ADAMTS 13 levels and von Willebrand Factor (vWF) collagen activity in Dengue fever (AVID Study)

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF M.D. GENERAL MEDICINE BRANCH I EXAMINATION OF THE TAMIL NADU DR. M.G.R. UNIVERSITY, CHENNAI TO BE HELD IN APRIL, 2018

CERTIFICATION

This is to certify that the dissertation **"ADAMTS 13 levels and von Willebrand Factor (vWF) collagen activity in Dengue fever (AVID Study)"** is a bonafide work of Dr. Jayastu Senapati carried out under our guidance towards the M.D. Branch I (General Medicine) Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in April, 2018

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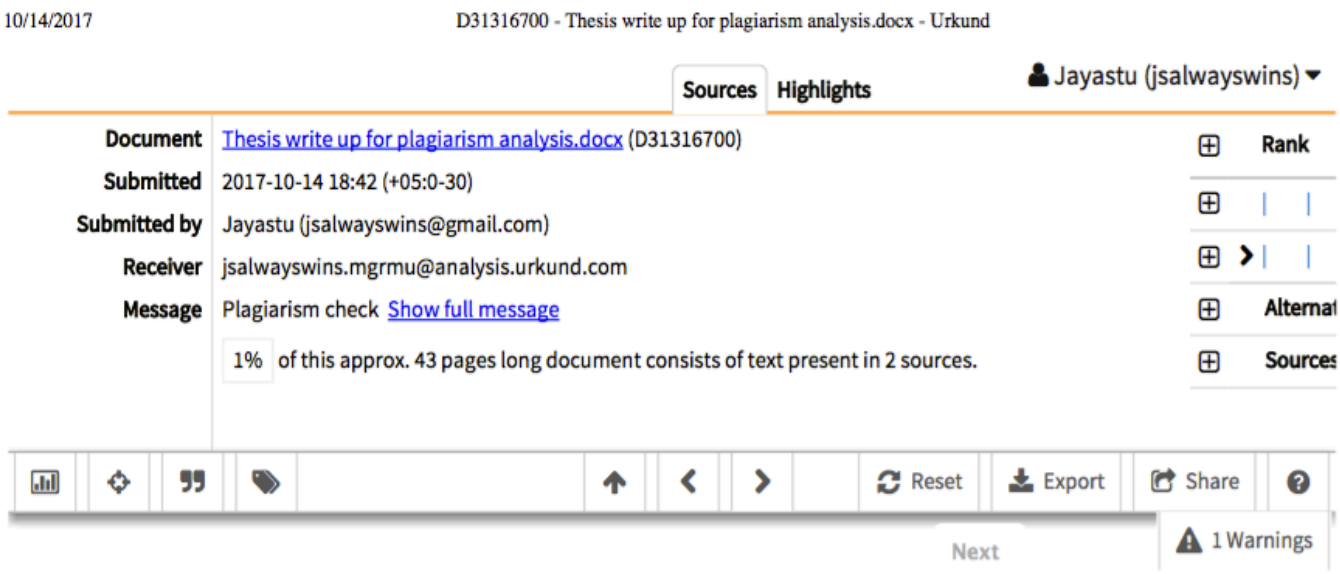


Introduction

Dengue fever is a major health problem in India and the world with a disease burden not matched to healthcare resource allocation, especially in resource poor countries like India. Dengue fever is caused by the bite of Aedes mosquito, which has a propensity to breed near household areas, and has a daytime biting preponderance. All 4 serotypes of Dengue have been reported from India, and it continues to be an ever-increasing healthcare problem. In

ACKNOWLEDGEMENT

I will take this opportunity to express my heartfelt acknowledgement to my guide Dr. Sowmya Sathyendra for her relentless support, constant inspiration and being the backbone for this thesis. Her support extends beyond this thesis and includes my entire residency programme. Any form of gratitude will fall short of her guidance that she had towards me.

I would also like to thank my co guides for the thesis, which includes Dr. J.V.Peter from the Department of Critical Care Medicine, Dr. Biju George from the Department of Clinical Haematology and Dr. Sukesh C. Nair from the Department of Transfusion Medicine and Immunohaematology. I also acknowledge the help of Dr. Thulasi and Mrs. Ramya who helped me with the laboratory work. I express my deepest gratitude to the heads of all Medicine units and to Dr. O.C. Abraham (Head of the Department of Medicine) to allow me to pursue this study and recruit patients from all the Medicine units and ICU. My acknowledgement extends to Dr. Vishalakshi and Mrs. Jyotilakshmi, our statisticians for this study who helped me in data compilation and the analysis. It will be incomplete to finish this without thanking my parents, family, friends who have been by my side and God, whose faith is never-ending.

Jayastu Senapati,

14th October, 2017

Abstract

Objective: To estimate the levels of ADAMTS 13 levels and vWF activity in adults with dengue fever at presentation and compare it to disease severity

Setting and design: This is a prospective observational study conducted in the Departments of Medicine, Medical ICU and Accident and Emergency Medicine at Christian Medical Hospital, Vellore, India. The study recruited participants who presented to the above departments from May 2106 to July 2017

Participants: Consecutive adult patients with acute febrile illness and thrombocytopenia with a platelet count less than 1lac/cu mm were selected. After analysis of inclusion and exclusion factors they were recruited to the study. Sample for ADAMTS 13 and vWF:CBA was collected on day 1 and patients were followed by till death, discharge or convalescence.

Results: A total of 62 patients were recruited over the above mentioned time period. The median age of participants was 22 years with 40 males and 22 females. A total of 15 patients had non-severe dengue with no warning signs, 36 had non-severe dengue with warning signs and 11 patients had severe dengue. We clubbed the latter two severity grades into “more severe dengue” and the former as “less severe dengue” and analysed the data as dichotomous outcomes. ADAMTS 13 levels did not correlate with disease severity according to W.H.O. grading or to SOFA scores. However, higher levels of ADAMTS 13 meant lesser transfusion requirement. On multivariate analysis SOFA score on Day 1 correlated to dengue severity.

TABLE OF CONTENTS

Introduction

Dengue fever is a major health problem in India and the world with a disease burden not matched to healthcare resource allocation, especially in resource poor countries like India. Dengue fever is caused by the bite of Aedes mosquito, which has a propensity to breed near household areas, and has a daytime biting preponderance. All 4 serotypes of Dengue have been reported from India, and it continues to be an ever-increasing healthcare problem. In the absence of a proper vaccine, the population at risk in India is large.

Clinical manifestations of Dengue are varied and can range from asymptomatic illness to a serious life threatening Multi organ dysfunction syndrome (MODS). The WHO in 2009 laid down concrete guidelines for Dengue fever classification and management. However, the basic pathogenesis of the hematological manifestations and organ dysfunction in Dengue fever, that accompanies the severe type remains to be elucidated.

ADAMTS 13 (A disintegrin like metalloproteinase and thrombospondin like activity motif 13) is a serine protease circulating in plasma, and produced mostly by the liver and also from the endothelial cells. One of the main functions of ADAMTS 13 is to cleave ultra large polymers of vWF (ULvWF) in circulation to small monomers, by binding to the A2 site of vWF, and regulating the activity of the latter. ADAMTS 13 deficiency is implicated in conditions like Thrombotic thrombocytopenic purpura, decompensated liver cirrhosis, and anecdotally in a handful of other conditions. It has been hypothesized that alteration of relative levels of ULvWF and ADAMTS 13 levels leads to platelet sequestration in

circulation, micro thrombi formation and organ dysfunction. Our study aims at proving that severe Dengue infections are associated with increased ADAMTS 13 depletion with a subsequent increased vWF activity secondary to increased levels of ULvWF.

Pregnancy is also known to be associated with an altered milieu of coagulation factors and relatively low levels of ADAMTS 13. Data on pregnancy outcomes after dengue infection is contradictory. However the overt severity of dengue infection in pregnancy maybe attributed to the already depleted ADMAMTS 13 levels in pregnancy. However, no studies have compared the levels of ADAMT S13 in dengue infected pregnant individuals to non- pregnant individuals. The present study also aims to do so.

This study thus aims to highlight the role of ADAMTS 13 in the pathogenesis of Dengue related thrombocytopenia and its association to disease severity. This might in the long run help us to formulate models for early assessment of disease severity, management and transfusion strategies in the more severe forms of the disease.

Aim and objectives

Aim:

To estimate the levels of **ADAMTS 13** (*A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member* 13) levels and **vWF activity** in adults with dengue fever at presentation and compare it to disease severity .

Objectives:

1) To assess if the severity of thrombocytopenia at presentation is correlated with ADAMTS-13 level and vWF collagen activity.
2) To assess if severity of illness is correlated with ADAMTS-13 level and vWF activity.
3) To determine if low ADAMTS-13 level and vWF activity are associated with increased mortality.
4) To analyze ADAMTS-13 levels and vWF collagen activity in pregnant individuals with dengue and compare their levels with non-pregnant individuals with dengue.

Review of literature

Dengue epidemiology:

Dengue is a common arboviral infection in the Indian subcontinent and most of South East Asia. It is caused by the bite of either *Aedes aegypti* or *Aedes albopticus* mosquito. The virus belongs to the family *Flaviviridae*, as does the causative agents of Chikunguniya, Japanese encephalitis and Yellow fever to name a few. Four serotypes of Dengue virus (DEN1-4) have been described till date, all of which are widely present in India. The earliest description of dengue goes back to the latter part of the 18th century when it was described as "*backbone fever"* (1). The term " Dengue" was coined in 1828 and thereafter many epidemics from different parts of the world have been reported.

The earliest report of Dengue fever in India dates back to 1946 (2). The first isolation of dengue virus in India was in 1956, shortly after the isolation of the same in Japan in 1944 (1). Subsequent reports had shown the presence of isolated serotypes of dengue virus. In the 1968 epidemic of Dengue, all four serotypes were described (3). This fuelled further epidemiological studies of dengue in India and helped draw the serotypes involved in subsequent epidemics. Over the years there has been significant variation in the prevalence of particular serotypes, patient demographics and the outcomes.

The menace of this arboviral illness is not limited to the tropical climates or the third world countries. The estimated global burden of Dengue is huge and WHO estimates around 50 million dengue infections every year with more than around 1% of them having

signs of disease severity requiring hospitalisation (4). The highest burden of the severe forms of disease are however in the Sub-Saharan Africa and South East Asia. Other than South East Asia, the South American countries are also seeing growing incidence of Dengue fever(5). In the 2017 biweekly report by WHO, an early increased incidence of disease was noted in Philippines and Vietnam, which seemed to be dying down by May 2017(6). Over the years there seem to be growing disease incidence, however this can also be because of more awareness and better reporting, than the actual increase in disease occurrence. In India, there exists significant discrepancy between actual disease incidence and reporting. Despite that, data from National vector borne disease control program has shown progressive increase in both the number of cases and deaths throughout the country over the last 10 years (7). The data quite obviously highlights that states, which have better healthcare framework and networking, have more reported dengue cases and death. This undermines the bulk of disease burden through reporting bias. Case definition of dengue has been standardized which leads to earlier diagnosis and confirmation with more readily available laboratory resources. This helps in better diagnosis at the primary care level, which ideally should translate into better practices and more favorable outcomes.

The epidemiology of dengue is evolving throughout the world and India. A close monitoring and pattern recognition of new cases in the community might help us in preventing major epidemics. As of now dengue remains a preventable burden for a vast majority of people in our country.

Dengue virus and pathogenesis:

Dengue virus belongs to the family *Flaviviridae* and genus *Flavivirus.* It was first isolated in Japan in 1944 (8). In India the virus, DEN 1 serotype, was first isolated in 1956 at Christian Medical College, Vellore (1). It exists in 4 serotypes that provide serotype specific lifelong immunity. There is significant genetic and molecular heterogeneity amongst the serotypes, with only around 65% of shared characteristics at the amino acid level (4,9,10). Within each serotype there are several genotypes that differ at the genetic level by about 3% (9). Structurally the virus has an icosahedral nucleocapsid, which encloses a single stranded RNA genome with the mature virions being around 50 nm in diameter (11). The virus contains three structural proteins and seven non-structural (designated as NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins, which is covered by the lipid envelope. The structural proteins namely, the capsid, pre-membrane/membrane (prM/M) and the envelope protein which provides the icosahedral symmetry(9). The structural proteins are primarily involved in cellular attachment and infection. Cryoelectron microscopy showed the virus to be smooth on the exterior and also elucidates the role of many of the structural proteins in viral pathogenesis.

The environment and transmission: Dengue infection more commonly results in inapparent infection, accounting for over 75% of infected cases, with an estimated around 3 million apparent infections in 2010 (9,12). The huge number of inapparent infections forms the most common natural reservoir of the disease in Nature. Disease transmission depends on host, environment and host factors as with most arboviral illnesses. The more important

factors pertaining to the host include their age, prior dengue infection, temporal difference from prior dengue infection and their immunological memory, presence of other comorbidities etc. (10). Overcrowding and presence of vector breeding grounds close to habitat have consistently shown to promote disease transmission (13). Breakthrough epidemics are often due to viral genomic assortment producing strains with more replicative capabilities in both humans and the vectors (14). Studies have shown that newer virulent strains often replace lesser virulent and replicative strains providing a survival advantage to the virus. Together with waning of population immunity might explain resurgences of dengue at an epidemic scale. This has been postulated to be one of the reasons for increased epidemicity of South Asian dengue compared to South America (14).

The Aedes mosquito dwells near human habitat. Female Aedes are the commoner vectors and become viremic after having a blood meal from a viremic human. Vertical transmission in mosquitoes has been reported, however it is not the common mechanism of viral transmission. Environmental factors like increased humidity, as is experienced in the monsoon and in the months following that is a strong factor in vector propagation (13). Increased diurnal variation of temperature lead to increased vector survival and dissemination of infection. The effects of this vary between the species of *Aedes* mosquito, but on the whole, positively affects disease propagation (15). Different models have been implicated in the interplay between environmental factors, vector, host and the virus that leads to Dengue infection. The following diagram (Fig. 1) depicts that these interplay are intricate and decide both vector propagation and disease transmission in the community that ultimately defines the disease incidence (16) .

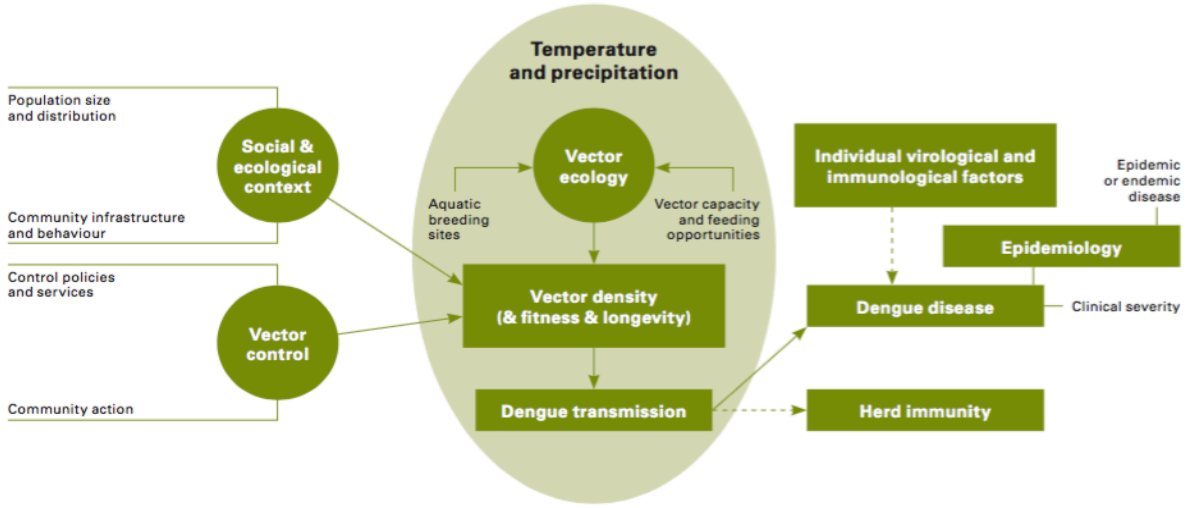


Fig. 1. Interplay of environment, vector and host factors in dengue transmission

Thus dengue infection depends on multiple factors. At each step there can be efforts to abort the passage of the virus downstream ultimately halting human infection.

