 2010. The complexity of antibody-dependent enhancement of dengue virus infection. *Viruses*, *2*(12), pp.2649–2662.

Ha, T.T.N. et al., 2011. Elevated levels of cell-free circulating DNA in patients with acute dengue virus infection. *PloS One*, 6(10), pp.e25969.

Henchal, E.A. & Putnak, J.R., 1990. The dengue viruses. *Clinical microbiology reviews*, 3(4), pp.376–396.

Honsawek, S. et al., 2007. Increased levels of serum hyaluronan in patients with dengue infection. *Journal of Infection*, 54(3), pp.225–229.

Horstick, O. et al., 2014. Comparing the usefulness of the 1997 and 2009 WHO dengue case classification: A systematic literature review. *The American Journal of Tropical Medicine and Hygiene*, 91(3), pp.621–634.

John, D. V, Lin, Y.S. & Perng, G.C., 2015. Biomarkers of severe dengue disease---a review. *Journal of Biomedical Science*, 22(83), pp.1–7.

Juffrie, M. et al., 2001. Inflammatory mediators in dengue virus infection in children: interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. *The American Journal of Tropical Medicine and Hygiene*, 65(1), pp.70–75.

Kalayanarooj, S. et al., 1997. Early clinical and laboratory indicators of acute dengue illness. *Journal of Infectious Diseases*, 176(2), pp.313–21.

Koraka, P. et al., 2004. Elevation of soluble VCAM-1 plasma levels in children with acute dengue virus infection of varying severity. *Journal of Medical Virology*, 72(3), pp.445–450.

Kuan, G.L.K., Hin, Y.M. & Isa, R.M., 2015. Comparison of vomiting and diarrhoea frequency among dengue-infected patients. *Journal of Coastal Life Medicine*, 3(8), pp.616–620.

Kumar, Y. et al., 2012. Serum proteome and cytokine analysis in a longitudinal

cohort of adults with primary dengue infection reveals predictive markers of DHF. *PLOS Neglected Tropical Diseases*, 6(11), pp.e1887.

Leo, Y.-S. et al., 2013. Utility of warning signs in guiding admission and predicting severe disease in adult dengue. *BMC Infectious Diseases*, 13(1), pp.498.

Libraty, D.H. et al., 2002. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *Journal of Infectious Diseases*, 186(8), pp.1165–1168.

Low, G.K.K., 2016. Changing pattern of symptoms in dengue patients over the years: A review and meta-analysis. *Journal of Coastal Life Medicine*, 4(9), pp.678–682.

Low, G.K.K., Gan, S.C. & Ho, S.C., 2015. Biomarkers in differentiating clinical dengue cases: A prospective cohort study. *Journal of Coastal Life Medicine*, 3(12), pp.967–970.

Mairuhu, A.T.A. et al., 2005. Elevated plasma levels of the long pentraxin, pentraxin 3, in severe dengue virus infections. *Journal of Medical Virology*, 76(4), pp.547–552.

Malavige, G.N. & Ogg, G.S., 2017. Pathogenesis of vascular leak in dengue virus infection. *Immunology*, 151(3), pp.261–269.

Mantovani, A., Garlanda, C. & Bottazzi, B., 2003. Pentraxin 3, a non-redundant soluble pattern recognition receptor involved in innate immunity. *Vaccine*, 21, pp.S43–S47.

Matsumoto, K. & Kanmatsuse, K., 2001. Elevated vascular endothelial growth

factor levels in the urine of patients with minimal-change nephrotic syndrome. *Clinical Nephrology*, 55(4), pp.269—274.

Michels, M. et al., 2011. High plasma mid-regional pro-adrenomedullin levels in children with severe dengue virus infections. *Journal of Clinical Virology*, 50(1), pp.8–12.

Ministry of Health, 2010. *Clinical practice guidelines: Management of dengue infection in adults*, rev 2nd ed. Putrajaya: Ministry of Health Malaysia.

Ministry of Health, 2015. *Clinical practice guidelines: Management of dengue infection in adults*, 3rd ed. Putrajaya: Ministry of Health Malaysia.

Ministry of Health, 2017. *Press release of dengue fever situation in Malaysia week 1/2017 (1 - 7 Jan 2017)*. Putrajaya: Ministry of Health.

Misra, U.K., Kalita, J. & Singh, A.P., 2014. Role of Vascular Endothelial Growth Factor (VEGF) in the Neurological Manifestations of Dengue: A Preliminary Study. *Inflammation*, 37(2), pp.611–614.

del Moral-Hernández, O. et al., 2014. Association DENV1 and DENV2 infection with high serum levels of soluble thrombomodulin and VEGF in patients with dengue fever and dengue hemorrhagic fever. *International Journal of Clinical and Experimental Medicine*, 7(2), pp.370–378.

Murgue, B., Cassar, O. & Deparis, X., 2001. Plasma concentrations of sVCAM-1 and severity of dengue infections. *Journal of Medical Virology*, 65(1), pp.97–104.

Nascimento, E.J.M. et al., 2009. Gene expression profiling during early acute febrile stage of dengue infection can predict the disease outcome. *PloS one*, 4(11), pp.e7892.

Nishiura, H. & Halstead, S.B., 2007. Natural history of dengue virus (DENV)–1 and DENV-4 infections: reanalysis of classic studies. *Journal of Infectious Diseases*, 195(7), pp.1007–1013.

Pal, S. et al., 2014. Evaluation of dengue NS1 Antigen rapid tests and ELISA kits using clinical samples. *PLoS One*, 9(11), pp.e113411.

Pawitan, J.A., 2011. Dengue virus infection: predictors for severe dengue. *Acta Medica Indonesiana*, 43(2), pp.129–135.

Phuong, C.X.T. et al., 2002. Evaluation of the World Health Organization standard tourniquet test and a modified tourniquet test in the diagnosis of dengue infection in Viet Nam. *Tropical Medicine & International Health*, 7(2), pp.125–132.

Potts, J.A. et al., 2010. Prediction of dengue disease severity among pediatric Thai patients using early clinical laboratory indicators. *PLoS Neglected Tropical Diseases*, 4(8), pp.e769.

Rathakrishnan, A. et al., 2012. Cytokine expression profile of dengue patients at different phases of illness. *PLoS One*, 7(12), pp.e52215.

Rigau-Pérez, J.G. et al., 1998. Dengue and dengue haemorrhagic fever. *The Lancet*, 352(9132), pp.971–977.

Rigau-Pérez, J.G. & Laufer, M.K., 2006. Dengue-related deaths in Puerto Rico, 1992-1996: diagnosis and clinical alarm signals. *Clinical Infectious Diseases*, 42(9), pp.1241–1246.

Rothman, A.L., 1999, *Pathogenesis of dengue virus infection* [Online]. Available at: https://www.uptodate.com/contents/dengue-virus-infection-pathogenesis [Accessed 4 April 2017].

Seet, R.C.S. et al., 2005. An outbreak of primary dengue infection among migrant Chinese workers in Singapore characterized by prominent gastrointestinal symptoms and a high proportion of symptomatic cases. *Journal of Clinical Virology*, 33(4), pp.336–340.

Soundravally, R. et al., 2015. Ferritin levels predict severe dengue. *Infection*, 43(1), pp.13–19.

Soundravally, R. et al., 2008. Oxidative stress induced changes in plasma protein can be a predictor of imminent severe dengue infection. *Acta Tropica*, 106(3), pp.156–161.

Souza, L. et al., 2004. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Brazilian Journal of Infectious Diseases*, 8(2), pp.156–163.

Srikiatkhachorn, A. et al., 2007. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. *The Pediatric Infectious Disease Journal*, 26(4), pp.283–290.

Srikiatkhachorn, A. et al., 2007. Virus-induced decline in soluble vascular endothelial growth receptor 2 is associated with plasma leakage in dengue hemorrhagic fever. *Journal of Virology*, 81(4), pp.1592–1600.

Srikiatkhachorn, A. & Green, S., 2010. Markers of dengue disease severity. *Current Topics in Microbiology and Immunology*, 338, pp.67–82.

Suaya, J.A. et al., 2009. Cost of dengue cases in eight countries in the Americas and Asia : A prospective study. *American Journal of Tropical Medicine and Hygiene*, 80(5), pp.846–855.

Suppiah, J. et al., 2018. Clinical manifestations of dengue in relation to dengue

serotype and genotype in Malaysia: A retrospective observational study. *PLOS Neglected Tropical Diseases* 12(9): pp.e0006817.

Tanner, L. et al., 2008. Decision Tree Algorithms Predict the Diagnosis and Outcome of Dengue Fever in the Early Phase of Illness. *PLoS Neglected Tropical Diseases*, 2(3), pp.e196.

Thakur, P. et al., 2016. Elevated levels of vascular endothelial growth factor in adults with severe dengue infection. *VirusDisease*, 27(1), pp.48–54.

Thein, T.-L. et al., 2013. Utilities and limitations of the World Health Organization 2009 warning signs for adult dengue severity. *PLoS Neglected Tropical Diseases*, 7(1), pp.e2023.

Tseng, C. et al., 2005. Elevated levels of plasma VEGF in patients with dengue hemorrhagic fever. *FEMS Immunology & Medical Microbiology*, 43(1), pp.99–102.