Viral entry and infection: Transmission of the virus via the vector to a susceptible individual leads to inoculation, which in proper circumstances leads to Dengue infection. This phase starts from the blood meal of the Aedes mosquito during which the virus is inoculated into the bloodstream of the human host, subsequent viral replication and maturation followed by transmission during a subsequent mosquito bite of the newly formed virions to another host.

Post inoculation into the host blood, the dengue virus binds to specific cell surface receptors like mannose receptors, glycosaminoglycans and certain members of the C type lectin family. The primary cell types that are targeted include the dendritic cells, hepatocytes, platelets, endothelial cells, though it can virtually infect any cell of the body. The dengue virus has a lipid bilayer membrane containing the M and E proteins, which are involved

primarily in the initial viral-host interaction (17,18). Once the virus has attached itself to the cellular membrane proteins it is internalized via clathrin mediated endocytosis (9,19) . Once viral entry inside the cell is successful there is fusion of the viral and endosomal membranes and the downstream cascade for generation of viral progeny is set forth.

Viral protein synthesis: The initially formed negative sense viral RNA drives the formation of the positive strand RNA. Multiple organelles of the infected cell are involved in this function, including but not restricted to the Endoplasmic reticulum, Golgi apparatus and the cell membrane. The entire genome is around 10 kb comprising of a positive sense RNA that codes for a total of 10 proteins (3 structural and 7 non-structural, designated as '*NS*') The positive strand viral RNA once formed is translated with the help of the host cellular machinery into a single poly-protein. This undergoes post-translational modification and is spliced into the 3 structural and 7 non-structural proteins, which have been mentioned before. The following diagram adapted from *Aruna et al*, schematically depicts dengue virus, entry, replication and association (19).

The newly formed RNA is assembled into progeny virions, which bud into enveloped immature virions. The newly budding virus can be either mature or immature. The former are infective and contains the processed M protein on the viral surface while the latter immature forms contain the uncleaved form of the protein denoted as 'prM'(9,17,19). Biochemical changes at the time of virus budding renders the immature virions into mature infective particles.

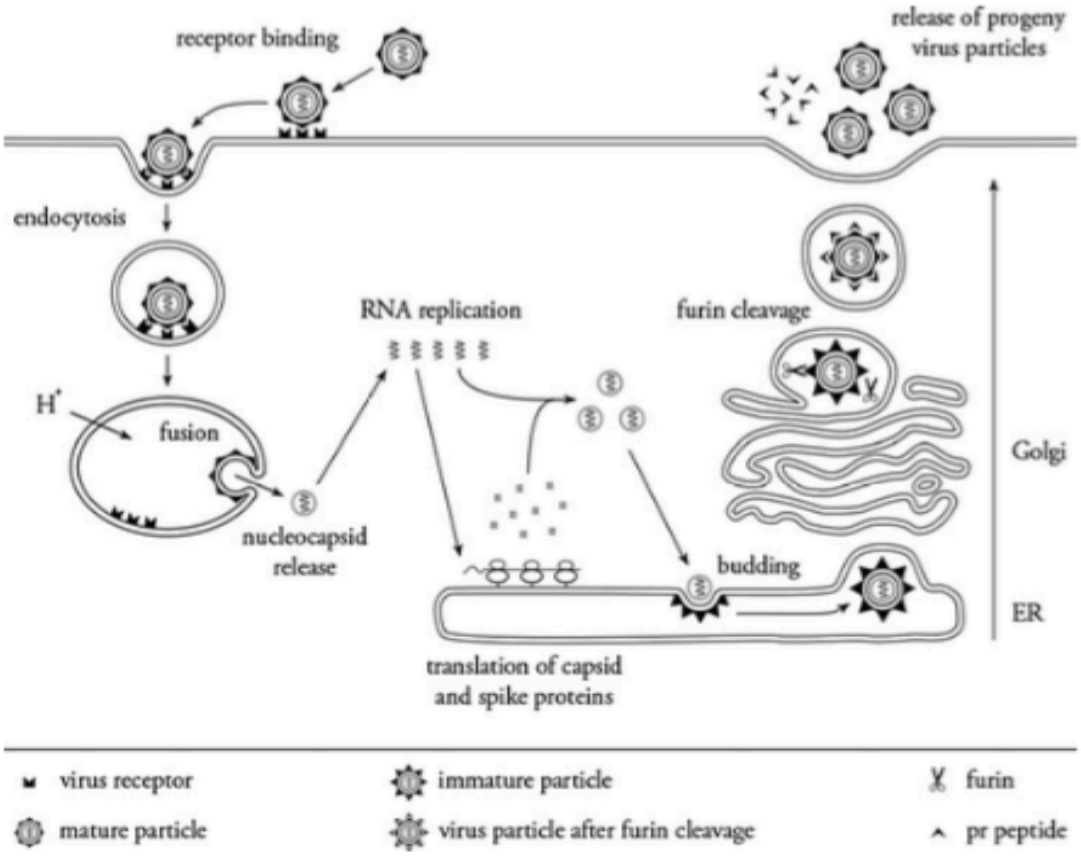


Fig. 2: The infection and replication of dengue virus

The differential confirmation of the E and M proteins provide the virions with the ability to infect different cell types and is a dynamic process (10). The structural proteins participate in viral entry and establishing infection while the NS proteins are involved directly in viral replication and packaging of new virions. The NS1 protein is transported subsequently to the cellular membrane and in the soluble, lipid-associated form is detectable in blood from the very early stages of infection (17). It thus plays an important role in early diagnosis of dengue infection in the community. NS1 has also shown to play an important

role in complement activation, while some of the other NS proteins have a direct association with the viral RNA polymerase, possibly as cofactors.

Immunopathogenesis: The pathogenetic landscape of Dengue virus is varied and includes evading both the innate and active immunity, while having a myriad of direct cytopathic effects. As the infection is set into motion and the virus uses the host cellular machinery to proliferate, the immune system and the virus plays a hand in hand role leading to organ damage.

At the onset the virus bypasses the innate immune system by directly infecting the cells of the innate immune system. These cells, which primarily include the epidermal macrophages (Langerhans cells), keratinocytes and blood monocytes carry the dengue virus to the local lymph nodes. Here more cells of the mononuclear macrophage cells are recruited resulting in viral amplification and propagation of the infection (20). The occurrence of prior infection with dengue determines serotype specific immunity and modulates the immune response. The final outfall depends on the adaptability of the immune response to the current infection and the virulence of the organism. The following factors have been shown to play primary roles in dengue pathogenesis:

a) Host factors:

Host immunity: The immune background plays the most determining role in dengue infection and extent of disease. The involved components of the innate immune system in this include the Toll like receptors (TLRs) and other intracellular sensors (Retinoic acid inducible

gene 1, Melanoma differentiation associated gene 5) that translate to increased production of IFN gamma, the main defense against dengue virus proliferation (21). The initial recognition of Pathogen associated molecular patterns (PAMPs) in the form of viral nucleic acids by the monocyte-macrophage cell lines form the initial defense to infection. The virus has multiple evasion techniques from the above surveillance, including proteases (NS2B/3) that down regulates the interferon response, to altering protein structure abetting cellular stress signals. In a study on 97 children with Dengue infection, *Singla et al* showed that while the severity of dengue infection did not correlate with the viral load, lower interferon responses did (22). The virus is astute at bypassing multiple innate immunological checkpoints and progresses through the infection, while at the same time offsetting the adaptive immune response into a flaw.

Post infection with any viral serotype, homotypic immunity is generated that last for a significant duration, while a short-lived immunity is generated against the other serotypes. The specificity of this protection evolves after a short time post infection, however it gives for over an year some amount of protection against severe diseases by other serotypes (17). The antibodies are directed against the viral structural proteins and whilst they are important for homotypic immunity, they drive the pathology in heterotypic infection (23). Most of these antibodies in circulation are non-neutralizing and accelerate viral entry into cells as well as impede interferon production in the absence of blocking neutralizing antibodies (24,25). This mechanism known as *Antibody Dependent Enhancement* (has been implicated behind the more severe forms of Dengue like *Dengue Hemorrhagic fever (DHF) and Dengue shock syndrome (DSS)* (21). This underlies the biology behind increased severity of delayed

heterotypic infection. Figure 3, adapted from *Whitehead et al* schematically depicts the mechanism of ADE. The ability of non neutralizing antibodies in aiding the virus to infect cells expressing the IgG FCγ receptors have been widely studied and have shown to increase viral infectivity, output and immune dysregulation (26). ADE leads to increased viral load while also up regulating pathways that culminate into more tissue damage and disease severity.

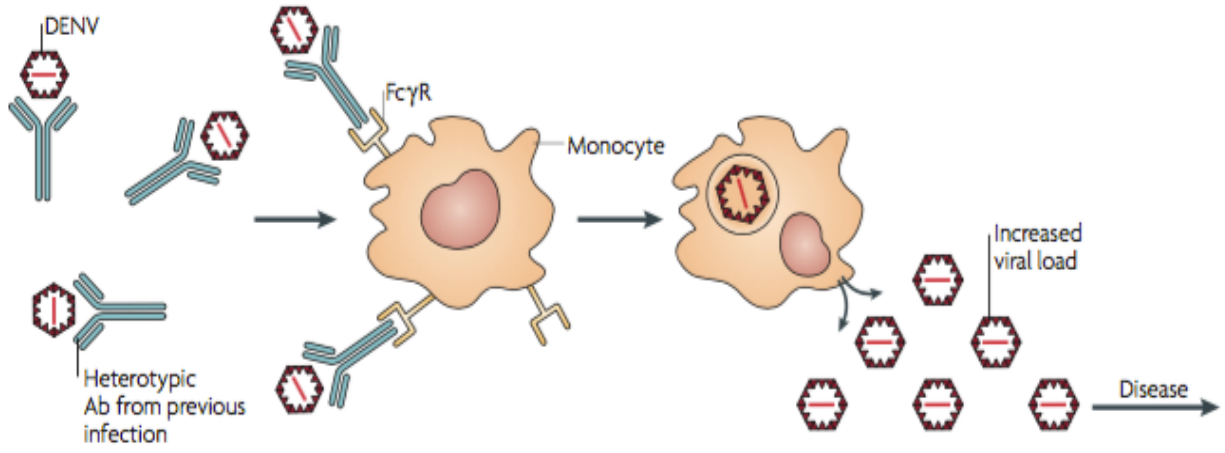


Fig. 3: Heterotypic antibody mediated enhancement of viral transformation and disease severity

Other mechanisms that have been implicated include direct complement activation, transient autoantibody generation to antigens like plasminogen, and deregulated auto-reactive T cell response, though these do not appear to be the primary pathologies (10,20). Thus host immunity plays an important role in pathogenesis of dengue infection and is strongly guided by any prior dengue/Flavivirus infection.

Host demography and genetics: The role of genetics in modulating dengue infection is less well known. Certain HLA types have shown increased preponderance to more severe disease (17,27). DC-SIGN a C-type lectin is used by all dengue serotypes to infect dendritic cells

(28). Polymorphisms of the same have been shown to predict increased dengue severity in one study in India. Given the homogeneity of its role across all dengue serotype infections, antibodies against it is being studied as a novel target for dengue vaccines (29). Age is a definite risk factor in dengue severity with both the children and elderly doing poorly. Children have increased vascular permeability following dengue infection leading to increased prevalence of DHF and DSS. In the elderly the presence of other comorbidities and poor organ reserves leads to early de-compensation and increased disease.

Pregnancy and dengue: Pregnancy is an added risk factor in dengue severity. The biological plausibility lies in pregnancy being a state of increased blood volume with decreased hemo-concentration and alteration in the milieu of coagulation factors and increased capillary permeability. *Machado et al in* a retrospective study from Rio de Janerio, Brazil compared severity of Dengue infections between pregnant and non-pregnant individuals (30). Amongst a total of 151064 cases of dengue infection over 2 years (Jan 2007-Dec 2008), they had 561 female patients in the age group 15-49 years (considered reproductive age group in this study) of which 99 patients were pregnant. Multivariate analysis showed pregnancy to be a risk factor for dengue severity with an Odds ratio of 3.38 (95% CI: 2.1-5.42). *Tan et al* in a prospective study from Malaysia showed that pregnant women who presented with miscarriage (upto 22 weeks) tested more commonly positive for dengue (Dengue specific IgM or NS1Ag) when compared to controls by an adjusted OR of 4.2 (95% CI 1.2-14), though the absolute difference was not large (31). In a retrospective cohort from Western French Guana, *Friedman et al*, showed that incidence of pre-term birth and low birth weight infants were modestly increased in mothers who had dengue infection during pregnancy (32).

A prospective study from South India looking at 73 pregnant women with Dengue fever over an 18 month period showed 4% of them to have fetal loss, 22% premature delivery with dengue in very early or late gestation having a poorer feto-maternal outcome (33). Pregnancy thus poses a heightened risk and early diagnosis with adequate management is important for both the mother and the fetus.

b) Viral factors:

The role of different viral serotypes and further subtypes of the same serotype attributing to disease severity has been widely studied. The virulence of these different serotypes range from their ability to infect mosquitoes establish human infection, infect human dendritic cells to modulating the immune response and direct cytopathic effect. *Cologna et al* developed laboratory dengue models and showed the DENV 2 South Asian genotype had increased ability to infect Aedes aegypti mosquito, human dendritic cells and viral output compared to the American genotype (14). Certain studies have shown the order of infections by different serotypes to be important while others have shown the time gap between primary and secondary infection to play an important role in disease severity. The role of the viral factors in disease severity is seldom in isolation and includes interplay between host immunity, demographics, and environment with the former. However the penetrance of a new viral serotype in a population naïve to it assumes significance and is probably the most important factor determining its virulence.

Pathogenic landscape: We have mentioned the role of the host, environment and immunity in establishing infection. The subsequent pathogenic processes that occur downstream

subsequently lead to organ injury and disease. The pathways involved are many and many approaches have been used to elucidate it. *Martina et al* in a seminal paper used an integrated approach to dengue infection and disease. The initial viral entry is associated with immune activation and dysregulation, viral multiplication in cells of the mononuclear and reticulo-endothelial system, subsequent cytokine storm leading to increased vascular permeability and endothelial cell dysfunction (20). There is direct virus mediated damage to hepatic cells, coagulopathy secondary to disruption of the intrinsic plasminogen-plasmin system as well as a consumptive thrombocytopenia. The effect of the above pathologies lead to the following:

a) Increased vascular permeability with capillary leak
b) Hepatotoxicity
c) Coagulopathy
d) Thrombocytopenia

Though the above list is in no way exhaustive, it forms the bedrock of the culminating effects of dengue infection in humans. Several mechanism have been described above that can explain the above pathologies, however most work in unison and becomes more prominent in the more severe forms of the disease. Fig. 4 depict a schematic diagram tries to put together the different pathways implicated in the disease process. Organ pathology is global and includes the central nervous system, lungs, liver, GI tract, hematopoietic system and most importantly the cardiovascular system. The exact mechanism involved is difficult to delineate but the final end point lies in diffuse endothelial cell dysfunction coupled with an abnormal coagulation response.