Vasilakis, N. et al., 2013. The daemon in the forest-emergence of a new dengue serotype in Southeast Asia. *3rd International Conference on Dengue and Dengue Haemorrhagic Fever. Global Dengue: Challenges and Promises*, 21-23 October 2013 Bangkok, Thailand. Bangkok: Ministry of Public Health, pp. 33.

Vaughn, D.W. et al., 1997. Dengue in the Early Febrile Phase: Viremia and Antibody Responses. *The Journal of Infectious Diseases*, 176(2), pp.322–330.

Vaughn, D.W. et al., 2000. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *Journal of Infectious Diseases*, 181(1), pp.2–9.

van de Weg, C.A.M. et al., 2012. Lipopolysaccharide levels are elevated in dengue virus infected patients and correlate with disease severity. *Journal of Clinical Virology*, 53(1), pp.38–42.

Widjaja, H. & Mantik, M.F.J., 2009. Effect of dengue hemorrhagic fever on thrombomodulin level. *Paediatrica Indonesiana*, 49(5), pp.259–263.

World Health Organization, 2016, *Dengue and severe dengue* [Online]. Available at: http://www.who.int/mediacentre/factsheets/fs117/en/ [Accessed 27 March 2017].

World Health Organization, 2009. *Dengue guidelines for diagnosis, treatment, prevention and control.* New ed. Geneva, Switzerland: WHO Press.

World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*, 2nd ed. Geneva: WHO Press.

World Health Organization, 2012. *Handbook for clinical management of dengue*, Geneva: WHO Press.

Yong, Y.K. et al., 2017. Aberrant monocyte responses predict and characterize dengue virus infection in individuals with severe disease. *Journal of Translational Medicine*, 15(1), pp.121.

Young, P.R. et al., 2000. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *Journal of Clinical Microbiology*, 38(3), pp.1053–1057.

APPENDIX A

List of publications during the Doctor of Philosophy (Ph.D.) candidature

1. Kuan, G.L.K., Yong, M.H., Isa, R.M., 2015a. Comparison of vomiting and diarrhoea frequency among dengue-infected patients. *Journal Coast Life Medicine*, 3(8), pp. 616–620.

2. Kuan, G.L.K, Yong, M.H., Isa, R.M., 2015b. Predicting severe dengue using quantified warning signs. A retrospective cohort study. *Journal Coast Life Medicine*, 3(9), pp. 708–712.

3. Low, G.K.K., Gan, S.C., Ho, S.C., 2015. Biomarkers in differentiating clinical dengue cases: A prospective cohort study. *Journal Coast Life Medicine*, 3(12), pp. 967–970.

4. Low, G.K.K., 2016a. Changing pattern of symptoms in dengue patients over the years: A review and meta-analysis. *Journal Coast Life Medicine*, 4(9), pp. 678–682.

5. Low, G.K.K., 2016b. The accuracy of newly proposed warning signs in the third edition of Malaysian guideline on the management of dengue infection in adult. *Journal Coast Life Medicine*, 4(8), pp. 652–654.

6. Low, G.K., et al., 2018a. Geographical distribution and spatio-temporal patterns of hospitalization due to dengue infection at a leading specialist hospital in Malaysia. *Geospatial health*, *13*(1), pp. 642.

7. Low, G.K., Ogston, S.A., Yong, M.H., Gan, S.C., Chee, H.Y., 2018b. Global dengue death before and after the new World Health Organization 2009 case classification: A systematic review and meta-regression analysis. *Acta Tropica*. 182, pp. 237–245.

8. Low, G.K.K., Yong, M.H., Looi, S.Y, Sharma, D., 2018c. Predictive and diagnostic test accuracy of ultrasonography in differentiating severe dengue from non-severe dengue, a systematic review. *Journal of Vector Borne Diseases*. 55, pp. 79-88.

9. Low, G.K.K., et al, 2018d. The predictive and diagnostic accuracy of vascular endothelial growth factor and pentraxin-3 in severe dengue, *Pathogens and Global Health*, 112(6), pp.334-341.

APPENDIX B

Data analysis of other variables not presented in the main text

1. **Analysis of Variance (ANOVA) of PTX-3 levels among serotype 1, 2 and 3 (N=52).**

Serotypes	Mean	Standard deviation	P value
1 (n=18)	68.7399	102.0563	
2 (n=26)	58.9883	93.663	
3 (n=8)	35.1811	23.0053	>0.05

Note: serotype 4 and mixed serotypes were not included into the analysis due to inadequate patients for comparison (one and two patients, respectively).

Interpretation: there is no statistically significant difference in the PTX-3 level among the three serotypes.

2. **Analysis of Variance (ANOVA) of VEGF levels among serotype 1, 2 and 3 (N=52).**

Serotypes	Mean	Standard deviation	P value
1 (n=18)	68.7399	102.0563	
2 (n=26)	58.9883	93.663	
3 (n=8)	35.1811	23.0053	>0.05

Note: serotype 4 and mixed serotypes were not included into the analysis due to inadequate patients for comparison (one and two patients, respectively).

Interpretation: there is no statistically significant difference in the VEGF level among the three serotypes.

3. **The first logistic regression model for prediction and diagnosis. (Model prior to development of the equation)**

Predictor variables	Beta (SE)
Prediction model	
Constant	-38.37 (145414.65)
Gender = Female	-51.15 (35144.84)
Age	0.38 (2642.39)
IgG status	15.26 (75133.03)
Race = Malay	30.76 (27200.61)
Race = Chinese	-102.07 (35523.46)
Race = Indian	76.10 (73344.36)
PTX-3	-1.26 (2819.20)
VEGF	0.39 (115.85)
WBC	-11.34 (14872.89)
PLT	0.68 (503.31)
HCT	-1.49 (2216.21)
ALT	0.53 (650.48)
AST	-0.07 (595.10)
Diagnostic model	
Constant	-8.11 (5.61)
Gender = Female	-1.24 (1.69)

Age	0.05 (0.05)
IgG status	2.01 (1.39)
Race = Malay	0.29 (1.27)
Race = Chinese	-3.70 (2.24)
Race = Indian	14.69 (20979.97)
Race = Other Malaysians	22.12(20979.55)
PTX-3	-0.01 (0.09)
VEGF	0.02 (0.01)
WBC	-0.87 (0.59)
PLT	0.04 (0.02)*
HCT	0.06 (0.14)
ALT	0.01 (0.02)
AST	-0.01 (0.01)

Abbreviation: WBC, white blood cell count; HCT, haematocrit; ALT, alanine aminotransferase; PLT, platelet; AST, aspartate aminotransferase; SE, standard error.

*p<0.05

Interpretation: All variables in both adjusted models are not statistically significant between non-severe dengue and severe dengue except platelet count. The results indicate that after controlling for potential confounding factors and each other, there is no difference in all the variables between non-severe dengue and severe dengue. Note: although platelet count is the only variable that is significant (p=0.32), the result is likely due to chance. Thus, it must be interpreted with caution.

APPENDIX C

1. **Master Data Set**
2. **Commercial and Optimised ELISA Comparison Data Set**
3. **Data For Constructing Figures**

Master Data Set

ID	Serotype	IgMEarly	IgMLate	IgG	Age	Gender	Race	PTX1	PTX2	PTX3	PTX4	PTX5	PTX6	PTX7	PTX8	PTX9	PTX10
269	1			0	15	0	0	NA	NA	4.81	5.66	7.02	5.56	8.07	6.7	NA	NA
1355	0		1	0	17	0	0	NA	NA	7.9	10.5	0	0	NA	NA	NA	NA
5014	0	0		0	41	1	0	NA	NA	11.5	NA	NA	NA	NA	NA	NA	NA
5027	0	0	1	0	44	0	1	NA	NA	0	2.16	1.49	2.51	NA	NA	NA	NA
5030	0	insufficien	1	0	31	1	0	NA	NA	9.73	insuffi	insuffi	NA	NA	NA	NA	NA
5047	0		1	0	20	0	0	NA	NA	1.02	7.19	8.68	16.5	5.11	NA	NA	NA
5054	1		1	0	33	1	0	NA	missin	10.8	NA	NA	NA	NA	NA	NA	NA
5068	0		1	0	63	1	0	NA	NA	7.87	missin	4.13	5.49	NA	NA	NA	NA
5069	1&2		1	0	28	0	1	NA	5.96	16.6	35.6	3.49	NA	NA	NA	NA	NA
5114	0		1	0	17	1	0	NA	NA	3.79	missin	5.96	6.56	NA	NA	NA	NA
5117	2			0	54	0	1	NA	2.02	3.82	2.54	1.6	3.23	3.07	2.22	3.27	NA
5129	2	0		1	19	0	0	NA	NA	0.34	missin	0.77	1.34	2.62	NA	NA	NA
5149	2	0	0	0	17	0	0	NA	NA	0.93	2.37	2.18	0.88	3.64	2.1	NA	NA
5168	0	0	1	0	23	1	1	NA	NA	5.18	4.42	2.68	NA	NA	NA	NA	NA
5169	3	0	1	1	28	0	0	NA	4.66	8.29	14	1.12	NA	NA	NA	NA	NA
5182	2			0	21	1	0	NA	3.3	3.27	1	NA	NA	NA	NA	NA	NA
5185	3			0	27	0	1	NA	5.59	NA	NA	NA	NA	NA	NA	NA	NA
5195	0		1	0	23	0	0	NA	NA	0	3.73	NA	NA	NA	NA	NA	NA
5206	insufficient			insu	71	1	1	NA	NA	4.98	NA	NA	NA	NA	NA	NA	NA
5207	1	0		0	28	0	0	NA	NA	1.97	NA	NA	NA	NA	NA	NA	NA
5217	0	0	0	0	17	0	0	NA	NA	8.06	7.92	6.11	NA	NA	NA	NA	NA
5219	2			0	20	0	0	NA	NA	1.75	3.29	4.04	4.79	2.94	2.43	missin	missing
5222	2	0		1	30	1	0	NA	NA	7.62	7.85	7.92	3.96	NA	NA	NA	NA
5261	2	1		1	34	0	0	NA	NA	6.33	5.89	14.9	3.24	NA	NA	NA	NA
5276	1&2			0	27	1	0	NA	0	2.96	1.77	NA	NA	NA	NA	NA	NA
5303	3			0	25	0	0	NA	3.29	5.61	20.3	NA	NA	NA	NA	NA	NA
5321	0		1	0	24	0	0	NA	4.27	4.23	18.8	20.3	6.04	NA	NA	NA	NA
5348	1			0	30	1	0	NA	NA	1.16	7.79	9.51	3.9	8.3	NA	NA	NA
5354	1			1	45	1	1	NA	NA	1.92	1.73	2.15	1.5	NA	NA	NA	NA
5362	0	0	1	0	46	1	0	NA	NA	4.91	4.45	5.46	4.3	3.49	NA	NA	NA
5368	2			0	32	1	0	NA	NA	0.94	4.68	7	3.59	2.43	NA	NA	NA
5373	2			0	21	0	0	NA	2.18	0.85	2.6	6.98	NA	NA	NA	NA	NA

Master Data Set

5398	3		1	0	33	1	0	NA	0.33	3.76	6.92	2.25	1.16	NA	NA	NA	NA
5405	0	1		1	33	0	0	NA	NA	6.56	5.31	9.38	7.1	NA	NA	NA	NA
5464	3		1	0	19	1	1	NA	NA	4.17	missin	missin	5.46	NA	NA	NA	NA
5473	1			0	28	0	0	6.08	3.63	7.43	1.27	3.96	3.14	4.02	NA	NA	NA
5474	1			0	46	1	0	NA	NA	0.42	1.67	0.87	0.76	6.72	NA	NA	NA
5506	2			0	34	1	0	NA	2.42	9.07	4	2.03	NA	NA	NA	NA	NA
5519	0	1		0	27	0	3	NA	NA	4.65	2.82	NA	NA	NA	NA	NA	NA
5587	0	1	1	1	54	0	1	NA	NA	5.02	7.35	8.2	1.17	NA	NA	NA	NA
5651	2			0	24	0	0	NA	NA	6	7.37	7.7	missin	missin	NA	NA	NA
5657	1	0		1	28	0	0	NA	NA	2.38	0.27	NA	NA	NA	NA	NA	NA
5672	2&3		1		1	49	1	0	NA	NA	1.83	3.51	NA	NA	NA	NA	NA	NA
5675	0	0	1	0	20	0	2	NA	NA	6.08	2.13	7.3	2.52	7.8	0.71	NA	NA
5696	1			0	25	1	1	2.55	6.28	6.9	11.7	11.5	3.72	NA	NA	NA	NA
5703	1			0	60	0	0	NA	4.86	1.55	8.84	NA	NA	NA	NA	NA	NA
5709	0		1	0	43	0	1	NA	NA	11.9	NA	NA	NA	2.94	3.61	4.6	NA
5718	2			0	16	1	0	NA	NA	1.75	1.6	1.89	5.19	NA	NA	NA	NA
5731	1			0	18	0	0	NA	NA	6.93	56.2	49.9	7.69	6.02	11.1	NA	NA
5735	3			0	22	0	2	NA	NA	5.38	missin	missin	missin	5.73	4.13	NA	NA
5792	0	0		0	25	1	2	NA	NA	7.24	NA	NA	NA	NA	NA	NA	NA
5793	1		1	0	17	0	0	NA	NA	11.5	8.97	6.66	missin	NA	NA	NA	NA
5874	2		1	0	41	1	3	NA	NA	9.38	7.9	1.31	2.05	3	4.58	9.71	7.85
5875	1	0	1	0	53	0	1	NA	NA	10.1	9.09	3.02	3.84	4.2	1.7	6.21	7.938
5878	1			0	35	1	0	NA	NA	4.36	missin	8.84	36.3	6.8	missin	missin	NA
5880	0	0		1	18	1	0	NA	4.39	6.84	2.22	2.9	4.36	Missin	3.62	NA	NA
5979	2		1	0	30	0	0	NA	4.34	6.24	1.58	3.48	3.26	NA	NA	NA	NA
5995	2			0	53	0	0	NA	NA	5.73	4.14	3.94	1.49	2.13	NA	NA	NA
6055	0		1	0	17	0	0	NA	NA	3.09	missin	missin	5.53	NA	NA	NA	NA
6203	0	0	0	0	20	0	0	NA	0.79	0	2.03	missin	missin	NA	NA	NA	NA
6292	3		1	0	56	1	0	NA	3.34	5.42	4.49	2.51	5.2	3.6	7.68	NA	NA
6489	1			0	23	0	0	NA	6.56	12.3	11.9	9.82	NA	NA	NA	NA	NA
6529	3			0	19	0	0	NA	NA	0.18	missin	4.66	3.64	5.14	NA	NA	NA
6536	0		1	0	24	0	0	NA	NA	0.06	1.83	3.11	NA	NA	NA	NA	NA
8925	2	0		0	22	0	0	NA	8.37	5.97	NA	NA	NA	NA	NA	NA	NA

Master Data Set

	170672	2	1	1	1	16	1	0	NA	NA	0	0	NA	NA	NA	NA	NA	NA	
	172081	1		1	0	33	1	2	NA	0	0	0	0	NA	NA	NA	NA	NA	
	177613	2	0		0	26	1	4	NA	NA	4.38	NA	NA	NA	NA	NA	NA	NA	
	549871	2	0	1	1	29	0	4	NA	NA	0.82	1.1	2.11	NA	NA	NA	NA	NA	
	550620	2		1	0	34	0	4	1.68	11.5	9.68	9.97	20.6	NA	NA	NA	NA	NA	
	557599	2		1	0	62	1	0	NA	3.11	1.21	0.66	1.65	4.34	8.7	NA	NA	NA	
	558117	2	insufficien	1	1	23	0	0	NA	NA	insuffi	missin	11.1	NA	NA	NA	NA	NA	
	558764	2	0	insufficier	0	76	0	0	NA	7.47	NA	NA	NA	NA	NA	NA	NA	NA	
	561064	1		1	0	69	1	0	9.45	15.6	7.26	6.5	5.19	5.7	NA	NA	NA	NA	
	562017	2	1		1	20	1	0	NA	NA	4	3.62	0.68	NA	NA	NA	NA	NA	
	562210	2		insufficier	0	23	1	0	NA	0	0	0	0	NA	NA	NA	NA	NA	
	562584	1		1	1	41	1	2	NA	NA	0	0	0	0	missin	0	NA	NA	
5657(Adhil)		4			0	17	0	0	NA	NA	9.19	2.97	4.25	4.95	NA	NA	NA	NA	
5875(Hadri)	insufficient			insu		25	0	0	7.69	NA	NA	NA	NA	NA	NA	NA	NA	NA	
SD875470	2,3	0		0		30	0	0	NA	NA	14.3	8.21	7.19	10.5	7.72	7.15	3.71	4.461	
SD875805(6139)	0	1		1		37	0	0	NA	NA	insuffi	2.17	1.63	2.76	1.38	1.34	2.85	3.413	
SD879358(5394)	2	1		1		32	1	0	NA	NA	2.97	insuffi	7.33	5.82	3.25	2.89	3.84	4.421	