Dengue induced thrombocytopenia: On the most consistent pathology in dengue infection is thrombocytopenia. It is as much an effect of the infection as it is a driver of the severe forms of infection, like DHF. Multiple mechanisms working in unison have been described in the pathogenesis of thrombocytopenia in dengue. Some of the possible mechanisms are:

a) Transient bone marrow suppression:

- Direct cytotoxicity by the virus on megakaryocytes
- Cytokines preventing maturation of megakaryocytes to platelets
- Antibodies directed against megakaryocytes
- Macrophage activation syndrome

b) Increased peripheral destruction: Immune mediated; Apoptosis

c) Consumptive thrombocytopenia

- Akin to disseminated micro-thrombi and platelet sequestration as in TMA

Dengue virus is known to directly infect platelets in circulation as well as their precursors in the bone marrow. The virus gains entry into cells via various receptors like the TLRs, DC-SIGNs and causes platelet activation (34). *Ojha et al* showed that dengue virus infection of platelets led to platelet activation and a linear relationship between the viral load and the level of activation leading to platelet micro particle, clot formation and a form of consumptive thrombocytopenia (35). They also showed that virus infected platelets were more readily phagocytosed by the monocytes. Thus multiple mechanisms are at play even when direct virus related cytotoxicity is considered. Anti platelet antibodies are also present in dengue infection and mediate opsonisation followed by complement-mediated lysis. IgM antibodies against platelets in dengue have been shown to prevent aggregation and promote

lysis (36). These antibodies have been also shown to be higher in the severe forms of dengue fever (DHF/DSS) compare to the non-severe forms. A lot of these autoantibodies generated by molecular mimicry between the dengue virus and coagulation factors function like de-novo anti-thrombin antibodies and promote fibrinolysis as well as propagate thrombocytopenia (37).

Thrombocytopenia alone is not the only platelet anomaly in dengue as it is usually accompanied by platelet dysfunction. Multiple studies have shown decreased platelet activation and aggregation in dengue to adequate stimuli, some mechanisms of which have been described above (38). Platelets play a vital role in viral propagation and endothelial dysfunction as well thereby acting as effectors in dengue pathogenesis as well. While no unified concept can be drawn several factors working in consort determine the level of platelet dysfunction and the severity of dengue.

ADAMTS 13 and Dengue: As new concepts get drawn to understand better the pathogenetic mechanisms in dengue, it becomes clearer that the virus has myriad effects and works through several pathways, with some more abrogated than the others.

ADAMTS 13 (A *Disintegrin And Metalloproteinase with Thrombospondin type 1 repeats*) is a zinc metalloproteinase that which is primarily produced by the stellate cells of the liver and in to a lesser extent by the endothelial cells and platelets (39,40). It belongs to the family of ADAM proteins, that function as zinc serine protease and has been implicated in multiple physiological processes like neural cell development and migration, fertilization as well as in pathologies like malignancy, asthma and cardiac hypertrophy (41,42).

ADAMTS 13 is secreted as a constitutively active enzyme into the plasma and its main function is to cleave ultra-large polymers of von Willebrand factors (ULvWF) into smaller lesser potent vWF structures (43). vWF is synthesized and secreted in large polymeric forms from the Webel Palade bodies of endothelial cells and platelets and have an important role in hemostasis, by acting as a physiological sensor in vessel injury and promoting hemostasis by platelet adhesion to the site of injury as well as binding to other matrix proteins (44). The thin line between hemostasis and thrombosis entails that the function of vWF needs to be regulated. The ULvWF have a higher hemostatic potential (often thrombogenic) with it correlating directly to the length and thickness of the polymers (45). This delicate balance is maintained by two mechanisms:

1) The tertiary structure of the vWF protein

- This reduces the accessibility of the protein to the intact vasculature and prevents its adhesion to the vasculature, platelet activation and thrombus formation in normal state. In physiology the vWF is present as a globular fold, which in presence of vessel damage undergoes a structural transition secondary to the shear stress, exposing the different binding sites (to platelets and vessel wall) and promoting hemostasis (46). This intricate housekeeping mechanism provides the first line of control on the function of vWF.

2) ADAMTS 13 mediated cleavage of ULvWF:

- As mentioned above, ADAMTS 13 cleaves ULvWF polymers into monomers, thereby reducing their thrombogenic potential to a more balanced hemostatic one. ADAMTS13 binds to the A2 portion of vWF protein, which is properly exposed on unfolding of its globular structure. Though ADAMTS 13 can bind to the globular ULvWF, only on

unfolding of the vWF on exposure to shear stress, does the bond strengthen and further sites become accessible leading to cleavage of the latter (47). The action of ADAMTS 13 on VWF thus occur at the following places (44):

a) ULVWF in circulation
b) Newly secreted vWF polymers from platelets and endothelial cells
c) Unfolded vWF at the site of platelet plug.

The function of ADAMTS 13 in physiology is as important as its role in pathological conditions. Congenital deficiency of ADAMTS 13 is associated with Upshaw-Schulman syndrome, which presents with a congenital form of thrombotic thrombocytopenic purpura (TTP) (48). Acquired deficiency of ADAMTS 13 leads to a the more commoner form of TTP, first described as Moschowitz's disease by *Singer et al* in 1942 (43). Acquired deficiency is seen secondary to the presence of autoantibodies inhibiting ADAMTS 13 function in majority of the cases, while around 10% is secondary to increased antibody mediated clearance of the same (49). TTP is defined by the presence of thrombocytopenia, microangiopathic hemolytic anemia, renal failure and central nervous system disturbances. Most studies have showed ADAMTS 13 deficiency in excess of 90% cases of idiopathic TTP (43). Severe deficiency of ADAMTS 13 is specific for TTP with values < 5% being shown to be discriminatory from other causes of thrombocytopenia (50). Treatment consists of plasma or cryosupernatant infusion, plasma exchange, and ADAMTS13 concentrate infusion, together with immunomodulation.

The role of ADAMTS 13 and dengue has recently come to light. As dengue as a disease is associated with thrombocytopenia with multiorgan dysfunction secondary to many reasons (hypoperfusion, immune dysregulation, micro-thrombi formation), a biological plausibility existed between the pathogenesis of ADAMTS 13 deficiency and dengue fever though not very apparent. Anecdotal reports exist about TTP in dengue, however in one report of a pregnant woman diagnosed with dengue infection related TTP by *Kadhiaravan et al*, ADAMTS 13 activity was normal (51,52). Different viruses (both RNA and DNA) have been shown to cause TTP, but most are rare and have a varied presentation, with a very few being directly attributed to ADAMTS 13 deficiency (53). *Rossi et al* reported a 45-year-old gentleman with diagnosed dengue fever and developed features of thrombotic microangiopathy (TMA) on the 11^{th} day of illness (54). The authors showed the presence of anti-ADAMTS 13 IgG antibodies, which correlated with a lower ADAMTS 13 activity. This was the first report that looked at ADAMTS 13 pathology in dengue related thrombocytopenia and microangiopathy. *Djamiatun et al* in a cohort of 73 patients from Indonesia with dengue (43= Non severe dengue, 30= DHF and DSS) compared the levels of ADAMTS 13, vWF:Ag (as a surrogate marker for vWF activity) amongst other to outcomes (55). ADAMTS 13 was done for a selected group of 15 patients from the subgroup of severe dengue. They found that high vWF:Ag levels were higher and ADAMTS 13 levels were lower in individuals with severe dengue compared with healthy controls, though the levels were not compared for with those having non-severe dengue. Though limited by numbers, its points towards the clinical relevance of ADAMTS 13 and vWF activity in propagating pathogenesis in severe dengue. In another prospective cohort study of 42 children with

dengue (20= Non severe dengue, 23= DHF), *Sosothikul et al* showed that DHF is associated with endothelial activation and injury, an aberrant hemostatic system and decreased levels of ADAMTS 13 when compared with patients with non severe dengue (56). They also showed the presence of abnormal vWF multimers only in those with DHF. These studies throw light on lesser-known mechanisms of thrombocytopenia in dengue and a potential new marker that can be used to assess severity early into the disease. As such if this is considered a major mechanism in pathogenesis of severe dengue, platelet transfusions can prove detrimental, because it might promote disseminate micro-thrombi formation and organ dysfunction (57). *Lee et al* did a non randomised retrospective observational study on 788 patients with dengue infection in Singapore and compared the outcomes between those who received prophylactic platelet transfusion (N= 486) to those who did not (N=302) (58). They showed while individuals who received platelet transfusions had a slower platelet increment than the other group, there was no difference in the incidence of ICU admission or death between the two groups. Large surveys have not shown consistency in indications for platelet transfusion (59). As such the guidelines published by the National Vector borne diseases control Programme (NVBDCP), India in 2008 laid down guidelines for platelet transfusion, which includes (60):

- Prophylactically when platelet count is <10,000/cu mm in the absence of any bleeding
- Major systemic bleeding manifestation, usually together with packed red cells
- Severe coagulopathy with prolonged shock.

Recommendations are still lacking about prophylactic plasma infusions in dengue patients with thrombocytopenia, however as our knowledge of the pathogenesis of severe dengue improves, it might soon dominate transfusion practices.

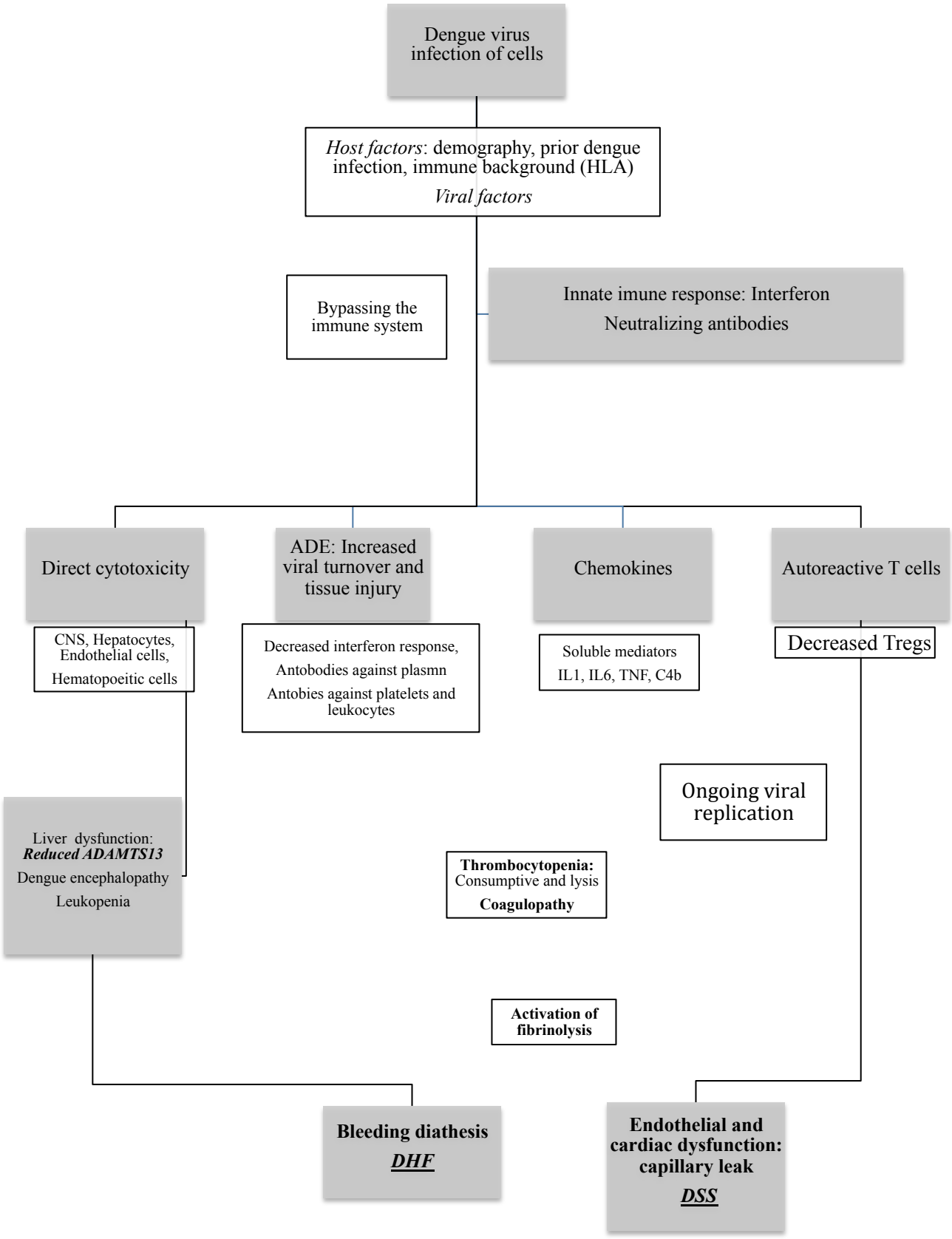


Fig.4: Pathogenic landscape in Dengue infection

Diagnosis and Management: The crux of dengue management lies in early clinical case recognition, risk stratification and initiation of management. As laboratory facilities adept for serological diagnosis of dengue are not widely available in resource poor settings, using the conglomerate of clinical signs and symptoms together with basic blood investigations are prudent in case identification.

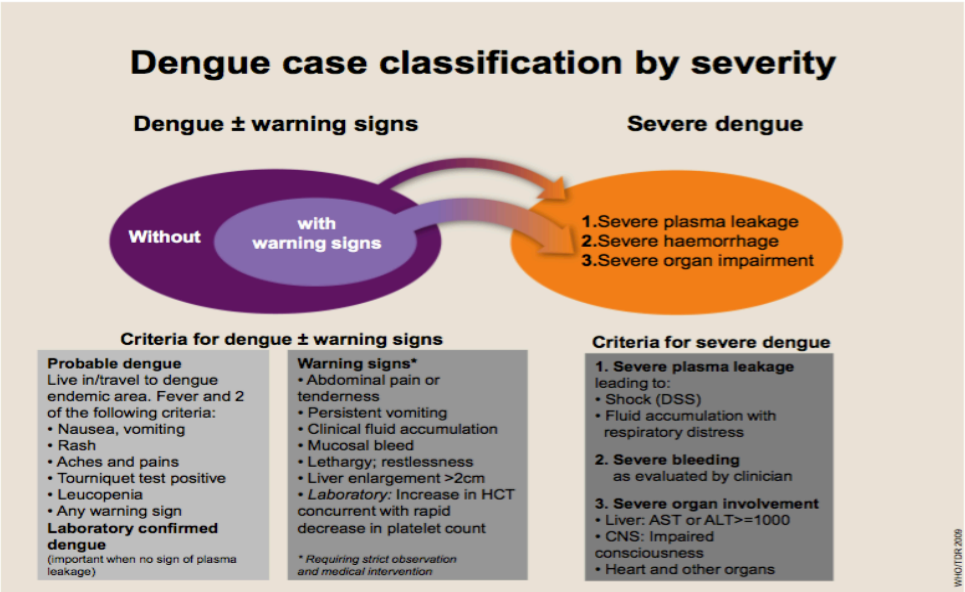
The WHO case definition of dengue includes an acute febrile illness in the correct epidemiological setting, which is then classified based on severity. The WHO guidelines from 2009 and initially replaced the prior terminologies of DHF and DSS and introduced the classification of dengue fever into severe and non severe forms (61). The rationale was to ensure early assessment of clinical severity and initiation of measures to prevent multiorgan dysfunction. Fig. 5 adapted from the WHO 2012 guidelines on dengue management shows the disease classification according to severity (62). The case classification includes a conglomerate of symptoms, clinical examination findings and basic laboratory tests, which are expected to be readily and widely available. Moving past the prior classification of dengue, this helps in an early assessment of disease and setting treatment goals before the onset of severity.