Master Data Set

PTX11	PTX12	PTX13	PTX14	PTX15	PTX16	PTX17	VEGF1	VEGF2	VEGF3	VEGF4	VEGF5	VEGF6	VEGF7	VEGF8	VEGF9	VEGF10	VEGF11	VEGF12
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	18.33	0	16.21	20.43	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	360.3	252	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	41.75	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	10	0	0	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	missing	76	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	5.333	missing	0	11.5	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	29.5	27.25	154.5	0	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	missing	25.67	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	missing	0	0	0	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	9.75	24.75	20	4.25	13.17	15.83	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	25	2.25	0	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	15.75	16.5	142.5	0	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	66	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	221	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	NA	NA	NA	NA	NA	NA	NA
0.161	NA	NA	NA	NA	NA	NA	NA	NA	0	0	16.33	7.667	0	0	missing	missing	0	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	23	23	18.75	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	16	17.75	0	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	77.4	15.5	0	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	14.5	17.5	128.5	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	56.67	0	55	95	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	70.57	41.14	0	0	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	8.75	NA	NA	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	0	20.56	20.56	135.6	76.11	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	25.25	21	24	32.25	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	6E-08	missing	missing	3.375	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	26.34	0	0	0	0	0	0	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	445.4	0	0	0	0	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	13.25	0	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	8.333	37.33	21.67	missing	missing	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	55.56	709.4	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	1.87	0	70.01	127.8	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	81.89	238.8	887.7	64.9	98.47	103.5	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	27.33	0	0	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	41	NA	NA	NA	0	19.5	19.5	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	7.071	75.33	81.33	0	27.75	35.25	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	97.67	missing	missing	missing	9	0	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	61	NA	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	57.5	32	49.75	missing	NA	NA	NA	NA	NA	NA	
12.43	7.031	4.444	5.444	12.41	26.11	12.65	NA	NA	172	133.5	89.25	109.5	89	117	119.5	92.75	125.25	119.75	
5.505	5.677	4.726	5.86	3.628	3.595	5.311	NA	NA	0	0	49.25	16	9.5	0	0	83.5	79.375	368	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	missing	0	56	19.5	missing	missing	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	70.5	22.75	47.75	43.89	54.44	Missing	107.2	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	15	16.67	11.11	6.667	10	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	22	missing	missing	0	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	missing	missing	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	15.75	21.13	28	75	83.33	22.75	185.6	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	37.5	missing	50.67	24.33	26.83	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	221.1	0	0	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	45.5	38.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	199.8	181	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	421.8	432.5	453.7	216	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	11.12	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	22	34	38.75	37	83	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	406	94.44	85.56	83.89	59.44	699.4	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	insuffici	missing	54.44	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	55.56	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	89.17	30	41.67	30	3.333	0	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	447.2	335.2	232.8	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	278	207.7	261.7	209.3	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	158.5	159.5	166.7	220	missing	113	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	31	30.69	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	81	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6.088	missing	5.118	3.329	NA	NA	NA	NA	NA	0	1.111	0	0	0	0	0	0	7.77778	missing
1.587	2.157	1.43	1.207	3.802	3.198	NA	NA	NA	insuffici	168.3	185	220	94.17	90.83	217.5	171.667	188.333	249.167
2.925	4.487	0	0	insuffic	3.066	2.618	NA	NA	201.7	60	127.8	135	111.1	0	99.44	45.5556	35	139.444

Master Data Set

VEGF13	VEGF14	VEGF15	VEGF16	VEGF17	WBC1	WBC2	WBC3	WBC4	WBC5	WBC6	WBC7	WBC8	WBC9	WBC10	WBC11
NA	NA	NA	NA	NA	NA	NA	5.6	6.3	4.3	3.3	3.6	4.9	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.7	2.5	2.4	5.5	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.3	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.6	2.6	5.1	5.7	9.1	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	2.5	1.4	1.7	5.8	5	3.3	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.8	2.8	3.1	3.8	6	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	18.7	22.8	19.7	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.4	4.9	8.8	9.7	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	3.8	2.6	2.2	4	5.5	11.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.1	14.3	2.9	4.8	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4.6	3.1	4.6	6.2	9.2	16.6	16.6	14.9	NA	NA
NA	NA	NA	NA	NA	NA	NA	5.3	3.5	2.9	4.6	7.1	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4	3.1	4.4	4.6	4.5	8.1	12.6	NA	NA	NA
NA	NA	NA	NA	NA	NA	1.4	1.1	1.4	3	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.3	1.6	2.2	4	4	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	3.7	3.8	2.6	2.7	5.6	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.9	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	6.7	5.4	8.1	9.5	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	7.2	5.5	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.9	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.9	4.1	3.6	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	5.3	5.1	4.6	4	5.4	4.7	missing	missing	6.2
NA	NA	NA	NA	NA	NA	1.7	2.9	3.8	5	3.5	4.2	NA	NA	NA	NA
NA	NA	NA	NA	NA	6.5	6.2	4.7	2.8	3.8	5.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	2.6	1.8	2.3	2.3	3	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4.28	2.7	3.1	4.4	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	3.1	2.2	2.1	2.1	2.8	3.5	2.4	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.3	1.8	2.2	2.3	5	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.1	5	6.2	5.6	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.1	2.6	2.7	5.3	6.8	6.9	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	2.6	1.4	2.7	3.4	6.4	5.5	NA	NA	NA	NA
NA	NA	NA	NA	NA	2.8	2.8	2.1	2.2	3.4	3.8	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	1.5	1.2	2.3	3	4.3	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	2.8	5.2	6.3	5.2	3.7	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.5	1.5	1.8	2.4	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	8.9	4.8	3	2.3	2.6	5.4	5.6	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4	3.4	1.3	1.8	3.7	NA	3.3	NA	NA	NA
NA	NA	NA	NA	NA	NA	2.7	1.8	2.4	3	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	2.2	4.3	7.4	10.4	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.6	5.5	8.3	10	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.7	5	5.2	missing	missing	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	6.7	5.4	8.8	14.6	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	16.1	9.1	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.5	2	1.6	2.2	3.3	4.9	4.9	4.7	NA	NA
NA	NA	NA	NA	NA	8.8	6.4	2.6	2.7	3.8	5.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	8.2	9.9	11.7	12.5	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.5	NA	2.3	11.9	7.3	5.3	7	NA	NA
NA	NA	NA	NA	NA	NA	1.8	1.8	2	1.5	3	4.3	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.6	1.5	1.1	3.8	5.1	4.5	3.1	NA	NA	NA
NA	NA	NA	NA	NA	NA	10.1	5.7	3.4	3.3	missing	4.1	4.8	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.8	3.1	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.5	1.6	2	3.4	3.3	NA	NA	NA	NA
83.75	149.25	351.667	343.333	390.833	NA	3.2	6.1	7.3	18.5	10.9	11.5	11.4	10.2	18.7	11
163.125	319.375	39	25.5	379	NA	NA	2.5	3.6	5.9	10.4	26.4	31.7	20	18.8	20.4
NA	NA	NA	NA	NA	NA	NA	3.2	2	2.3	11.7	3.5	3.9	4.8	NA	NA
NA	NA	NA	NA	NA	3.7	3.3	1.6	1.8	2.1	4.2	5.8	6.6	NA	NA	NA
NA	NA	NA	NA	NA	4.78	2.9	2.3	3.2	4.4	7.8	5.2	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4.9	3.4	3.8	5.6	7.2	7.3	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.4	missing	missing	1.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	4.2	4.5	3.9	3.9	3.2	3.7	4.7	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.4	3.6	2.6	2.4	10	12.1	9.7	NA	NA	NA
NA	NA	NA	NA	NA	3.7	3.9	2	1.9	2.5	2.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	3.6	3.9	2.8	3.7	6.4	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.3	2.1	2.3	6.2	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	7.8	4.9	NA	NA	NA	NA	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	4.7	4.9	4.4	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	4.2	2.4	2.6	4.3	6.4	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.5	2.6	2.7	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	7.47	9.5	7.3	7.5	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	7.7	2.7	2.1	2.9	3.1	3.9	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	7.1	13.6	9.8	14.6	22.8	26.8	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	5.3	5.7	4.7	4.5	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	13.5	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	3.9	3.6	2.5	3.1	4.7	7.2	6.9	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	5.5	9.1	9	6.8	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	3.5	4.2	4.7	5.3	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	2.9	5.1	7.3	8.2	5.1	4.4	5.1	NA	NA
NA	NA	NA	NA	NA	NA	4.7	1.6	1.8	3.7	6.3	6	NA	NA	NA	NA
NA	NA	NA	NA	NA	4.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3.88889	0	NA	NA	NA	NA	NA	7.6	6.8	4.5	4.3	3	4.4	6.4	7.6	11.1
79.1667	50.8333	26.6667	13.3333	NA	NA	NA	3.3	7.6	10.8	10.6	12.5	12.5	NA	NA	NA
138.333	57.2222	36.6667	18.8889	27.7778	NA	NA	2.8	6.5	13.4	22.1	15.6	10.5	7.6	NA	NA

Master Data Set

WBC12	WBC13	WBC14	WBC15	WBC16	WBC17	Plt1	Plt2	Plt3	Plt4	Plt5	Plt6	Plt7	Plt8	Plt9
NA	NA	NA	NA	NA	NA	NA	NA	132	126	94	90	92	123	NA
NA	NA	NA	NA	NA	NA	NA	NA	44	39	11	20	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	107	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	49	33	15	21	56	NA	NA
NA	NA	NA	NA	NA	NA	NA	47	72	44	20	35	52	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	149	95	40	24	38	NA	NA
NA	NA	NA	NA	NA	NA	NA	19	5	113	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	29	30.7	33	42	NA	NA	NA
NA	NA	NA	NA	NA	NA	164	88	68	28	37	63	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	88	47	31	37	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	39	10	7	8	17	37	83	99
NA	NA	NA	NA	NA	NA	NA	NA	141	95	56	38	42	NA	NA
NA	NA	NA	NA	NA	NA	NA	138	128	120	117	110	130	194	NA
NA	NA	NA	NA	NA	NA	NA	95	89	73	86	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	65	39	32	39	41	NA	NA	NA
NA	NA	NA	NA	NA	NA	114	141	120	100	84	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	117	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	205	123	202	228	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	63	292	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	155	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	144	114	129	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	134	99	92	82	77	79	missing
NA	NA	NA	NA	NA	NA	NA	43	19	14	12	20	88	NA	NA
NA	NA	NA	NA	NA	NA	140	106	49	28	21	49	NA	NA	NA
NA	NA	NA	NA	NA	NA	105	107	109	93	46	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	145	107	110	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	61	45	34	21	26	23	34	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	145	160	133	112	109	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	37	16	14	54	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	157	96	24	25	32	82	NA	NA
NA	NA	NA	NA	NA	NA	NA	101	122	91	86	75	85	NA	NA
NA	NA	NA	NA	NA	NA	97	119	120	73	92	74	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	168	137	135	124	128	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	55	28	16	15	58	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	158	134	117	113	NA	NA	NA
NA	NA	NA	NA	NA	NA	188	146	82	87	69	77	85	NA	NA
NA	NA	NA	NA	NA	NA	NA	75	75	38	11	24	NA	58	NA
NA	NA	NA	NA	NA	NA	NA	126	84	96	91	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	28	28	30	56	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	27	22	29	83	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	128	122	75	missing	missing	NA	NA
NA	NA	NA	NA	NA	NA	NA	150	87	81	100	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	75	20	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	168	78	49	9	8	14	35	93
NA	NA	NA	NA	NA	NA	318	208	133	83	54	58	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	68	15	23	51	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	138	NA	24	12	9	7	67
NA	NA	NA	NA	NA	NA	NA	137	130	120	104	72	68	NA	NA
NA	NA	NA	NA	NA	NA	NA	141	93	57	17	29	16	51	NA
NA	NA	NA	NA	NA	NA	NA	113	121	98	47	missing	37	46	NA
NA	NA	NA	NA	NA	NA	NA	NA	130	135	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	189	35	18	20	35	NA	NA
9.6	11	11.9	10.4	10.6	9	NA	33	17	13	32	66	50	56	50
20.4	15.3	13.5	12.8	15.5	17.9	NA	NA	17	9	11	19	55	95	71
NA	NA	NA	NA	NA	NA	NA	NA	117	104	99	56	72	89	227
NA	NA	NA	NA	NA	NA	123	119	55	18	18	49	80	198	NA
NA	NA	NA	NA	NA	NA	153	116	69	72	85	117	170	NA	NA
NA	NA	NA	NA	NA	NA	NA	136	69	27	9	16	38	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	124	missing	missing	186	NA	NA	NA
NA	NA	NA	NA	NA	NA	177	158	143	101	91	78	79	NA	NA
NA	NA	NA	NA	NA	NA	NA	165	127	82	64	49	33	136	NA
NA	NA	NA	NA	NA	NA	131	115	77	41	42	49	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	177	79	69	29	35	NA	NA
NA	NA	NA	NA	NA	NA	NA	90	72	62	49	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	225	189	NA	NA	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA		52	22	34	NA	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA		161	77	63	19	34	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA		33	27	51	NA	NA	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA	NA		5	11	18	45	NA	NA	NA									
NA	NA	NA	NA	NA	NA	204		124	80	60	58	55	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA		31	66	46	47	43	58	NA	NA									
NA	NA	NA	NA	NA	NA	NA	NA		8	7	16	44	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA		28	NA	NA	NA	NA	NA	NA	NA									
NA	NA	NA	NA	NA	NA	125		83	60	23	16	17	38	NA	NA									
NA	NA	NA	NA	NA	NA	NA	NA		39	16	18	40	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA		12.2	10	17	32	NA	NA	NA	NA									
NA	NA	NA	NA	NA	NA	NA	NA		90	29	20	29	41	65	132									
NA	NA	NA	NA	NA	NA	NA		80	103	44	32	41	70	NA	NA									
NA	NA	NA	NA	NA	NA	125	NA	NA	NA	NA	NA	NA	NA	NA	NA									
14.1	15.2	14.8	14	NA	NA	NA	NA		103	83	51	35	17	8	21									
NA	NA	NA	NA	NA	NA	NA	NA		47	26	30	59	94	150	NA									
NA	NA	NA	NA	NA	NA	NA	NA		20	16	24	45	79	136	191									

Master Data Set

Plt10	Plt11	Plt12	Plt13	Plt14	Plt15	PLT16	PLT17	HCT1	HCT2	HCT3	HCT4	HCT5	HCT6	HCT7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44.5	41	39.7	40.3	40.2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	45.2	42.9	46.7	43.1	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	38.7	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	51.7	45.7	45.9	45.1	43.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	41.9	37.7	39.5	39.7	41.8	38
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	45.8	44.2	47.3	45.4	47.3
NA	NA	NA	NA	NA	NA	NA	NA	NA	46.5	46.9	24.6	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31	29	36	28.5	NA
NA	NA	NA	NA	NA	NA	NA	NA	45.1	40.6	43.3	41.5	40.8	41.7	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42.7	44.8	39.8	37.5	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	45.3	45.1	45.7	44.3	40.3	36.6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	45.2	47.4	41.9	41.6	44.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	43.1	40.2	41	41.8	38.7	41.3
NA	NA	NA	NA	NA	NA	NA	NA	NA	35.1	37.1	37.2	35.6	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	42.4	43.2	41.6	39.4	42.1	NA
NA	NA	NA	NA	NA	NA	NA	NA	30.4	32.9	29.6	30.6	29.9	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	44.3	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	44.6	45.9	46.8	46.8	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35.9	40.5	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44.2	48	47.3	NA	NA
missing	305	NA	NA	NA	NA	NA	NA	NA	NA	43.2	46	46.2	47.5	50.9
NA	NA	NA	NA	NA	NA	NA	NA	NA	43.5	40.7	42	37.4	34.4	35.5
NA	NA	NA	NA	NA	NA	NA	NA	43	42.8	39.6	38.1	39.7	44	NA
NA	NA	NA	NA	NA	NA	NA	NA	39.3	39.3	42.6	38.9	43.5	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	44.8	39.1	41.4	42.3	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	42.3	37.2	39	41.6	38.4	42.8	40.9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	39.2	42.9	45.6	44.7	44.2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	40.7	41.7	39.4	34.8	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	39.6	36.3	37.8	38.8	38.4	37.4
NA	NA	NA	NA	NA	NA	NA	NA	NA	39.6	35	38.3	40.1	36.4	32.8
NA	NA	NA	NA	NA	NA	NA	NA	38	39.6	39.2	42.8	42.4	42.2	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	13.2	37.5	38.4	40.6	37.2	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	46.4	48.2	52.7	46.3	41.9	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	33.6	34.4	33.1	34.8	NA
NA	NA	NA	NA	NA	NA	NA	NA	42.1	44.5	4.5	37.1	40.8	38.8	41.5
NA	NA	NA	NA	NA	NA	NA	NA	NA	38.6	38.5	39.2	39.3	30.3	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	38.8	35.5	35	35.1	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	42.6	43.2	39.3	39.8	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	46.5	45.3	45.4	44	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	53	52.5	51.5	missing	missing
NA	NA	NA	NA	NA	NA	NA	NA	NA	27.9	24.5	24.4	23.9	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42.6	34.7	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	52	43.7	40.8	47.9	44.1	39.8
NA	NA	NA	NA	NA	NA	NA	NA	43.3	43.1	36	36	36.7	37.1	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	37.2	36	34.6	31.3	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41.5	NA	45.6	41.4	46.5
NA	NA	NA	NA	NA	NA	NA	NA	NA	40.6	38.7	35.5	37.2	36.6	34.5
NA	NA	NA	NA	NA	NA	NA	NA	NA	42.9	40.3	42.7	42.2	41.5	36.8
NA	NA	NA	NA	NA	NA	NA	NA	NA	48.3	54.7	53.6	48	missing	46.9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35.6	34.9	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	46.2	38.9	39.9	37.8	40.2
47	46	54	65	90	112	147	195	NA	50.4	47	48.9	28.6	21.9	22.8
73	75	85	74	62	71	114	137	NA	NA	39.2	37.7	39.1	33.7	23.1
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	37.3	38	36.9	33.7	35.8
NA	NA	NA	NA	NA	NA	NA	NA	36.6	33.2	38.3	42.7	40.1	36.4	33.7
NA	NA	NA	NA	NA	NA	NA	NA	41	44.4	46.2	45.2	45.2	44.6	44.3
NA	NA	NA	NA	NA	NA	NA	NA	NA	46.9	49.3	50.5	40.1	43.2	41.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31.8	missing	missing	45.4	NA
NA	NA	NA	NA	NA	NA	NA	NA	39.3	35.7	35.9	38.4	36.8	37.4	34.9
NA	NA	NA	NA	NA	NA	NA	NA	NA	30.5	32.9	34.1	33.6	34.2	34.6
NA	NA	NA	NA	NA	NA	NA	NA	45.2	43.3	40.6	41.4	45.9	42.1	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	45.8	47.1	46.5	45.3	40.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	42.7	39.3	43.9	46.6	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	41.2	38.2	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41.3	33.7	34	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	35.7	38.2	39.8	40.2	40.7	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	36.1	37.4	35.6	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	45.9	40.4	41.8	40	NA
NA	NA	NA	NA	NA	NA	NA	NA	36.9	36.9	39.7	39.5	36.4	40.2	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	33.3	31.3	27.3	26.2	25.8	24.4
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	49.2	44.7	40.3	40.3	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	52.2	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	46.7	44.2	45.5	41.8	41.7	42.6	41.2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50.6	24.7	43.4	36.6	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	49.3	41.3	40.5	43.4	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43.2	43.9	38.1	34.4	31.8
NA	NA	NA	NA	NA	NA	NA	NA	NA	39.4	39.5	43.2	42.3	40.4	45.3
NA	NA	NA	NA	NA	NA	NA	NA	48.8	NA	NA	NA	NA	NA	NA
46	80	100	122	139	146	NA	NA	NA	NA	43.3	41.4	46	48.2	45.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	46.7	33.5	26.5	24.7	27.7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	44.3	45.8	34.7	30.8	28.2