The clinical presentation of dengue fever is of a continuous spectrum that can extend from a short duration flu like illness to a severe condition associated with multi-organ dysfunction. Most patients present with an acute febrile illness, accompanied by headache, myalgia and fatiguability. Clinical diagnosis is thus the bedrock while laboratory evaluation helps assessing the severity of organ dysfunction and confirming the diagnosis.

The initial assessment also should consider other differentials, which in our setting includes the following, but is not limited to;

- Other viral hemorrhagic fevers: Chikunguniya, Hantavirus
- Malaria - Rickettsial infections: Scrub typhus
- Leptospirosis -Viral coryza, Influenza
- Bacterial infections: Community acquired pneumonia, enteric fever

The knowledge of the patient's of comorbidities like diabetes, hypertension, cardiovascular disease, renal diseases are important in guiding treatment, as these conditions can be associated with poorer outcomes (63,64). Dengue infection can present with superadded bacterial infection, thus thorough history and examination is mandatory for a comprehensive management. Most patients present after the febrile period, which is when the manifestations of capillary leak and other severe features become more apparent (61).



ALT = alanine aminotransferase; AST = aspartate aminotransferase; CNS = central nervous system; DSS = dengue shock syndrome; HCT = haematocrit

Fig. 5: The WHO 2009 classification of Dengue fever

The WHO has divided the course of the illness into three phases:

1) ***Febrile phase:*** This phase is associated with high-grade fever associated with myalgia, headache, retro-orbital pain, fatiguability, loss of appetite, flushing and a rash. It mimics undifferentiated viral fever and flu like illness. Some patients may have conjunctival injection, sore throat resembling a coryza. Patients may often not seek medical attention in this phase.
2) ***Critical phase:*** This is the phase of organ dysfunction that starts with increased capillary permeability and all the other pathogenic mechanisms that have been discussed above. Not all patients will have a critical phase, but those who do might manifest the warning signs or progress to severe dengue.

   The onset of the critical phase is usually heralded by defervescence with additional new symptoms, which are attributable to the capillary leak, thrombocytopenia and coagulopathy. With adequate therapy the disease progression can often be halted. However rarely severe disease ensues which is accompanied by multi-organ dysfunction and high mortality. An outline of the symptoms and organ involvement has been outlined in Fig. 5. The major manifestations can be:

a) CNS: Dengue encephalopathy - Altered sensorium, seizures
b) Liver dysfunction: Severe transaminitis jaundice
c) Acute kidney injury: Pre-renal failure, acute tubular necrosis, and interstitial nephritis
d) Bleeding: Coagulopathy, thrombocytopenia
e) Respiratory distress: Pleural effusion, pulmonary edema, ARDS, secondary to myocarditis

A progressive drop in thrombocytopenia usually heralds the critical phase. This is usually accompanied by rising hematocrit, leukopenia, and transaminitis with tender hepatomegaly. All patients who reach this stage require in patient therapy with parenteral hydration, monitoring of cardio-respiratory and bleeding parameters and a close watch for any overt organ failure.

3) ***Recovery phase:*** This phase marks the onset of remedial measures by the immune system towards homeostasis. There is a progressive improvement of platelet and leukocyte counts, couple with improvement in plasma volume and endothelial integrity. However an aftermath of the capillary leak might proceed onto the recovery phase, especially in those who have been aggressively resuscitated leading to persistent respiratory distress. Most patients however have a steady improvement in clinical and laboratory parameters.

Laboratory diagnosis:

The laboratory diagnosis of dengue is based on either indirect evidence of the viral infection through serologies or direct assessment of viral RNA and proteins (Table 1) (61). The use of serology in the diagnosis of dengue is based on the understanding of the immune response to dengue infection and the duration of illness. Some markers are used for routine clinical purpose, while some are predominantly for research work.

The uses of these markers are guided by the duration of illness and resource availability. WHO has detailed the use of these diagnostic tests as mentioned in the following table:

		Methodology	Time to detection after infection	Results turnaround time
Direct detection of virus and viral products	Virus isolation	Cell culture: Mosquito based inoculation	First week	> 1 week
	Nucleic acid detection	PCR based assays	Mostly from Day1	1-2 days
	Antigen detection	NS1Ag - Rapid card test - ELISA based assay		Few minutes to hours 2-5 days
Detection of serological response	Single serum analysis	IgM and IgG detection: - Rapid card test - ELISA based assay	Mostly After Day 4	Few minutes to hours 2-5 days
	Paired sera	Comparison of acute (1-5 days) and convalescent sera (15-21 days) for IgM and IgG		

Table 1: Direct and serological assessment of dengue infection

The positivity and titres of the different tests also depend on primary vs. secondary dengue infection (65). Fig. 6 adapted from *Peeling et al* depicts the variable response of different serologies in primary and secondary dengue infection.

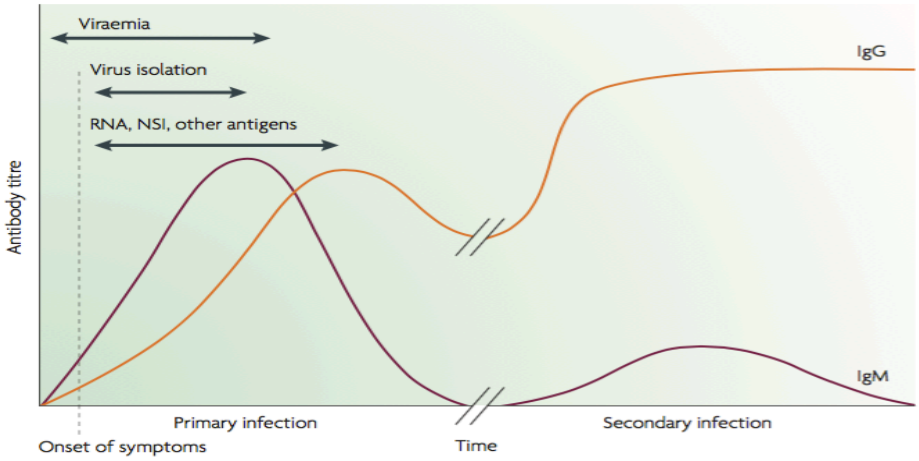


Fig. 6: Serological response in primary and secondary dengue

The laboratory diagnosis of dengue infection is also guided by the purpose of testing:

- Individual case detection: Early vs. late
- Epidemiological surveillance
- Vaccine efficacy studies

The modality used varies accordingly. The WHO in the guidelines of 2009 and 2012 have segregated the diagnostic tests according to the level of care and also categorized the interpretation of tests into confirmed and possible dengue infection. Table 2 and Table 3 adapted from the WHO 2012 handbook of clinical management guidelines of dengue highlights the same. The uses of diagnostic tests are always a supplement to clinical diagnosis and to eliminate other disease differentials. Hence it is not always necessary especially when the clinical picture is sufficiently clear. Local disease epidemiology and cost benefits should be assessed before any diagnostic test is used routinely.

		Primary health-care centres	District health centres	Reference centres
Virus isolation				Yes
Genome detection				Yes
NS1 Ag detection	Rapid tests	Yes	Yes	Yes
	ELISA		Yes	Yes
IgM detection	Rapid tests	Yes	Yes	Yes
	ELISA		Yes	Yes
IgG detection	ELISA			Yes
	IHA			Yes
	Neutralization assay			Yes

ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; IgM = immunoglobulin M; IHA = indirect haemagglutination; NS1 Ag = non-structural protein 1 antigen

Table 2: Laboratory service level recommendation for diagnostic tests for dengue infection

	Method	Interpretation	Sample characteristics
Confirmed dengue infection	Viral isolation	Virus isolated	Serum (collected at 1–5 days of fever) Necropsy tissues
	Genome detection	Positive RT-PCR or positive real-time RT-PCR	
	Antigen detection	Positive NS1 Ag	
		Positive immunohistochemical	Necropsy tissues
	IgM seroconversion	From negative IgM to positive IgM in paired sera	Acute serum (days 1–5) and convalescent serum (15–21 days after first serum)
	IgG seroconversion	From negative IgG to positive IgG in paired sera or 4-fold increase IgG levels among paired sera	
Probable dengue infection	Positive IgM	Positive IgM	Single serum collected after day 5
	High IgG levels	High IgG levels by ELISA or HI (≥ 1280)	

ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; IgM = immunoglobulin M; NS1 Ag = non-structural protein 1 antigen; RT-PCR = reverse transcriptase polymerase chain reaction

Table 3: Diagnostic tests and their role in diagnosis of dengue infection

Management principles:

Different international and local bodies from time to time formulate management strategies and algorithms for management of dengue fever with the focus being on available resources and epidemiological patterns. The WHO latest in 2012 laid down guidelines for management of the different forms of dengue fever and its complications. These algorithms address the commonly faced problems and provide the backbone on which further titration of therapies should be done locally and by the attending physician on a case-to-case basis. The management principles are based solely on supportive care with early fluid resuscitation, organ support and rarely transfusion support.

In the febrile phase and those not progressing to the critical phase, patients can be managed on an outpatient basis. Adequate oral hydration should be ensured and patients should be warned about the danger signs that herald the critical phase. All patients who show

any of the danger signs ideally need admission for close monitoring. On of the commonest manifestation of the critical phase is shock. This closely accompanies organ dysfunction in the form of encephalopathy, ischemic hepatitis, respiratory distress and renal failure. The shock can be:

- Transient compensated shock
- Profound, prolonged uncompensated shock

In dengue, unlike other etiologies of septic shock, there is early and severe endothelial dysfunction coupled with the hypotension. This makes fluid resuscitation in shock challenging as it leads to third spacing and adds to ongoing respiratory distress and liver dysfunction. Cardiac dysfunction is has been often described in dengue infection and can range from mild tachycardia to severe myocarditis and cardiogenic shock (66,67) . This makes fluid resuscitation a closely orchestrated management, but all the same the most important of the supportive measures. In the non-severe forms of dengue oral hydration is often sufficient. However with the onset of more severe disease the added gastrointestinal symptoms leading to poor oral intake coupled with the severe intravascular depletion warrants parenteral hydration. Fluid resuscitation in severe dengue should be monitored considering the amount of third spacing, respiratory fluid overload and cardiac dysfunction that often accompanies the severe forms. Early and adequate fluid resuscitation might prevent organ injury, need for vasopressors and hospital stay. Serum electrolytes and renal function should be monitored while such therapies are continued.

The WHO guidelines classifies the management approaches into three groups of patients:

- **Group A**: Patients with non severe disease with no warning signs, who adequately tolerate oral feeds, have no other contraindication to outpatient care and can come easily for follow ups. They form the crux of community-based care.

- **Group B**: Patients who have warning signs and have high and early chance of progression to the critical phase. Pregnant patients, young children, elderly and those with comorbidities belong to this category. They warrant in-patient management and need parenteral fluids with close monitoring of organ systems.

- **Group C**: Patients with severe dengue who have significant single organ or multi-organ dysfunction. They often require significant critical care and organ support. They are better referred to centres equipped to handle sicker patients. The mortality increases significantly with delayed identification of these patients.

Fig. 7 depicts the outlines of fluid resuscitation in patients with compensated shock. In accordance with other guidelines of care in shock, like the Surviving sepsis guidelines, goal directed therapy helps in preventing over-resuscitation and worsening cardio-respiratory parameters. (68). All patients should be examined for superadded infections that might co-exist over and above the dengue infection and might need broad-spectrum antibiotics if a focus is found (69). Certain scoring systems, like the *DDIS* (Dengue Dual Infection Score) has been constructed to diagnose early patients with superadded bacterial infection (70). As thrombocytopenia and coagulopathy are common in severe dengue, any major clinical bleeding or significant/progressive drop in hemoglobin should warrant transfusion. Transfusion guidelines for platelets by the NVDCP India have been highlighted before.

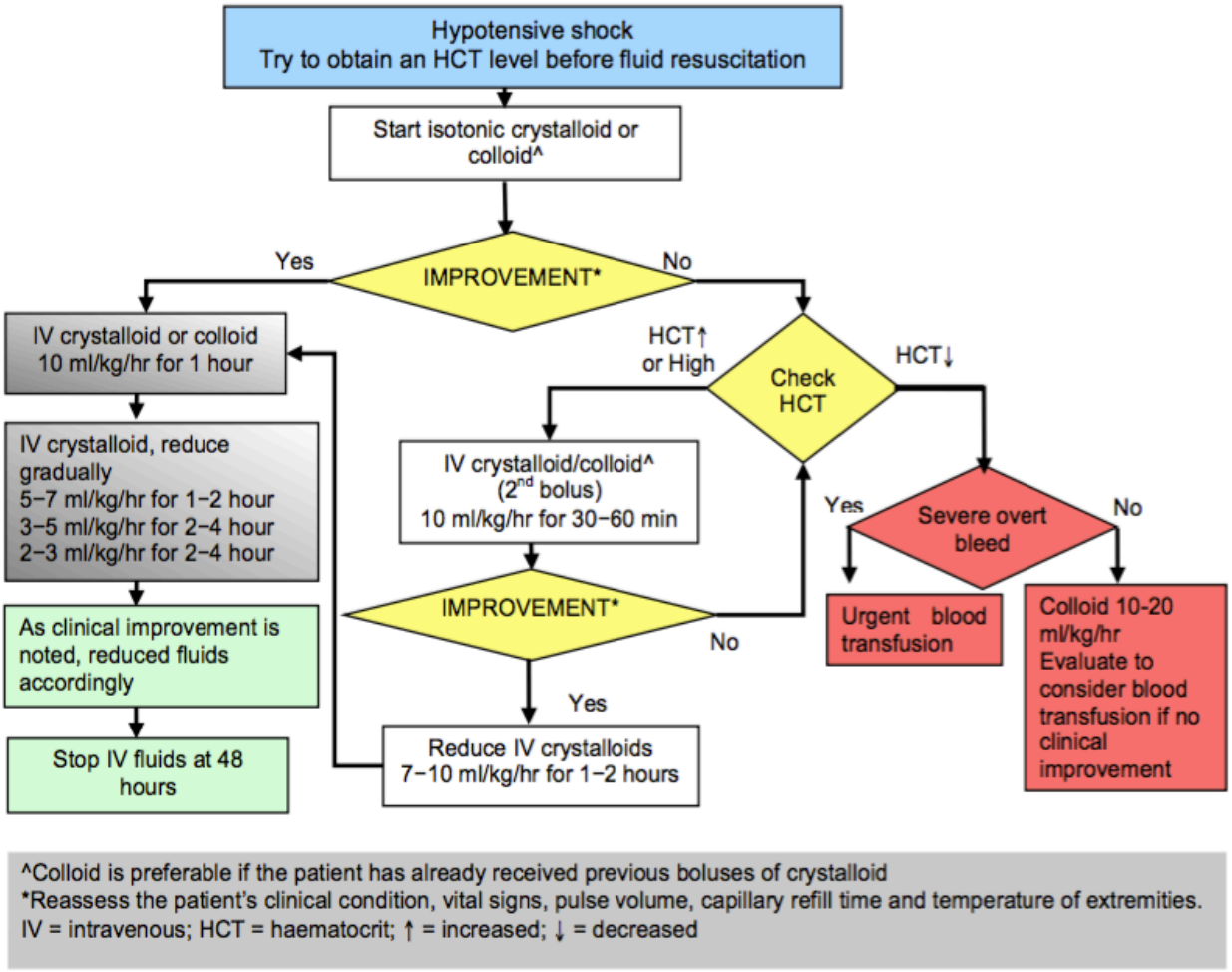


Fig 7: Algorithm for management of compensated shock in adults with dengue fever. Adapted form the WHO 2012 guidelines on management of dengue

The specifics of organ support and transfusion have been dealt with by the WHO and modified by local guidelines. In principle, management of dengue fever requires proper classification of patients into different risk groups, assessment of early signs of severity and protocol based management of shock and other complications. Transfusion of platelets and packed cells are reserved for special conditions and rampant use of antibiotics is not warranted. Early referral to higher centres is prudent in cases with severe dengue.