Master Data Set

HCT8	HCT9	HCT10	HCT11	HCT12	HCT13	HCT14	HCT15	HCT16	HCT17	ALT1	ALT2	ALT3	ALT4	ALT5	ALT6	ALT7	ALT8
39	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	13	25	31	32	38
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18	28	30	38	35	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42	38	99	211	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	84	70	74	72	81	83	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	90	84	75	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42	55	1078	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	167	359	222	97	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	32	NA	NA	NA	NA	67	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	17	16	35	NA	NA
36.2	34.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	100	NA	187	NA	222	238	182
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	55	56	46	70	NA
41.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12	8	10	10	14	16	21
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41	67	160	155	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43	50	52	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	15	14	17	17	22	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42	42	48	56	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	26	25	NA	NA	NA
47.1	missing	missing	46.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	23	19	16	NA	22	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	72	74	67	73	88	106	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	72	56	65	212	219	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	14	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	103	128	101	102	129	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18	23	32	42	34	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	66	97	157	142	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	NA	32	NA	NA	81	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	25	50	52	59	42	27	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	19	32	37	43	42	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	36	29	NA	29	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	211	221	216	148	132	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	27	26	45	83	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	40	11	62	NA	
29.9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	38	36	45	151	147	NA	110	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31	26	49	73	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	64	69	63	82	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	75	79	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	84	77	114	97	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	158	327	NA	NA	NA	NA	
37.7	39.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	148	131	108	171	169	139	183	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	29	32	24	27	32	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	63	68	67	78	NA	NA	NA	
44.2	45.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	22	23	35	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	18	17	36	48	48	45	NA	
36.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42	33	39	61	NA	81	88	
49.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	200	242	missin	209	215	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	12	12	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	36	25	27	36	NA	
25.3	24.2	26.5	24	25.3	25.6	26.5	25.8	25.7	23.8	NA	117	126	861	NA	1391	675	580	
23.9	24.3	27.7	29.7	31.4	30.5	32.8	32.1	33.1	35.2	NA	NA	217	182	576	1772	1531	1431	
34.4	36.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41	NA	62	77	
33.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	37	NA	30	39	45	57	61	53	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28	NA	NA	29	52	74	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43	NA	41	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	13	14	14	20	16	26	NA	NA	
31.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79	73	82	79	69	59	54	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	53	NA	52	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	113	118	120	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	24	26	32	35	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	34	25	28	NA	NA	NA	NA	NA	

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	73	46	44	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31	38	50	52	47	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79	53	41	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	727	443	338	269	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	25	41	67	73	118	102	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1167	946	602	378	288	231	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	89	97	119	183	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1333	1140	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	NA	64	NA	111	95	79	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	52	30	39	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	22	19	23	25	NA	NA	NA	
35.3	41.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	81	70	74	61	54	52	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31	78	192	181	181	191	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
51.6	48.3	41	39.2	36.7	38.3	42.2	43.5	NA	NA	NA	NA	154	182	201	256	190	198	
27.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	132	192	171	185	161	131	
30.9	29.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	775	644	386	279	NA	156	

Master Data Set

ALT9	ALT10	ALT11	ALT12	ALT13	ALT14	ALT15	ALT16	ALT17	AST1	AST2	AST3	AST4	AST5	AST6	AST7	AST8	AST9	AST10	AST11	AST12	AST13
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	37	44	73	77	56	54	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43	72	69	67	59	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	117	81	191	284	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	72	119	120	120	107	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	182	108	86	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	96	195	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	400	166	138	144	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	35	NA	NA	NA	76	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	54	81	NA	NA	NA	NA	NA	NA	NA
179	NA	NA	NA	NA	NA	NA	NA	NA	NA	107	NA	322	NA	300	NA	173	133	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	57	62	61	74	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	34	33	28	30	30	32	38	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	42	64	143	103	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	40	32	31	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	21	27	32	NA	45	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	21	21	23	26	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	35	29	31	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	65	61	52	47	NA	46	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	68	NA	111	113	123	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	66	65	89	254	245	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	35	41	158	166	99	100	141	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	29	44	63	75	52	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	134	147	206	152	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	56	NA	88	NA	116	129	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50	145	111	133	79	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	36	36	53	57	52	44	NA	NA	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	30	NA	31	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	318	358	308	206	151	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	49	48	72	114	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50	50	86	40	101	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	64	87	138	439	259	NA	150	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	71	65	100	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	116	91	104	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	31	NA	83	66	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	116	103	NA	103	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1339	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	73	123	119	249	272	196	244	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	49	23	45	53	53	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79	86	36	78	NA	NA	NA	NA	NA	NA	NA	NA
34	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	155	63	73	79	69	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50	37	62	83	74	61	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	61	57	93	187	NA	137	129	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	180	235	missin	170	172	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	20	21	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	71	104	72	62	66	NA	NA	NA	NA	NA	NA
433	NA	NA	216	169	133	113	96	79	NA	398	NA	331	NA	4880	1290	771	515	NA	NA	NA	138
1752	140	948.6	1181	436	55	56	139	112	NA	NA	323	296	NA	3926	NA	NA	140	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	125	133	104	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	49	NA	51	78	96	86	78	50	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	27	34	NA	NA	NA	80	76	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	63	NA	65	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	20	18	22	50	37	55	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	77	61	99	152	105	84	98	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	43	75	NA	NA	91	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	219	196	191	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	47	63	80	85	NA	NA	NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	30	25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Master Data Set

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	92	72	67	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	69	NA	182	230	133	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	82	58	65	NA	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1289	664	446	299	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	28	36	63	83	135	120	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	10648	6128	2580	1722	1242	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	140	154	199	273	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	795	2163	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	76	NA	191	NA	291	197	144	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	131	75	81	NA	NA	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	65	67	61	NA	NA	NA	NA	NA	NA	NA	NA	
56	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	192	131	140	NA	91	81	88	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	50	134	278	230	216	192	NA	NA	NA	NA	NA	NA	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
315	223	217	NA	209	197	171	NA	NA	NA	NA	131	183	235	344	399	443	479	200	147	NA	129	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	116	226	205	220	158	106	NA	NA	NA	NA	NA	
127	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1844	1822	709	316	NA	107	84	NA	NA	NA	NA	

Master Data Set

AST14	AST15	AST16	AST17	Diagnosis	DIagDaySD	SDCat	SDSubcat	AfebrileDay	Admission
NA	NA	NA	NA	0	NA	NA		7	3
NA	NA	NA	NA	1	3	0	Compensated shock	5	3
NA	NA	NA	NA	0	NA	NA		NA	NA
NA	NA	NA	NA	0	NA	NA		4	3
NA	NA	NA	NA	1	2	0	compensated shock	4	2
NA	NA	NA	NA	0	NA	NA		5	6
NA	NA	NA	NA	1	2	0	decompensated shock	NA	3
NA	NA	NA	NA	0	NA	NA		5	3
NA	NA	NA	NA	0	NA	NA		6	2
NA	NA	NA	NA	0	NA	NA		4	4
NA	NA	NA	NA	0	NA	NA		3	2
NA	NA	NA	NA	0	NA	NA		5	5
NA	NA	NA	NA	0	NA	NA		5	2
NA	NA	NA	NA	0	NA	NA		4	2
NA	NA	NA	NA	0	NA	NA		3	2
NA	NA	NA	NA	0	NA	NA		3	1
NA	NA	NA	NA	0	NA	NA		NA	NA
NA	NA	NA	NA	0	NA	NA		3	3
NA	NA	NA	NA	0	NA	NA		4	NA
NA	NA	NA	NA	0	NA	NA		NA	NA
NA	NA	NA	NA	0	NA	NA		3	3
NA	NA	NA	NA	0	NA	NA		4	NA
NA	NA	NA	NA	0	NA	NA		3	2
NA	NA	NA	NA	0	NA	NA		4	1
NA	NA	NA	NA	0	NA	NA		2	1
NA	NA	NA	NA	0	NA	NA		3	2
NA	NA	NA	NA	0	NA	NA		4	2
NA	NA	NA	NA	0	NA	NA		5	3
NA	NA	NA	NA	0	NA	NA		4	3
NA	NA	NA	NA	0	NA	NA		4	2
NA	NA	NA	NA	0	NA	NA		4	2
NA	NA	NA	NA	0	NA	NA		4	1