The signs of adequate resuscitation and progress to the recovery phase in patients with severe dengue can be manifested as the following:

- Improvement in blood pressure and reduction in heart rate
- Improvement in urine output and normalisation of electrolytes, creatinine if had been deranged before
- Reduction in transaminitis
- Normalisation of hematocrit and improvement in platelet counts
- Improvement in sensorium
- Reduction of abdominal pain and improvement in appetite

If the achievements of these variables are delayed, a superadded infection or other pathology should be considered. Clinical improvement in dengue is usually steady and any worsening after initial improvement should arouse suspicion of secondary pathologies.

In pregnant dengue patients, as has been already mentioned; the disease can be often severe with early capillary leak, respiratory distress and hypotension. Disease severity can vary with the pregnancy trimester, with studies showing increased evidence of preterm delivery and low birth weight in infections prior to third trimester. Ruling out pregnancy related morbidities like hyperemesis gravidarum, severe pre-ecclampsia and HELLP syndrome should be ruled out, as a lot of symptoms like vomiting, pedal edema, respiratory distress, altered sensorium, thrombocytopenia, renal failure, dyselectrolemia and transaminitis are shared by these conditions. In the presence of resources it might be better to admit and manage these patients. There is no difference in the fluid management between

pregnant patients and others. If delivery is inadvertent, platelets and other blood product support may be required. All pregnant women with dengue infection in their last trimester should be treated as a high-risk pregnancy.

Disease outcome assessment: It has been discussed above in detail show the dengue fever is classified based on its severity, the clinical care groups and models of management. However these classification systems though helpful in designing management algorithm fail to prognosticate adequately. Several scoring systems such as SOFA (Sequential organ failure assessment), APACHE II (Acute physiology and chronic health evaluation) and others have been used to assess dengue severity especially in ICU patients. Individual immunological markers, laboratory parameters alone or isolated organ failure assessment might not depict the entire picture (71). Scoring systems that include clinical and laboratory parameters may be able to assess better the status in severe dengue with a higher prognostic yield. There is no uniform accepted prognosticative scoring system available for dengue fever. *Lee et al* used a retrospective cohort of 69 patients with severe dengue and 1184 patients with non severe dengue from Taiwan and made scoring systems for early assessment of severity in dengue (72). Validations of the same in other cohorts are awaited.

Amâncio et al in a series of 97 patients from Brazil admitted to the ICU with dengue fever found that those with lower albumin, elevated creatinine, leukocytosis and elderly had an increased mortality (73). They also showed the utility of SOFA and APACHE II scores and found higher scores to co-relate with in-hospital mortality. *Jog et al* from India in a retrospective study of 113 patients with dengue fever and at least 2 organ dysfunction according to SOFA, found low serum albumin, high arterial lactate levels and SOFA scores

correlated with mortality (74). This study did not include pregnant women. In a retrospective study of 4787 patients with dengue admitted to the ICU from Taiwan, *Chen at al* showed that lower GCS scores, thrombocytopenia and higher APACHE II scores among other variables were associated with increased mortality (69). Smaller prospective studies also from Taiwan had shown female gender, prolonged aPTT, higher levels of transaminitis and cardiac arrest before hospital admission to be associated with increased mortality (75). Thus many parameters in different studies have been shown to correlate well with outcomes, especially in sicker patients, however scoring systems like APACHE II and SOFA may be more consistent in dynamic assessment of status and prognostication.

The road ahead: Despite increasing idea about the epidemiology, virology, immunopathogenesis, and better diagnostics with management, dengue infection is a global health burden with hyperendemicity in South East Asia. Its toll on healthcare costs is huge. Prevention of infection is the ultimate goal and can range from vector control measures, better protection from mosquito bites to vaccination. Much study is going on about the latter , with newer epitopes being recognized that are relatively more conserved across the different serotypes thereby promising to be vaccine targets. The current vaccine approaches against dengue include (24,76):

1) Live attenuated viruses:
   - Viruses attenuated by cell culture or mutagenesis
   - Chimeric live viruses (Yellow fever-dengue chimera, Dengue-dengue intertype chimera)
2) Inactivated and pure whole virion

3) Recombinant subunit proteins

4) DNA vaccines

5) Virus like particles

6) Viral vectors expressing DENV antigen

These vaccines are at different phases of study with some having made remarkable progress, though none have yet been licensed or included in the management guideline. It is probably a matter of time when efficient and potent vaccines will be included in guidelines and readily available. The road ahead in dengue management is promising though much research still needs to be done to both disease prevention and to optimize management especially in the severe forms of the disease.

Materials and Methods

Study setting and duration:

This study was conducted in Christian Medical College and Hospital, Vellore, a tertiary care teaching hospital in South India with around 2700 beds. The hospital serves the population of Tamil Nadu and the neighboring state of Andhra Pradesh, besides being a referral center for patients from other parts of the country and the Indian subcontinent.

Patients were recruited for this study from May 2016 to August 2017. As the present study is dependent of seasonal variations we expected to recruit significant numbers over the stipulated time period as mentioned above. The study recruited patients from the following departments in our hospital:

- Department of Medicine
- Department of Accident and Emergency Medicine
- Medical Intensive care unit

Study design:

This is a prospective study aiming to look at the association of ADAMTS 13 levels and vWF activity to dengue severity and outcomes. It was approved by the Institutional review board (Blue) and the Ethics committee prior to its initiation. (Annexure 1)

Study participants:

Selection set: All adults (Age >18) who present with an undifferentiated acute febrile illness with thrombocytopenia (Platelet count on Coulter < 1,00,000/cu mm)

Inclusion criteria: All individuals in the above set who are tested positive for Dengue IgM/ NS1 Antigen.

Exclusion criteria:

1) All individuals from the "Selection" set who are negative for both Dengue IgM and NS1Ag.

2) All individuals from the "Selection" set who have any diagnosed hematological condition

3) All individuals from the "Selection" set who have received any form of transfusion from the onset of fever to presentation

4) All individuals from the "Selection" set who are seropositive for HBV, HCV or HIV.

5) All individuals from the "Selection" set who have any known autoimmune condition/ collagen vascular disease/ prior or present malignancy/ diagnosed chronic liver disease/ on Aspirin, Clopidgrel, other antiplatelets/ Warfarin, Acitrom, other anticoagulants

6) All individuals from the "Selection" set who have an eschar

The present study did not necessitate controls as the comparison was done amongst the study population with different severity of dengue fever.

Case definition and ascertainment: The WHO 2009 definition was used for initial case selection as "probable dengue" and was included in the selection set as mentioned above. Once they were tested positive for NS1 antigen or IgM for Dengue, they were included in the study.

Data sources and collection: For all patients from the " Selection" set blood samples were collected for ADAMTS 13 assay and vWF collagen activity at presentation, while the demographic and clinical data were noted. The individuals who tested positive for Dengue IgM/ NS1Ag had the samples processed for the above. Ward/ OPD/ casualty notes and daily direct assessment by the principal investigator was used for following up the patient.

The demographic and clinical data was collected on a clinical pro-forma validated by the participating departments and the Institutional review board. Follow up of those individuals who get admitted were done as following:

1) SOFA (Sequential organ failure assessment) score at admission and during their follow up till convalescence

2) Total transfusion support needed (Separate cumulative for Packed red cells/ Platelets as Platelet rich concentrate / Fresh Frozen plasma/ Cryoprecipitate)

3) Organ supports needed during admission : Renal replacement therapy : Ventilatory support as - 1) Non invasive 2) Invasive etc.

4) Outcome: Death/ Discharge- Cured/ Against medical advice

Outcome Assessment:

Primary outcome: To estimate the levels of ADAMTS 13 levels and vWF activity in adults with dengue fever at presentation and compare it to disease severity by the highest SOFA score documented in the subjects

Secondary outcomes:

1) To assess if the severity of thrombocytopenia at presentation is correlated with ADAMTS-13 level and vWF collagen activity. Such correlation may suggest a mechanism for the thrombocytopenia

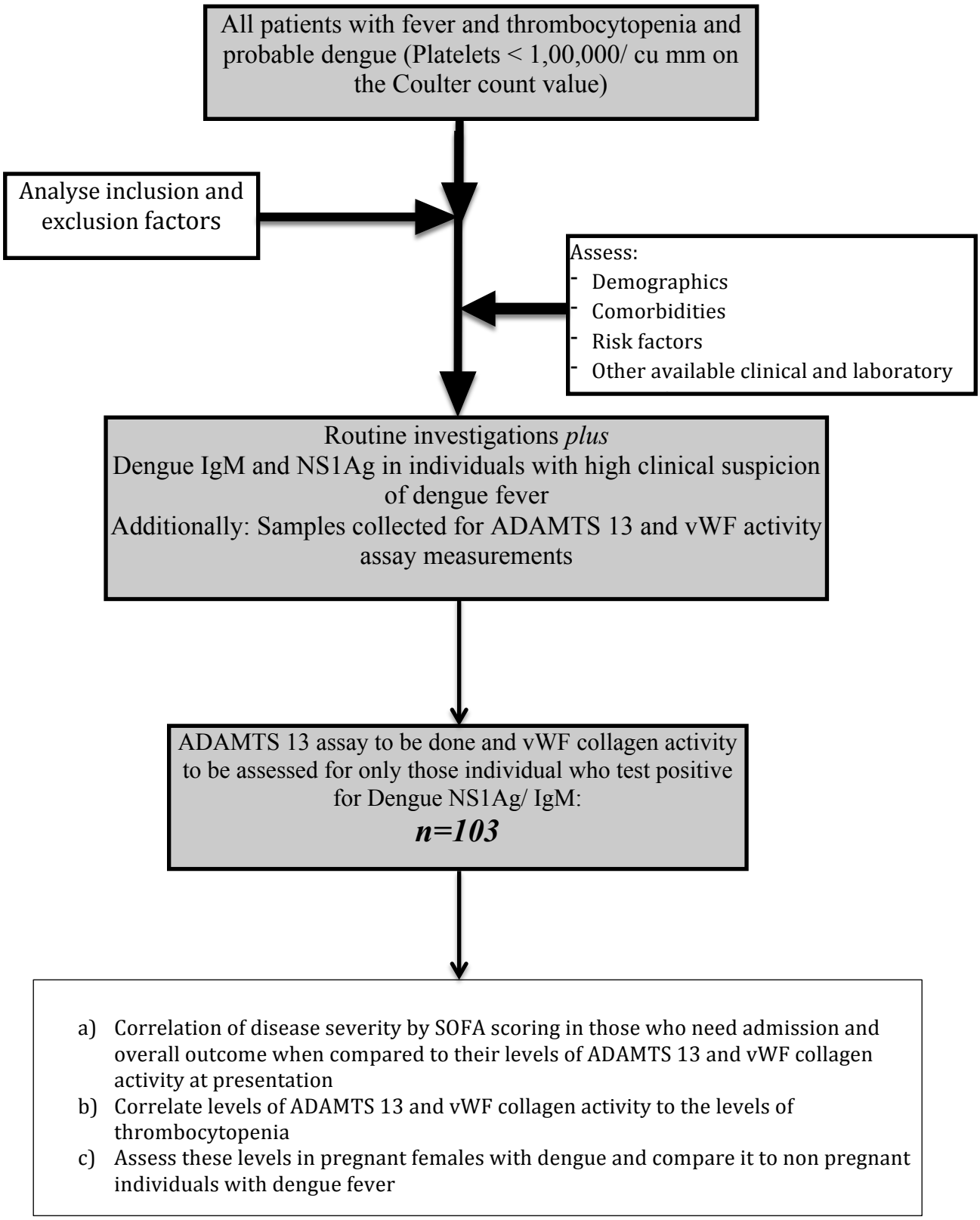
2) To assess if severity of illness is correlates with ADAMTS-13 level and vWF activity.

3) To determine if low ADAMTS-13 level and vWF activity are associated with increased mortality

4) To analyze ADAMTS-13 levels and vWF collagen activity in pregnant individuals with dengue and compare their levels with non-pregnant individuals with dengue

Sample size: The required sample size to show that SOFA score will correlate with ADAMTS13 and VWF was found to be **103 subjects** with a power of 80%, 5% level of significance and an anticipated correlation of about 0.7 between the two measures. As there is no study mainly focused to look at the correlation of SOFA scores with ADAMTS 13 and VWF, however, as it was expected to be good, the anticipation of correlation was considered to be 0.75 (55) .

Study Algorithm:



Assessment of laboratory variables:

ADAMTS 13 assay:

ACTIFLUOR ADAMTS13 activity assay is a fluorescence resonance energy transfer (FRET) assay for measurement of ADAMTS13 in human plasma. Principle: The assay measures the amount of ADAMTS13 activity in human plasma (citrated sample) using recombinant VWF86-ALEXA FRET substrate. Proteolytic cleavage of the VWF86-ALEXA FRET substrate between Tyr/Met residues by ADAMTS13 uncouples ALEXA fluorochromes resulting in increased fluorescence. The increase in Fluoroscence over time (Vmax) is monitored at 37 C using spectroflurometer (Ex =485nm; Em = 535nm) A standard curve is constructed using normal plasma with a known concentration of ADAMTS13 (provided in the kit). The activity in the plasma is determined by interpolation of the Vmax values from the standard curve.

Reagents:

- 6x8 fluorescence microwell strips plus frame (white)
- 2 vials of ADAMTS13 standard, 250ul
- 1 vial of positive control
- 1 vial of DMSO
- 1 vial of ALEXA488-VEF86 FRET substrate
- 2 vials of Assay buffer
- 3 vials of ADAMTS13 inactivated plasma

Von Willebrand Factor collagen binding activity (vWF: CBA):

To estimate the Collagen binding assay level in citrated plasma by using ELISA method:

Principle: The assay tests the ability of patient plasma derived from von Willebrand factor to bind collagen, which has previously been coated onto 96 well plates. Low VWF: CBA levels will be present in individuals with either quantitative defects or with qualitative defects.

Sample: Citrated sample. Samples and control run in duplicate. Quality control: Normal control (pooled normal plasma) and low levels abnormal control (Dade P).

Normal values: 50-400%

Data entry and statistical analysis:

All data were entered into the clinical proforma and subsequently into EpiData software. The data was extracted onto Microsoft Excel and analysed by IBM SPSS software. Univariate Analysis -For the normal data, we did independent T-test and for the non-normal data, Mann-Whitney U test by the outcome variable based on dengue severity. For the variables that were significant on univariate analysis we did a multivariate analysis by logistic regression.

Results

This prospective observational study was conducted from May 2016 through August 2017. During this period 105 patients met the selection criteria. Of these 105, 74 had confirmed dengue, of which 12 were excluded according to the exclusion criteria as mentioned above. A total of 62 patients were included for whom the ADAMTS 13 and vWF: CBA was processed and the data was used for final analysis. Fig. 8 describes the STROBE diagram for the present study. Due to seasonal and yearly variation in the incidence of dengue cases, the required number of patients as mentioned in the sample size calculation could not be met in the present study.

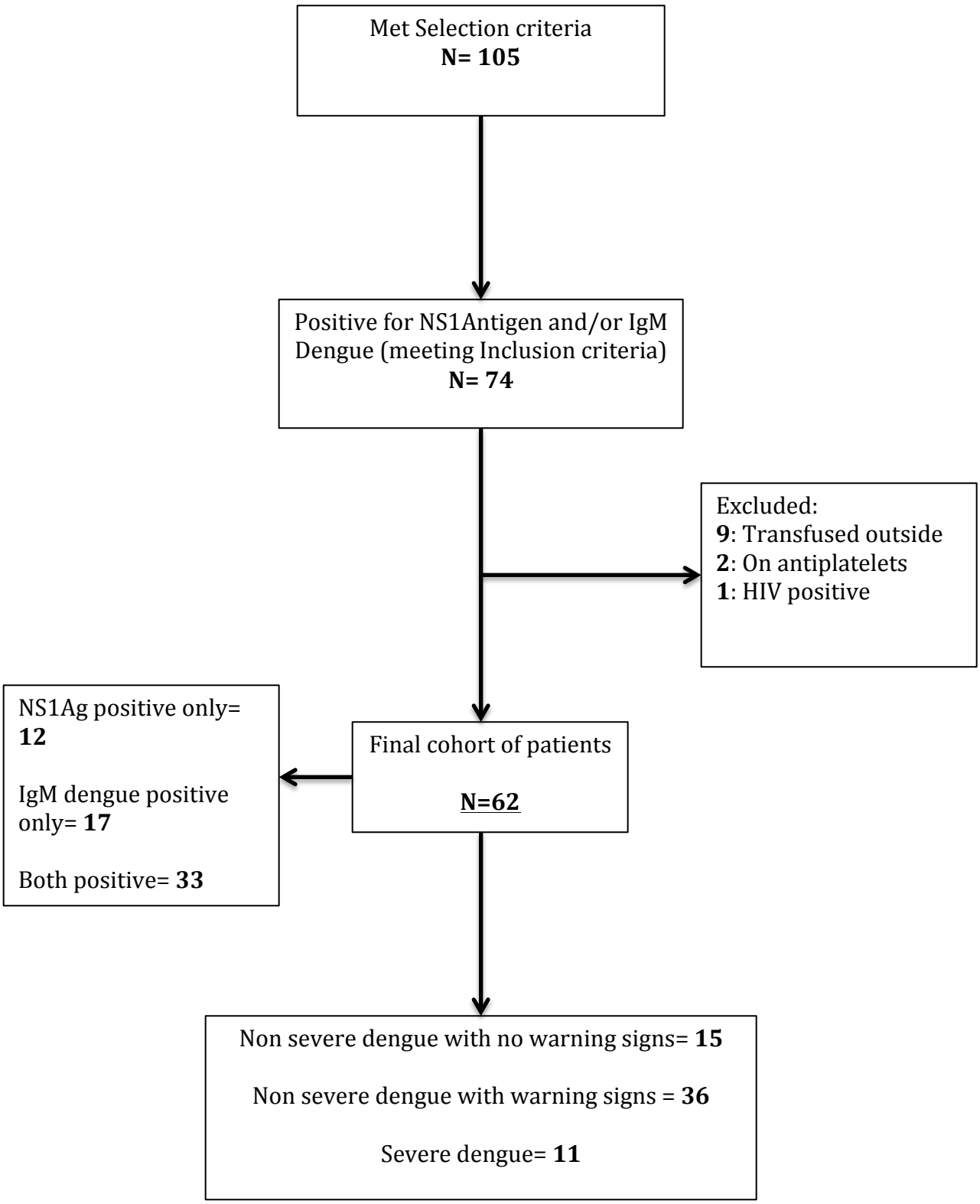


Fig. 8: STROBE diagram of AVID study

Baseline characteristics:

The cohort consisted of 62 patients who presented in the above mentioned time period with an acute febrile illness, thrombocytopenia with a platelet count of less than 1 lac/cu mm and tested positive for NS1 antigen or Dengue IgM. Of the 62 subjects who were included for the final analysis there were 40 males (64.5%) and 22 females (35.5%), showing a clear male preponderance. The median age was 22 years (Range 16-84 years) reflecting larger epidemiological studies. Majority of the male subjects were unskilled or semi-skilled labourers while most of the female subjects were homemakers. Majority of the patients had no known comorbidities. Four patients (6.4%) had Type 2 Diabetes, 3 (4.8%) had essential hypertension of which 2 had both the above. None of the patients had any prior history of prior dengue infection and none of them had received any form of transfusion prior to their inclusion in the study. None of them had any significant history of prior bleeding diathesis or any family history of the same. No patients had any significant history of substance abuse.

Eight patients (12.9%) were managed on an outpatient basis (OPD or Casualty) while the remaining 54 patients (87.1%) patients were admitted for further management. The median days post symptom onset after which the patient presented was 5 (2-15 days). We considered fever, myalgia or any complaint attributable to the dengue infection as symptom while calculating the date of presentation. Thirty-two patients (51.6%) were afebrile at the time of presentation to our centre. Forty-eight patients (77.4%) did not have any complaints of bleeding manifestation at presentation, while 14 patients (23.6%) had some form of bleeding. We considered oral (mucosal), nasal and injection site bleeding as minor bleeding diathesis,

while hemoptysis, hematemesis, hematuria, maleana/haemtochezia as major bleeding manifestations. Six patients (9.6%) had major and 8 (12.9%) had minor bleeding manifestations. Table 4a and 4b elaborates the demographic and clinical findings at presentation respectively.

Parameters	**Median/ N (Range) {%}**
Age	22 (18-64)
Gender distribution	
Males	40 {64.5}
Females	22 {35.5}
State of residence	
Tamil Nadu	44 {70.9}
Andhra Pradesh	10 {16.1}
Others	8 {13}
Co-morbidities	
Diabetes mellitus	2 {3.2}
Systemic hypertension	1 {1.6}
Both	2 {3.2}
Substance abuse	Nil
Prior dengue infection	Nil
Prior transfusion	Nil
Prior history or family history of bleeding diathesis	Nil

Table 4a: Demographic characteristics of study subjects

Clinical parameters		**Median value (Range) {%}**
Day of presentation post symptom onset		5 (2-15)
Subjects afebrile at presentation		32 {51.6}
Bleeding manifestation:		*14 {23.6}*
	Minor	8 {12.9}
	Major	6 {9.6}
General Symptomatology at presentation:		
	Headache	60 {96.7}
	Myalgia	62 {100}
	Fatiguability	62 {100}
Respiratory symptoms: *		*15 {24.2}*
	Cough	13 {21}
	Breathlessness	7 {11.3}
	Chest pain	1 {1.6}
Gastrointestinal symptoms: *		38 {61.3)
	Nausea/vomiting	33 {53.2}
	Loose stools	13 {21}
	Abdominal pain	16 {25.8}
CNS symptoms: *		*1 {1.6}*
	Altered sensorium	1 {1.6}
	Seizures	0 {0}
Miscellaneous symptoms:		
	Decreased urine output	0 {0}

Table 4b: Symptomatology at presentation

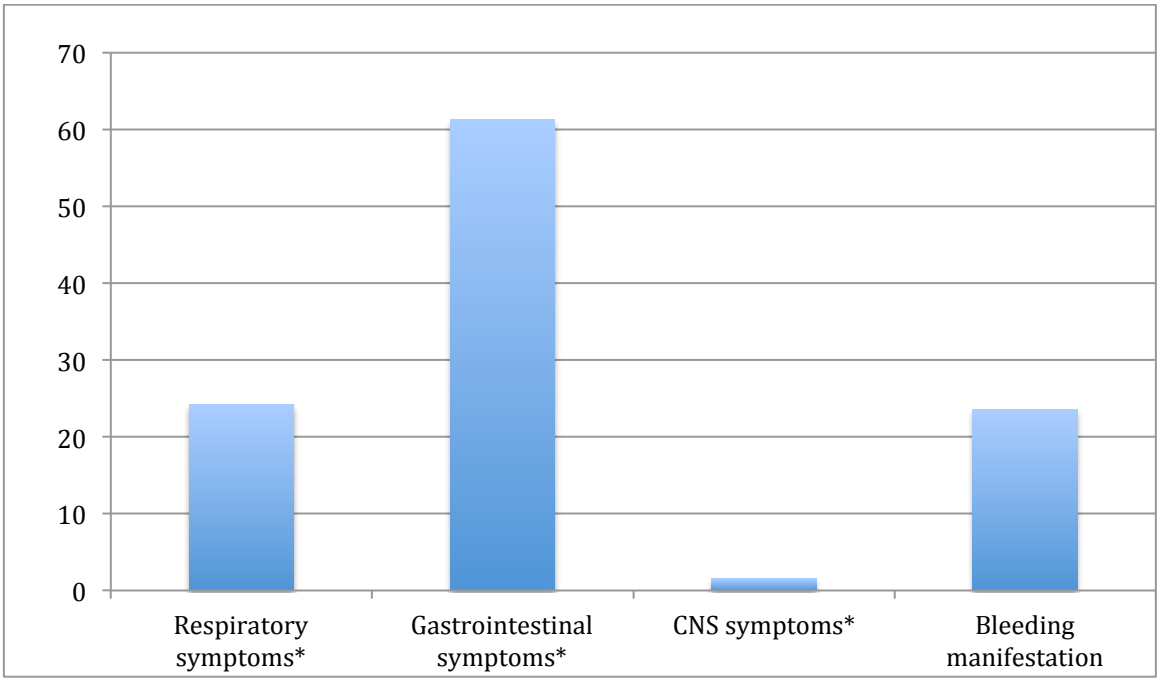


Fig. 9: Bar graph depicting the frequencies of symptoms at presentation

As is visible from the above bar diagram, most patients had GI symptoms in the form of nausea/vomiting, loose stools followed by respiratory symptoms. However almost all patients had headache while all patients complained of some form of myalgia and fatiguability at presentation.

All patients at admission were evaluated by the attending physician and vital signs and detailed clinical examination done. Most patients had tachycardia at presentation with a median pulse rate of 93/min (68-130 beats /minute). The median systolic blood pressure at presentation was 100 mm of Hg (70-130 mm of Hg) and diastolic blood pressure of 60 mm of Hg (50-90 mm of Hg). Table 5 depicts the clinical findings at presentation documented at admission.

Clinical parameters/findings	**Median value (Range) {%}**
Heart rate (beats/min)	93 (68-130)
Blood pressure (mm of Hg):	
SBP	100 (70-130)
DBP	60 (50-90)
Respiratory parameters:	
Rate (per minute)	20 (14-40)
Saturation (% in room air)	98 (88-100)
Dermatological manifestation:	
Erythematous rash	55 {88.7}
Petechiae/Ecchymotic spots	2 {3.2}
Respiratory findings:	
Positive findings [#]	9 {14.5}
Pleural effusion (reduced air entry)	9 {14.5}
Crackles	2 {3.2}
Increased conducted sounds	2 {3.2}
Abdomen findings:	
Positive findings [#]	11 {17.7}
Free fluid	7 {11.3}
Guarding	1 {1.6}
Tenderness	9 {14.5}
CNS findings:	
Positive findings [#]	1 {1.6}
Altered sensorium	1 {1.6}

Table 5: Clinical findings at presentation

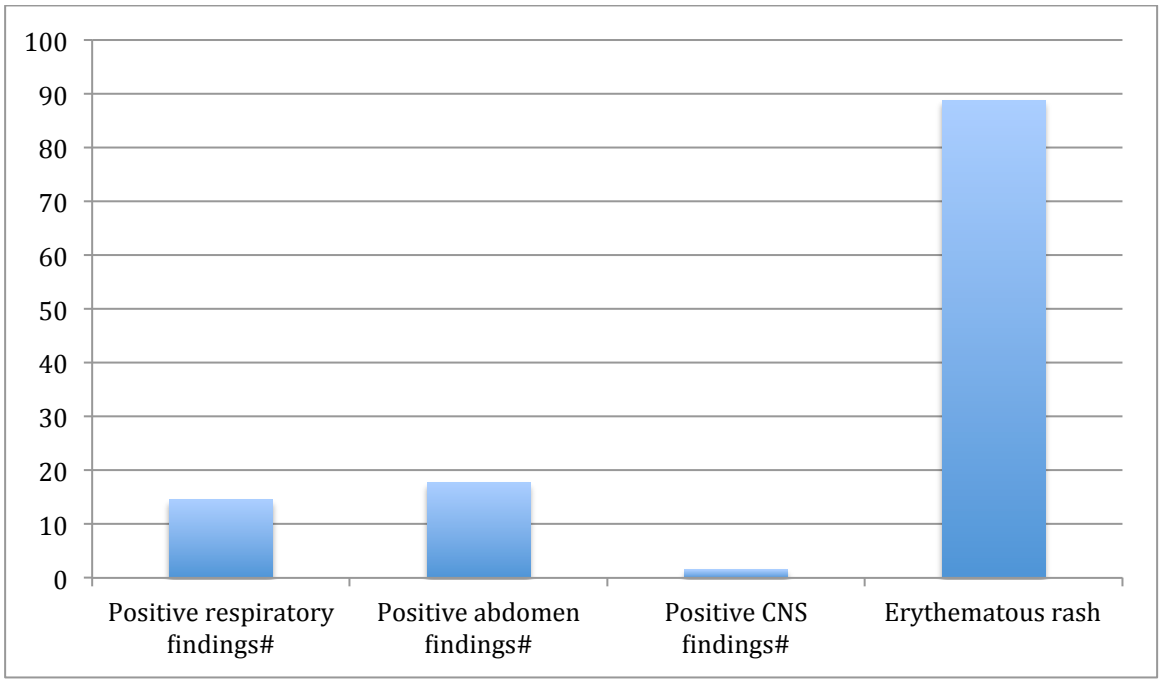


Fig. 10: Bar graph depicting the frequency of positive findings at presentation

All patients had basic blood investigation including complete blood count (CBC), renal and liver function tests, serum electrolytes at admission. Some patients had coagulation parameters, blood cultures and other serologies (Scrub typhus, leptospira IgM) according to the clinical presentation. Most patients had urinalysis and a chest skiagram. Malarial smears were done for all patients. As mentioned above the diagnosis of dengue was based on positivity for NS1 Antigen or IgM dengue positivity. Both of these were done for all patients together with IgG dengue. Further investigation as mandated by the clinical findings and the physician's discretion were conducted. Table 6 provides the baseline investigations at presentation/ enrollment of our study subjects. Investigations were repeated as required during their course of treatment and will be discussed below.

Laboratory parameters	**Median value/(Range) {%}**
Hemoglobin (gm/dl)	14.45 (3.5-19.2)
Total WBC (per cumm):	4350 (1700-37,600)
Neutrophils (% of WBC)	60 (23-92)
Lymphocytes (% of WBC)	25 (2-64)
Monocytes (% of WBC)	9 (2-29)
Platelet (per cumm):	
Automated via Coulter	31000 (4000-95,000)
Manual on smear	41000 (4000-95,000)
Creatinine (mg/dl)	0.90 (0.45-2.18)
Serum electrolytes:	
Sodium (mmol/L)	134 (125-142)
Potassium (mmol/L)	3.8 (2.8-5.7)
Liver function tests:	
Total bilirubin (mg./dl)	1.23 (0.2-9.1)
Direct bilirubin (mg./dl)	1.06 (0.1-7.8)
Total protein (gm./dl)	6.6 (4.2-8.3)
Serum albumin (gm./dl)	3.6 (1.8-4.7)
SGOT (IU/L)	193 (23-6860)
SGPT (IU/L)	93 (12-2958)
ALP (IU/L)	71 (34-361)

Table 6: basic laboratory parameters at presentation

A majority of patients presented with hemoconcentration and normal to low WBC counts. The median platelet count on automated counters was 31,000/cu mm, which was lower than when checked by manual smear count. Hyponatremia was common and more than 90%

patients had transaminitis, the SGOT being universally more than SGPT in our cohort of patients. Proteinuria (not shown in the above table) was the most common abnormality on urinanalysis, accounting in 60 patients (96.8%). Hematuria and pyuria were rare observed in 3 and 2 patients respectively.