Master Data Set

NA	NA	NA	NA		1		3		0	Compensated shock			5	3
NA	NA	NA	NA		0	NA		NA					3	2
NA	NA	NA	NA		0	NA		NA					4	3
NA	NA	NA	NA		0	NA		NA					5	3
NA	NA	NA	NA		0	NA		NA					4	2
NA	NA	NA	NA		0	NA		NA					4	2
NA	NA	NA	NA		0	NA		NA					2	2
NA	NA	NA	NA		0	NA		NA					4	3
NA	NA	NA	NA		0	NA		NA					3	5
NA	NA	NA	NA		0	NA		NA					4	3
NA	NA	NA	NA		1		3		0	DSS	NA			3
NA	NA	NA	NA		0	NA		NA					5	3
NA	NA	NA	NA		1		2		0	Compensated shock			4	2
NA	NA	NA	NA		0	NA		NA					3	2
NA	NA	NA	NA		0	NA		NA					4	5
NA	NA	NA	NA		0	NA		NA					5	2
NA	NA	NA	NA		0	NA		NA					6	2
NA	NA	NA	NA		0	NA		NA					6	4
NA	NA	NA	NA		0	NA		NA					3	2
NA	NA	NA	NA		1		3		0	compensated shock			4	3
96	81	67	62		1		3		0	fluid accumulation with			2	2
NA	NA	263	NA		1		4	0,2		Resp Distress(Ascites,Pl			4	3
NA	NA	NA	NA		1		5		0	Decompensated shock			5	5
NA	NA	NA	NA		1		1		0	Compensated shock			5	1
NA	NA	NA	NA		1		1		0	Compensated shock&m			3	1
NA	NA	NA	NA		0	NA		NA					4	4
NA	NA	NA	NA		0	NA		NA					3	NA
NA	NA	NA	NA		0	NA		NA					3	2
NA	NA	NA	NA		1		1		0	Compensated shock			5	1
NA	NA	NA	NA		0	NA		NA					4	1
NA	NA	NA	NA		1		5		0	Compensated shock			6	4
NA	NA	NA	NA		0	NA		NA					3	2
NA	NA	NA	NA		1		2		0	Compensated shock			2	1

Master Data Set

NA	NA	NA	NA	1	3	0	Compensated shock		3	3	
NA	NA	NA	NA	0	NA	NA			5	1	
NA	NA	NA	NA	1	2	0	compensated shock	missing		2	
NA	NA	NA	NA	1	3	0,2	fluid accumulation with		4	3	
NA	NA	NA	NA	1	1	0	decompensated shock+		4	1	
NA	NA	NA	NA	1	2	0,1,2	ALL 3 categories		2	2	
NA	NA	NA	NA	1	3	NA	NA		3	3	
NA	NA	NA	NA	1	2	0,1,2	severeplasmaleak, seve		3	2	
NA	NA	NA	NA	1	1	0	fluid accumulation with		3	1	
NA	NA	NA	NA	1	3	0	Compensated shock		3	3	
NA	NA	NA	NA	1	2	0	compensated shock		2	2	
NA	NA	NA	NA	1	3	0	Decompensated shock,		3	3	
NA	NA	NA	NA	0	NA	NA			4	2	
NA	NA	NA	NA	0	NA	NA		NA	NA		
111	95	NA	NA	1	5	2	Encephalopathy		7	3	
NA	NA	NA	NA	1	5	0,1	compensated shock, ble		5	3	
NA	NA	NA	NA	1	3	0	compensated shock and		3	3	

Master Data Set

Co-morbids	Death	Transfusio	DialysisDa	Others
				no plain tube(both)
				Last day no blood (D7)
				IgM positive at day 4 No last tube IgM positive IgM positive at day 5 fever spike at D4 due to CAP
				Antibiotic , Tonsilits at D6, thrombophlebitis D8
				No last tube IgM positive no plain tube on last day only one day, no further follow up
				insufficient sample to confirm dengue status Only follow up for one day IgM negative
				No last plain tube D3 treated as Lepto, increase CK HPT, hypothyroid, gastritis No plain tube on last day
				thrombophlebitis D5

Master Data Set

				no plain tu Bidayuh
Anemia				
		5		Subsequent pleural effusion, pericardial effusion, hepat
				no plain tu Lepto and dengue IgM +
				CCU D2 aft treated for Metabolic acidosis, resp distress
				No plain tube early
				No last blood both EDTA and plain
		6,7,32		Iban
HPT		18 7,8,9	15	
				Thrombop No blood at D7
			IgM negati	Thrombophlebitis D5
				Thrombophlebitis D6

Master Data Set

Diabetes
Philipine
Bangladeshi
Iraqi

7 3

3

DM and HPT

4

insufficien only one day, no further follow up

9

5,6

4

Master Data Set

Master Data Set

titis/failure, myocarditis, ascites.

(COPD?)

Commercial and Optimised ELISA comparison Data Set

PTXCom	PTXOptimi	VEGFCom	VEGFOptimized
0	1	1370	1314.4
6	19	209	587
8	10	342	112
6	5	1500	1897
6	2	227	210
5	4	607	1800
14	13	430	698
18	20	765	1500
17	16	1042	1671
6	2	427	789

Data for constructing figures

PTX	PTXDay	VEGF	VEGFDay	DIAGPTX	DIAGVEGF
1.678715	1	22	1	1	1
9.446281	1	89.16667	1	1	1
2.54709	1	81.89286	1	1	1
4.393574	2	70.5	2	1	1
3.341365	2	15.75	2	1	1
4.342466	2	15	2	1	1
11.5	2	34	2	1	1
15.61983	2	30	2	1	1
6.281091	2	238.8079	2	1	1
8.367133	2	45.5	2	1	1
3.111888	2	406	2	1	1
7.473684	2	55.55556	2	1	1
0	2	278	2	1	1
0.33105	2	0	2	1	1
6.843373	3	22.75	3	1	1
5.421687	3	21.125	3	1	1
6.237443	3	16.66667	3	1	1
9.681818	3	38.75	3	1	1
7.26033	3	41.66667	3	1	1
6.895065	3	887.7474	3	1	1
10.83916	3	76	3	1	1
5.968531	3	38.5	3	1	1
4.381579	3	0	3	1	1
1.214912	3	94.44445	3	1	1
9.728071	3	0	3	1	1
0	3	207.6667	3	1	1
1.833333	3	55.55556	3	1	1
0.821285	3	0	3	1	1
0	3	199.8333	3	1	1
4	3	447.1667	3	1	1
0	3	158.5	3	1	1
2.973684	3	201.6667	3	1	1
9.384615	3	172	3	1	1
3.762557	3	20.55556	3	1	1
11.48252	3	57.5	3	1	1
7.899122	3	0	3	1	1
10.11855	3	0	3	1	1
0.181452	3	37.5	3	1	1
4.356574	3	0	3	1	1
14.30702	3	0	3	1	1
2.216868	4	47.75	4	1	1
4.491968	4	28	4	1	1
1.584475	4	11.11111	4	1	1
9.968531	4	37	4	1	1
6.504132	4	30	4	1	1
11.70196	4	64.90323	4	1	1
0.662281	4	85.55556	4	1	1
0	4	0	4	1	1
3.513699	4	261.6666	4	1	1
1.096386	4	709.4444	4	1	1
0	4	0	4	1	1
3.616667	4	181	4	1	1
0	4	335.1667	4	1	1
7.895105	4	159.5	4	1	1
6.915525	4	60	4	1	1
8.972028	4	133.5	4	1	1
10.5	4	20.55556	4	1	1
9.092533	4	32	4	1	1
2.165289	4	0	4	1	1
8.210526	4	0	4	1	1
2.901826	5	168.3333	4	1	1
2.511416	5	1.111111	4	1	1
3.479452	5	43.88889	5	1	1
20.57343	5	75	5	1	1
5.194215	5	6.666667	5	1	1
11.53696	5	83	5	1	1
1.649123	5	3.333331	5	1	1

PTX	PTXDay	VEGF	VEGFDay	DIAGPTX	DIAGVEGF
5.016807	3	0	3	0	0
6.555555	3	16	3	0	0
0.335341	3	0	3	0	0
16.58411	3	0	3	0	0
2.956962	3	0	3	0	0
9.193622	3	25.25	3	0	0
5.609813	3	0	3	0	0
5.379032	3	27.25	3	0	0
5.726591	3	15.5	3	0	0
3.816961	3	0	3	0	0
0.936975	3	17.5	3	0	0
9.067113	3	97.66667	3	0	0
0.854271	3	0	3	0	0
3.265416	3	0	3	0	0
0.934152	3	0	3	0	0
1.751256	3	0	3	0	0
1.745968	3	0	3	0	0
6	3	0	3	0	0
7.433229	3	9.749998	3	0	0
1.554439	3	0	3	0	0
4.810887	3	0	3	0	0
1.159371	3	8.333333	3	0	0
12.26347	3	0	3	0	0
6.927771	3	0	3	0	0
0.415094	3	0	3	0	0
4.907916	3	0	3	0	0
0	3	0	3	0	0
6.079545	3	7.07143	3	0	0
5.181919	3	445.4	3	0	0
4.648438	3	0	3	0	0
4.226968	3	0	3	0	0
0	3	0	3	0	0
0.056604	3	25	3	0	0
11.87879	3	13.25	3	0	0
11.4697	3	0	3	0	0
1.015936	3	0	3	0	0
7.866935	3	221.1	3	0	0
3.78629	3	41	3	0	0
7.241935	3	41.75	3	0	0
3.08871	3	0	3	0	0
1.971887	3	5.333333	3	0	0
4.170683	3	0	3	0	0
0	3	61	3	0	0
8.0642	3	22	3	0	0
0	3	0	3	0	0
4.984064	3	5.59E-08	3	0	0
0.16129	11	432.5	3	0	0
13.96262	4	0	3	0	0
7.852803	4	0	3	0	0
5.893939	4	221	3	0	0
1.732244	4	142.5	4	0	0
0.267263	4	23	4	0	0
7.345523	4	17.75	4	0	0
5.306397	4	0	4	0	0
35.56075	4	0	4	0	0
1.767722	4	0	4	0	0
2.970387	4	21	4	0	0
20.27103	4	154.5	4	0	0
4.137608	4	0	4	0	0
2.540802	4	0	4	0	0
4.67648	4	128.5	4	0	0
4.004019	4	0	4	0	0
2.600503	4	0	4	0	0
1.002681	4	0	4	0	0
2.368304	4	0	4	0	0
1.596315	4	0	4	0	0
3.294355	4	0	4	0	0