Chest skiagram was done in all patients with 47 patients (74.2%) having a normal image, 7 (11.3%) having pleural effusion, 5 (8.1%) opacities and 4 (6.5%) having non-specific bilateral infiltrates. USG of the abdomen was done in 17 patients (27.4%) with 12 of them (19.4%) showing free fluid. The usual triggers for USG abdomen included severe abdominal pain, significant tenderness/guarding, persisting loose stools or persisting fever. The following pie chart shows the distribution of serology positivity fro dengue in our patients.

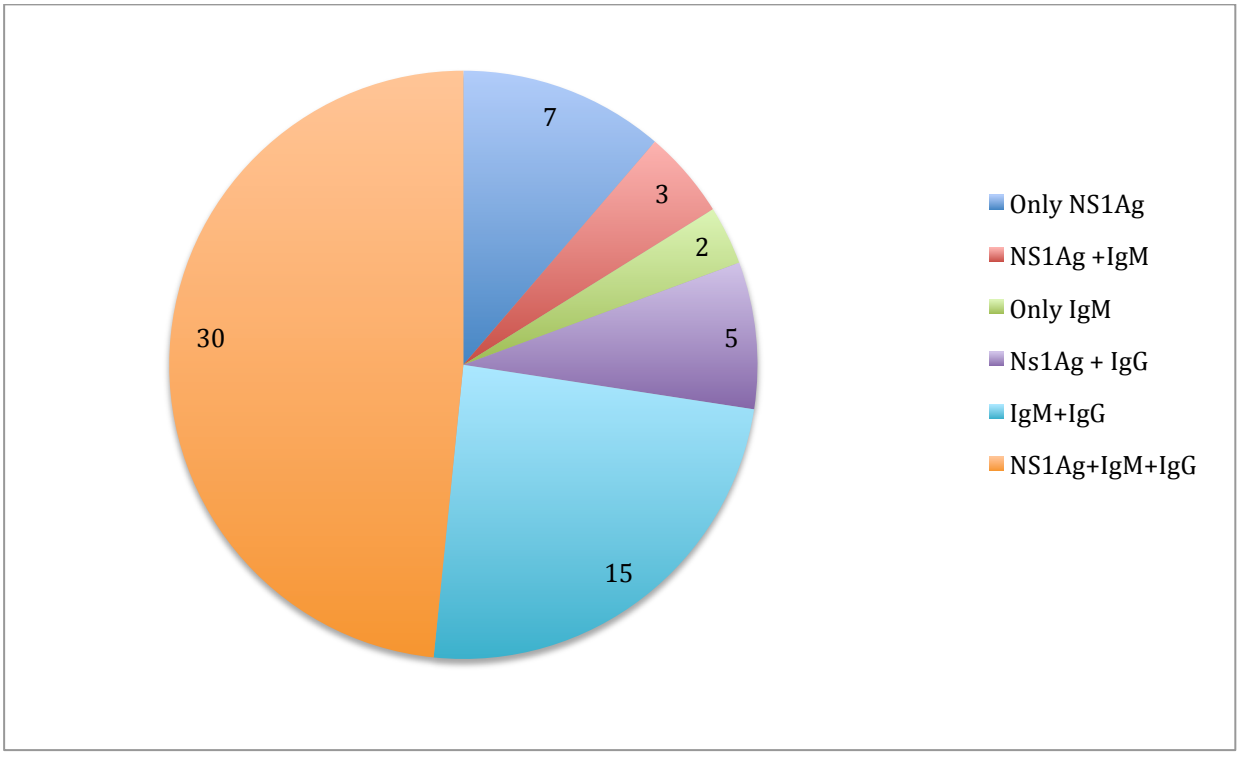


Fig. 11: Pie chart depicting the different combination of dengue serology positivity in study subjects

Pregnant subjects:

In the present study we aim to assess the differences in ADAMTS 13 levels and vWF:CBA in pregnant women with dengue when compared to the no pregnant dengue women. A total of 6 patients (9.6%) were pregnant at the time of study enrollment. 4 women were primigravida while 1 each were gravida 2 and gravida 3. One woman presented in the first trimester at 8 weeks, 2 women in the second trimester (weeks 16 and 18) and 3 women in their third trimester (weeks 32,34 and 38). None of them had any prior pregnancy related complications or any present pregnancy related comorbidities. Table 7 provides the baseline characteristics of the above subjects.

Parameters	**Median value/N (Range) {%}**
Number of pregnant women with dengue	6 (9.6% of patients, 27.2% of female patients)
Age (in years)	25 (21-36)
Gestational score: Gravida	 1 (1-3)
Gestational age: First trimester Second trimester Third trimester	 1 2 3

Table 7: Baseline characteristics of pregnant patients with dengue

Outcomes:

For all patients we assessed the SOFA score at the time of presentation or enrollment. Patients who had presented already prior to the define thrombocytopenia cutoff, were enrolled once the platelet counts dropped to less than 1 lac/ cu mm provided they fulfilled all the other criteriae. The SOFA score was calculated everyday during their period of treatment for the dengue infection based on the latest available laboratory values and daily clinical examination. The highest SOFA scores attained by each subject were also calculated.

We did univariate analysis to look at factors that had a significant association with dengue severity. We used dichotomous outcomes to do the same by clubbing non-severe dengue with warning signs and Severe dengue as "More severe dengue" and non-severe dengue without warning signs as "less severe dengue." From the univariate analysis we chose factors that appeared significant and used a multivariate analysis.

The following factors were looked at:

1) Association between ADAMTS13 levels to dengue severity and highest SOFA scores
2) Association of any positive systemic sign to disease severity
3) Association of initial and lowest platelet values, highest transaminitis documented, need for ICU care to disease severity
4) Gender specific difference between ADAMTS 13 levels
5) Difference in ADAMTS13 levels between pregnant and non pregnant females

Table 8 provides the baseline levels of ADAMTS 13 and vWF:CBA, SOFA scores at presentation and highest SOFA scores.

Parameters	Median value/N (Range) {%}
ADAMTS 13 (%)	76 (22-121)
vWF:CBA (%)	135 (38-309)
SOFA scores:	
At presentation	3 (2-8)
Highest SOFA	4 (2-21)
Liver enzymes:	
Highest SGOT (IU/L)	246 (40-6860)
Highest SGPT (IU/L)	138 (18-2958)
Lowest platelet (per cu mm)	17,000 (4000-78,000)

Table 8: Baseline ADAMTS 13 and vWF:CBA values with certain outcome parameters

SOFA was used in our study for initial assessment of disease severity. However, as it includes thrombocytopenia as one of the parameter and thrombocytopenia happens to be a disease manifestation of dengue, with the present inclusion criteria including patients only with platelet count less than 1 lac/cu mm, all patients at least had a SOFA score of 2 at presentation. However the daily use of SOFA score as a dynamic monitoring tool and correlation of highest SOFA attained in subjects to outcome might undermine this caveat. The following tables and diagrams depict the proportion of non-severe and severe dengue in our study, overall outcomes, organ system involvement, transfusion requirement and mortality.

Parameters	N {%}
Acute kidney injury	11 {17.7}
Requirement of RRT	1 {1.6}
ICU care	4 {6.4}
Vasopressor requirement	2 {3.2}
Respiratory support	3 {4.8}
Transfusion requirement	17 {27.4}
Platelets	16 {25.8}
Packed red cells	3 {4.8}
Fresh frozen plasma (FFP)	10 {16.1}
Cryoprecipitate	2 {3.2}

RRT: Renal replacement therapy (Hemodialysis/ ultrafiltration), ICU: Intensive care unit
Respiratory support included all forms of positive pressure ventilation, invasive and non-invasive

Table 9: Outcome parameters in our cohort of patients

Despite having a larger proportion of patients with severe dengue only about a quarter of patients required any form of transfusion support. Platelets were most commonly transfused. While the median value of platelets transfused were 0 units, the maximum platelet requirement were 17 units of random door platelets. The median units of FFP transfused were also 0 while the maximum transfused to patient was 20 units. In our institution guidelines have been laid for transfusion in dengue patient, which usually includes, but is not limited to

- Ongoing major bleed with significant thrombocytopenia
- High grade fever/ shock with platelets less than 10,000/cu mm

FFP / cryoprecipitate requirement were guided by the levels of coagulopathy.

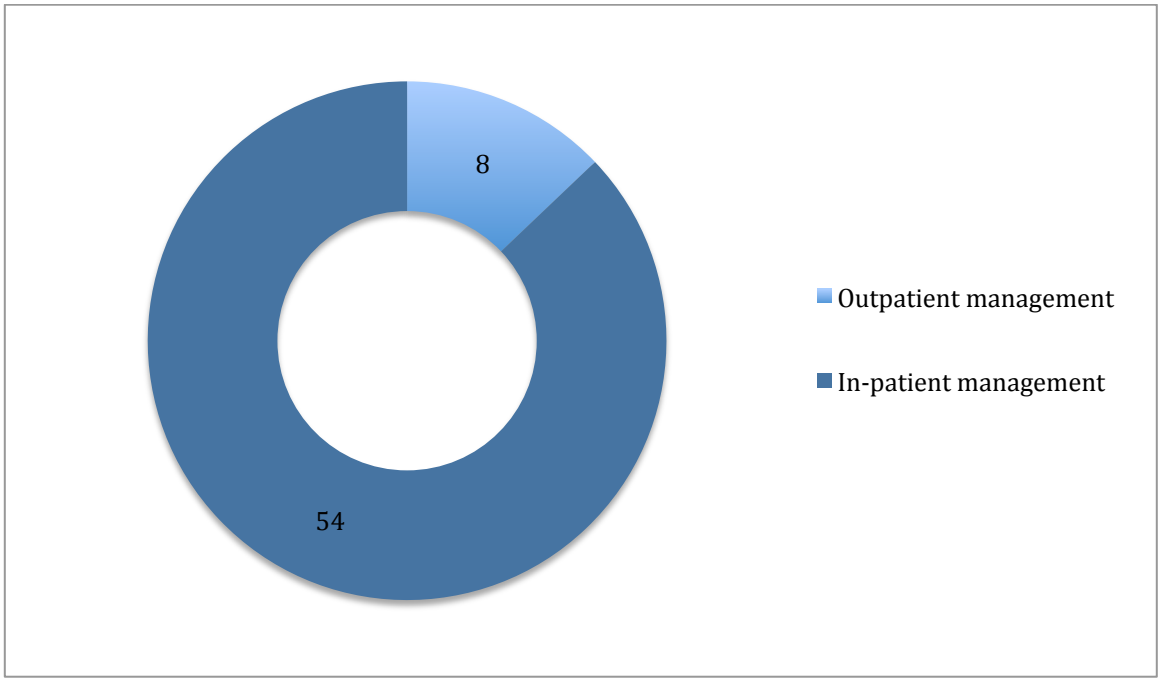


Fig 12: Pie diagram depicting the point of management of our patients

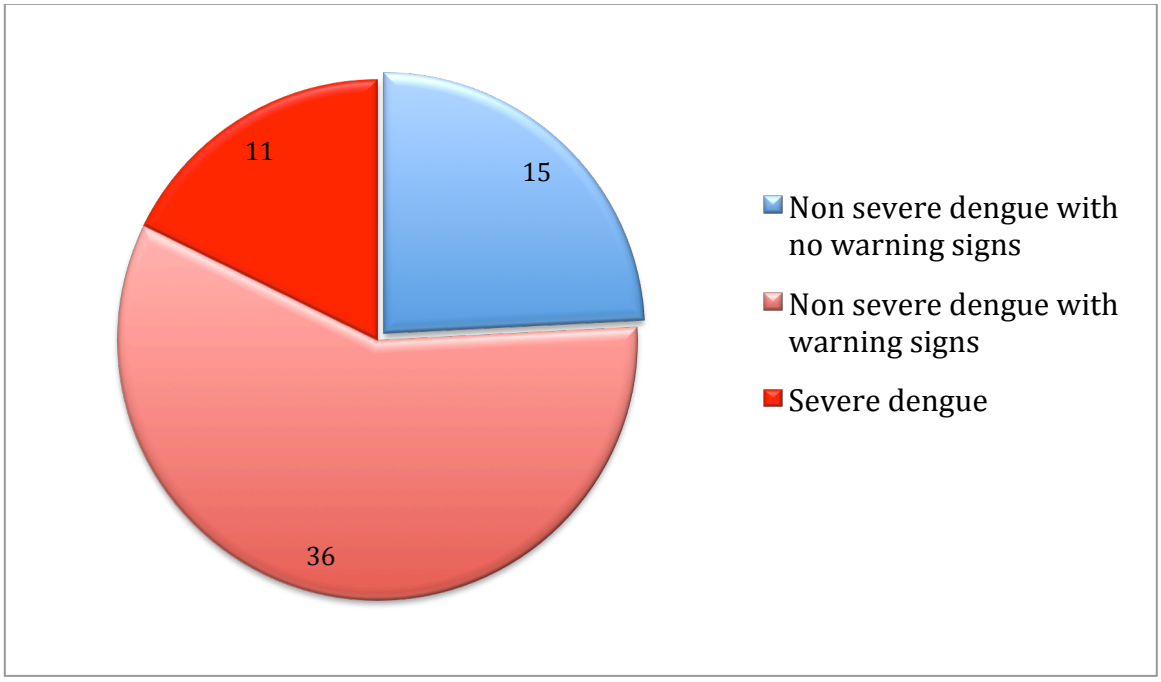


Fig 13: Pie chart depicting the dengue cases according to the WHO grading for severity

Overall in our study, out of the 62 patients, 60 (96.8%) were cured discharged. Two patients (3.2%) succumbed to the disease. One patient, a 48-year-old lady had presented with severe dengue in hypotension but had shown improvement with resuscitation and non-invasive positive pressure ventilation due to ensuing respiratory distress. On Day 2 of her admission she had a sudden cardiac arrest, probably due to an arrhythmia and could not be resuscitated. The second patient was an 18-year-old girl, who presented with manifestation of severe dengue, (hypotension, respiratory distress and altered sensorium). She was intubated, admitted in ICU and succumbed to progressive respiratory compromise and superadded infection. We presume the second patient had a superadded bacterial respiratory infection at presentation, and was started on adequate antibiotics. Both patients had presented late into disease onset and after prior treatment at other hospitals. Table 10 highlights some important parameters of the two deceased patient in our study.

The median duration of hospital stay in our study for the patients who were admitted were 4 days (3-30 days). For most patients with thrombocytopenia, we usually discharge once the platelet counts start to show an increment with amelioration of other major symptoms. Patients followed up in the outpatient department had at least one follow up after normalisation of platelet counts and symptom amelioration.