Data for constructing figures

0	5	98.47046	5	1	1	7.370968	4	24.75	4	0	0
2.114458	5	83.88889	5	1	1	1.271237	4	0	4	0	0
11.05263	5	0	5	1	1	8.836493	4	0	4	0	0
0.683333	5	209.3333	5	1	1	5.657688	4	37.33333	4	0	0
0	5	11.125	5	1	1	7.789539	4	0	4	0	0
7.328947	5	54.44444	5	1	1	11.86078	4	0	4	0	0
1.311189	5	232.8333	5	1	1	56.20958	4	0	4	0	0
2.246575	5	166.6667	5	1	1	1.667722	4	70.57143	4	0	0
6.664336	5	127.7778	5	1	1	4.449605	4	0	4	0	0
0	5	89.25	5	1	1	2.159446	4	75.33334	4	0	0
3.016414	5	135.5556	5	1	1	2.128551	4	0	4	0	0
1.632232	5	49.75	5	1	1	4.415179	4	0	4	0	0
4.657258	5	360.3333	5	1	1	2.81808	4	0	4	0	0
8.842629	5	49.25	5	1	1	18.82464	4	0	4	0	0
7.192982	5	185	5	1	1	3.734597	4	2.249999	4	0	0
4.356164	6	50.66667	5	1	1	1.826582	4	0	4	0	0
5.196347	6	0	5	1	1	7.191235	4	55	4	0	0
3.26484	6	0	5	1	1	0	4	66	4	0	0
5.702479	6	54.44444	6	1	1	7.923238	4	0	4	0	0
3.724398	6	83.33333	6	1	1	2.034161	4	0	4	0	0
4.342105	6	10	6	1	1	1.122396	5	453.6667	4	0	0
0	6	0	6	1	1	7.915888	5	0	4	0	0
5.824562	6	103.5415	6	1	1	14.90438	5	0	4	0	0
2.052448	6	59.44444	6	1	1	2.149015	5	0	5	0	0
1.157534	6	220	6	1	1	8.19823	5	23	5	0	0
0	6	135	6	1	1	9.378486	5	0	5	0	0
3.843434	6	109.5	6	1	1	0.769076	5	0	5	0	0
2.764463	6	76.11111	6	1	1	3.494792	5	95	5	0	0
3.637097	6	252	6	1	1	3.27057	9	0	5	0	0
36.3064	6	16	6	1	1	4.248016	5	24	5	0	0
10.52193	6	220	6	1	1	3.943122	5	0	5	0	0
3.604418	7	24.33333	6	1	1	1.603201	5	0	5	0	0
8.701754	7	56	6	1	1	7.001204	5	0	5	0	0
3.25	7	0	6	1	1	2.028234	5	31	5	0	0
2.996503	7	22.75	7	1	1	6.979296	5	0	5	0	0
4.200758	7	699.4444	7	1	1	2.177455	5	0	5	0	0
1.376033	7	111.1111	7	1	1	1.886097	5	0	5	0	0
5.137097	7	89	7	1	1	4.03629	5	0	5	0	0
6.79798	7	9.499999	7	1	1	7.701613	5	8.750005	5	0	0
7.723684	7	94.16667	7	1	1	3.95709	5	20	5	0	0
3.621005	8	26.83333	7	1	1	7.018519	5	0	5	0	0
7.684932	8	19.5	7	1	1	9.510585	5	16.33333	5	0	0
0	8	0	7	1	1	9.815868	5	21.66667	5	0	0
2.890351	8	107.2222	8	1	1	49.85629	5	0	5	0	0
4.583916	8	185.5556	8	1	1	0.867722	5	18.33334	5	0	0
1.69697	8	113	8	1	1	5.458643	5	41.14286	5	0	0
1.342975	8	0	8	1	1	1.486151	5	0	5	0	0
7.153509	8	117	8	1	1	7.304688	5	81.33333	5	0	0
3.837719	9	0	8	1	1	2.68192	5	0	5	0	0
9.706294	9	90.83333	8	1	1	4.59596	9	0	5	0	0
6.206434	9	0	8	1	1	20.34123	5	0	5	0	0
2.85124	9	99.44445	9	1	1	3.113924	5	1.870024	5	0	0
3.710526	9	119.5	9	1	1	8.681452	5	0	5	0	0
4.421053	10	0	9	1	1	4.133065	5	10	5	0	0
7.84965	10	217.5	9	1	1	5.959677	5	0	5	0	0
7.938338	10	0	9	1	1	0	5	25.66667	5	0	0
3.413223	10	45.55555	10	1	1	6.109213	5	216	5	0	0
4.460526	10	92.75	10	1	1	3.955608	6	0	5	0	0
2.925438	11	83.5	10	1	1	3.241036	6	18.75	6	0	0
12.43007	11	171.6667	10	1	1	1.501167	6	0	6	0	0
5.505176	11	0	10	1	1	1.170905	6	0	6	0	0
1.586777	11	35	11	1	1	7.099327	6	0	6	0	0
6.087719	11	125.25	11	1	1	1.341365	6	32.25	6	0	0
4.486842	12	79.375	11	1	1	4.952381	6	0	6	0	0
7.031469	12	188.3333	11	1	1	1.493117	6	30.6875	6	0	0
5.677019	12	7.777777	11	1	1	3.230514	6	4.249998	6	0	0
2.157025	12	139.4444	12	1	1	3.588065	6	0	6	0	0
0	13	119.75	12	1	1	0.883929	6	7.666667	6	0	0

Data for constructing figures

4.444056	13	368	12	1	1
4.725673	13	249.1667	12	1	1
1.429752	13	138.3333	13	1	1
5.118421	13	83.75	13	1	1
0	14	163.125	13	1	1
5.444056	14	79.16666	13	1	1
5.860248	14	3.888889	13	1	1
1.206612	14	57.22222	14	1	1
3.328948	14	149.25	14	1	1
12.40909	15	319.375	14	1	1
3.627877	15	50.83334	14	1	1
3.801653	15	0	14	1	1
3.065789	16	36.66667	15	1	1
26.10744	16	351.6667	15	1	1
3.594629	16	39.00001	15	1	1
3.198347	16	26.66667	15	1	1
2.618421	17	18.88889	16	1	1
12.65289	17	343.3333	16	1	1
5.310742	17	25.5	16	1	1
6.081218	1	13.33333	16	0	1
7.685484	1	27.77778	17	0	1
4.661215	2	390.8333	17	0	1
5.962617	2	379	17	0	1
0	2	26.3421	1	0	0
3.287383	2	81	1	0	0
5.585657	2	15.75	2	0	0
2.018815	2	29.5	2	0	0
2.42437	2	77.4	2	0	0
2.178392	2	14.5	2	0	0
3.300268	2	0	2	0	0
3.634128	2	0	2	0	0
4.860972	2	0	2	0	0
6.564134	2	0	2	0	0
4.266332	2	0	2	0	0
0	2	0	2	0	0
0.792225	2	56.66667	2	0	0
8.294392	3	421.8333	2	0	0
7.615269	3	0	2	0	0
6.326599	3	0	2	0	0
1.920455	3	27.33334	2	0	0
2.379795	3	16.5	3	0	0

5.191943	6	0	6	0	0
4.78629	6	0	6	0	0
3.139508	6	0	6	0	0
5.557239	6	0	6	0	0
3.89726	6	0	6	0	0
7.69012	6	0	6	0	0
0.757595	6	0	6	0	0
4.300508	6	0	6	0	0
2.512074	6	0	6	0	0
2.520329	6	0	6	0	0
6.040284	6	0	6	0	0
16.49004	6	0	6	0	0
5.487903	6	9	7	0	0
6.564516	6	0	7	0	0
5.528226	6	0	7	0	0
5.463855	6	9	7	0	0
2.624498	7	0	6	0	0
5.729839	7	11.5	6	0	0
2.134087	7	0	6	0	0
3.068631	7	0	6	0	0
2.433081	7	3.375	6	0	0
3.643216	7	0	7	0	0
2.939516	7	13.16667	7	0	0
4.01623	7	0	7	0	0
8.066002	7	0	7	0	0
8.302395	7	16.21429	7	0	0
6.018692	7	0	7	0	0
6.719818	7	27.75	7	0	0
3.489655	7	0	7	0	0
7.799056	7	0	7	0	0
2.944223	7	70.01203	7	0	0
5.105578	7	0	7	0	0
4.125	8	0	7	0	0
2.216377	8	0	8	0	0
2.097152	8	0	9	0	0
2.431452	8	0	8	0	0
6.699253	8	15.83333	8	0	0
11.11215	8	0	8	0	0
0.711202	8	20.42857	8	0	0
3.607744	8	35.25	8	0	0
		127.81	8		0
		19.5	8		0
		0	11		0
		19.5	9		0