Parameters	**Patient 1**	**Patient 2**
Age/Gender	48/female	19/female
Comorbidities	Nil	Nil
Date of presentation	10	14
Temp	Afebrile	Afebrile

Clinical manifestations:	Myalgia, headache Rash/ Vomiting Progressive breathlessness in ward	Myalgia, headache No rash, vomiting Breathlessness Altered sensorium
Clinical findings:	Pulse-112/min BP- 100/60 mmHg RR-30/min, Sat- 96% RS/PA/CNS/CVS: Normal	Pulse-119/min BP-94/60 mmHg RR-34/min Sat-90% R/S: Bilateral mild pleural effusion CNS: Altered
Laboratory parameters: Hb (gm/dl) Total WBC (/cu mm) Initial platelet count (/cu mm) Initial creat (mg/dl) Initial Total/direct bilirubin (mg/dl) Initial SGOT/SGPT (IU/L) Dengue serology Day1 SOFA Highest SOFA Highest SGOT/SGPT (IU/L) AKI/RRT Transfusion requirement Respiratory support ICU care **ADAMTS 13** **vWF:CBA**	 17.2 7400 17000 1.94 1/0.4 2800/920 NS1Ag/IgM/IgG + 6 7 2800/920 Yes/No Yes Yes (Non invasive) Yes 24.2 % 180.5%	 10.8 37600 47000 1.7 9.1/7.8 1284/400 IgM/IgG + 8 21 1287/400 Yes/Yes Yes Yes (Invasive) Yes 22.8 % 297.5%

Table 10: Clinical data and laboratory parameters of the 2 deceased patients in our study

Analysis of outcomes:

The statistical methods used for analysis have been described before. We looked at demographic parameters, presence of comorbidities, clinical manifestation and their association with dengue severity. We also looked at how levels of ADAMTS affected dengue severity, the highest SOFA scores and lowest platelet values. As the mortality was very low, statistical analysis of factors affecting mortality could not be analysed in the present study.

None of the demographic parameters showed any significance to disease severity. Presence of any erythematous blanching rash at presentation had an association with disease severity. The other clinical symptoms were not significantly associated. The subsequent tables will depict the significance of different demographic, symptoms, signs and laboratory parameters to dengue severity.

Parameters	***p* value**	**Parameters**	***p* value**
Gender	1	Abdominal pain	0.088
Type 2 DM	0.564	Nausea/vomiting	0.136
Hypertension	0.8	Loose stools	0.493
Erythematous rash	**0.008**	CNS symptoms	1
Bleeding	0.2	Petechiae	1
Cough	0.159		
Breathlessness	0.180		

Table 11a: Depicting the significance of demographic parameters and clinical symptoms to dengue severity on univariate analysis

Blanching rash seemed to herald a more severe form of dengue with an OR of 17 (95% CI= 1.9-66.5). Lower systolic/diastolic blood pressure, respiratory rate at presentation were not associated with increased dengue severity.

Parameters	***p* value**
Ascites	0.18
Abdomen tenderness	0.098
Pleural effusion	0.1

Table 11b. Abnormal clinical findings and their association with dengue severity on univariate analysis

We evaluated multiple laboratory and outcome parameters and analysed their association with dengue severity. On univariate analysis, the following parameters had a significant odds ratio:

- Platelet count at presentation
- Lowest platelet values
- Urea at presentation
- Total and direct bilirubin (TB/DB) at presentation
- Highest SGOT and SGPT levels

Parameters	***p* value**	**Parameters**	***p* value**
Platelet counts at presentation	0.001	SGOT/SGPT (highest)	0.001/0.008
Lowest platelet counts	0.001	TB/DB at presentation	0.001/0.021
Urea	0.002		

Table 11c: Basic laboratory parameters and its association with disease severity on univariate analysis

Other factors like hemoglobin at presentation, total WBC counts, serum electrolytes, serum albumin did not have a significant bearing on disease severity. Presence of proteinuria appeared to be significant (*p=0.010).* We also evaluated the presence of acute kidney injury, need for transfusion requirement and vasopressor use, Day 1 and highest SOFA scores to disease severity. On factors significant on univariate analysis we did a multivariate analysis.

Parameters	***p* value**	**Parameters**	***p* value**
SOFA Day 1	**0.0001**	AKI/RRT	**0.039**/1
SOFA highest	**0.0001**	Transfusion support	**0.048**
ICU care	0.247	Vasopressor requirement	1

Table 11d: Monitoring score and organ failure scores and their association with dengue severity on univariate analysis

SOFA values both on Day 1 and the highest SOFA obtained by each patient strongly associated with the severity of dengue. The presence of an acute kidney injury at any point or the requirement of any form of transfusion support was also associated with more severe disease. Finally neither ADAMTS 13 values on the day of presentation/enrollment, nor pregnancy had any bearing on disease severity. ADAMTS 13 values were also not significantly associated with the lowest platelet count achieved. Though a negative

correlation existed between ADAMTS 13 values and the highest SOFA score, it was not statistically significant. Fig 14a and 14b depict the scatter plots showing the relation of ADAMTS 13 to lowest platelet and highest SOFA respectively.

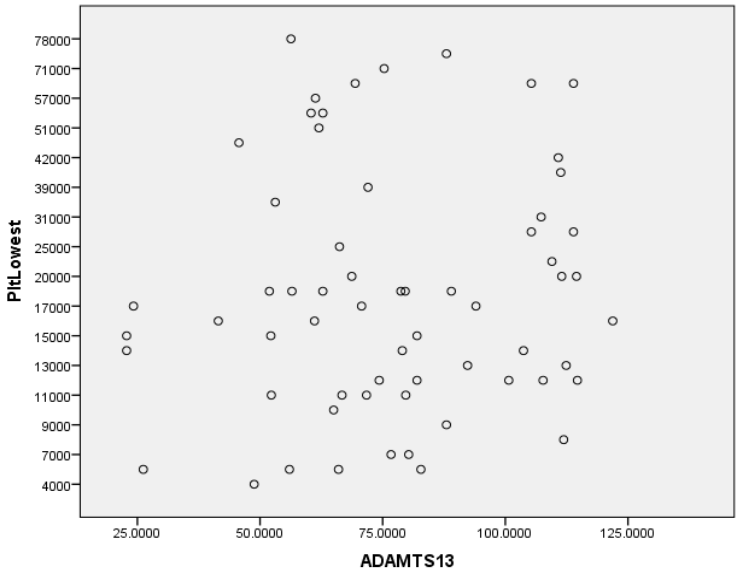


Fig 14a

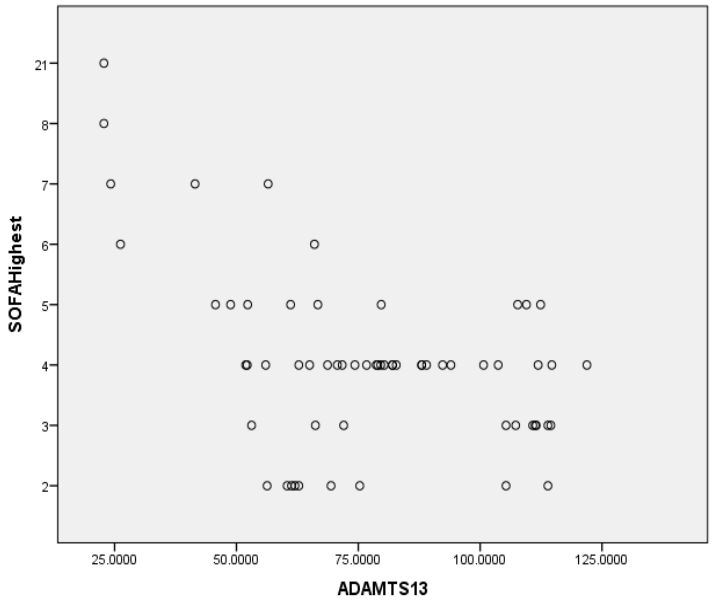


Fig 14b

Fig 14a and 14b: Scatter plots depicting the association of ADAMTS 13 values with the lowest platelet counts and highest SOFA scores

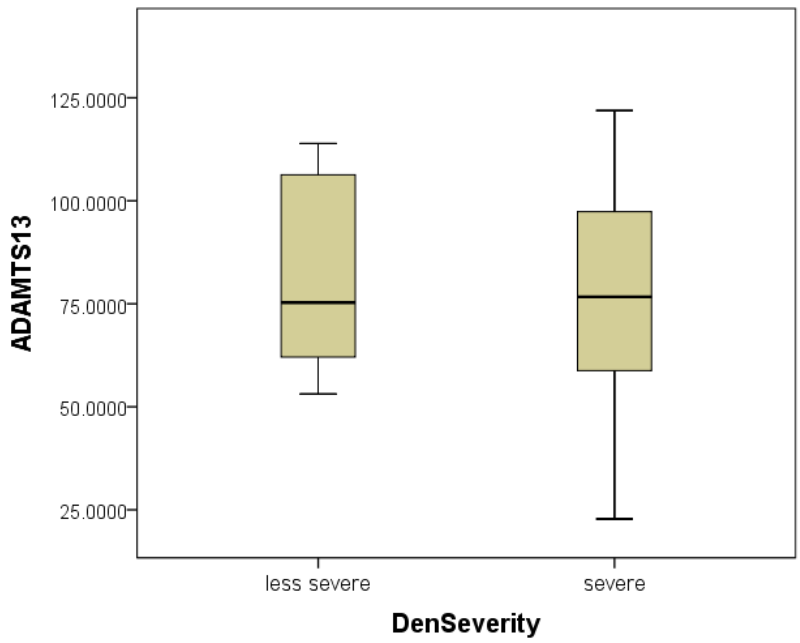


Fig. 15: Box plot diagram showing no significant difference in ADAMTS 13 levels between the less and dengue severity (DenSeverity)

Summarizing, ADAMTS 13 levels did not have any association with dengue severity, lowest platelet levels attained or the highest SOFA scores. However higher levels of ADAMTS 13 were associated with lesser transfusion requirement: OR of 0.97 (95% CI: 0.94-0.99, p= 0.02).

We also analysed the role of dengue diagnostic serology in predicting disease severity. Presence of IgG positivity was not associated with increased dengue severity, nor was any combination of NS1Ag or IgM positivity. Though secondary dengue infection has been described to be more commonly severe, our study failed to show the same based on IgG positivity.

In the following table we discuss the factors described above that were significant on multivariate analysis.

Parameters	Odds ratio (OR)	95% C.I
Rash	11.25	1.9-66.5
Proteinuria	5.4	1.5-18.8
SOFA Day 1	4.7	1.87- 12.2
Total bilirubin (On day of presentation)	133.1	4.6-3852.3

Table 12: Parameters significantly associated with dengue severity *on multivariate analysis*

As depicted in the preceding table SOFA on Day 1 was associated with dengue severity. The wide confidence intervals were secondary to the small numbers in our study. Certain other parameters like highest levels of transaminitis also showed an association but were not statistically significant on multivariate analysis. All these factors have been previously showed to predict dengue severity and negatively affect outcomes.

Analysis of pregnancy related factors:

Pregnancy was not associated with increased dengue severity. Comparison of ADAMTS 13 values between pregnant and non-pregnant women did not show any difference. The median ADAMTS 13 level was 70.3 (60.4-113.9), compared to 78.65 (22.8-121.9) in non-pregnant women with dengue. However with only 6 pregnant women in our cohort of patients it is impossible to come to any form of significant conclusion.

Discussion

The present study though limited by small numbers is the first study in adult dengue patients looking at ADAMTS 13 levels and its association with dengue severity. In this study we looked at several parameters that have been previously associated with dengue severity; from demographic variables, clinical features and laboratory findings. Though we had only 6 pregnant patients we evaluated the levels of ADAMTS 13 levels in this group of patients and compared the same to non-pregnant women with dengue in our cohort.

A total of 105 patients were initially selected who had presented to us with an acute febrile illness and thrombocytopenia. Amongst these 74 were positive for either NS1 antigen and/or IgM Dengue. Of these 74 patients, 12 were excluded as mentioned in the STROBE diagram, and 62 were included for the analysis. Based on a previous study we had assumed a total of 104 patients would be required to achieve an 80% power for this study to analyze the association between ADAMTS 13 levels and dengue severity. However in the 15-month time period we were unable to get enough subjects. As has been mentioned in the literature review before, seasonal variations of vector propagation, prior immunological background of the community plays an important role in yearly disease incidence and severity.

In the year 2015, the district of Vellore had seen a surge in dengue cases especially in the peri monsoon months. However 2016 had significantly lesser incidence of dengue despite heavier rainfall. Early April 2017 onwards there were surges in dengue cases at our centre.

We considered the following possibilities for the same:

1) Significant control measures to curb vector propagation at community levels post the 2015 dengue epidemic
2) Potent immunological memory in the community on recent dengue exposure attributing also to relatively lesser severity of established cases
3) In view of lesser severity (as mentioned above), lesser presentation to hospital and reporting appearing as lesser disease incidence

Demographic patterns:

Our study did not show any significant relation of disease severity to age, gender and presence of diabetes/hypertension. Multiple prior studies have shown elderly and presence of co-morbidities to correlate with dengue severity. However, only a small proportion of our patients had comorbidities and that too without any significant organ dysfunction. This might undermine the role of the above. The median age of dengue in our study replicates most large epidemiological studies. The disease commonly affects young adults and males; individuals who spent a considerable amount of time outdoors in our country, leading to more contact with vectors and disease incidence (77). No patients had any prior history of dengue, malaria or any other mosquito borne arboviral illness. Bigger numbers of study subjects can help us in identifying more demographic parameters that can determine outcome.

Clinical presentation:

In the absence of laboratory facilities, clinical presentation is the strongest determinant in the assessment of dengue severity. Dengue being more often a disease of the tropics and developing nations, clinical judgment drives treatment decision and prognostication. Our study showed that almost all patients had headache, myalgia and fatiguability, manifestations that are common in most viral infections. Other studies from both South and Northern India have shown similarly (78–80). However the symptoms are more severe and protracted in dengue.

The median duration of presentation of subjects in our study was at 5 days after symptoms onset. Individual who presented late had mostly been under treatment elsewhere and had presented in view of either worsening symptoms or rapidly dropping platelet values. Our centre being a referral tertiary care centre often have patients who come after having been diagnosed elsewhere and treatment initiation. This delays their index visit, alters symptomatology and laboratory parameters. However our median duration of 5 days is early enough given that most dengue fever symptoms start occurring usually towards the end of the first week nearing defervescence.

A significant proportion of patients had an erythematous blanching rash, characteristic of dengue. In our study, the presence of an early rash showed a significant association with disease severity. Most bleeding manifestations were minor, while GI bleeding in the form of maleana was the most common major bleeding manifestation. Respiratory symptoms were present only in a minority of patients, mostly in the form of cough. Cough can be a

manifestation of capillary leak in dengue and can predict severity, however our study failed to show so. A significant proportion of patient had GI symptoms manifesting as nausea/vomiting and loose stools. Both of these contribute to dehydration secondary to poor oral intake and increased fluid losses and can push non-severe dengue to more severe forms and cause renal dysfunction. Non-specific abdominal pain is also a manifestation of capillary leak and can quickly progress to shock. Though these are salient clinical signs of severity, in our study they did not show any significant association with severe dengue.

Most patients had tachycardia at presentation with the median heart rate being 93/min. Median systolic and diastolic blood pressure values were low normal as is expected with dengue. A clinical rash was discernable in 88.7% patients with significant odds for severe dengue ($p=0.002$). The presence of any positive respiratory, abdominal or CNS finding did not correlate with dengue severity. Severe forms of dengue are usually associated with capillary leak and subsequent pleural effusion, free fluid in the abdomen and their antecedent symptoms. Possibly the initiation of early resuscitation ameliorated the progression of the pathology in these patients and thus these factors did not have a significant association in our study with dengue severity. For most patients the majority of such symptoms and signs were present at admission, and a very few (6.4%) developed new symptoms or findings during their ward stay. Clinical evaluation was done by the principal investigator and the attending physician on a daily basis and SOFA scores calculated.

We compare in the following table the clinical presentation of sour study subjects with other studies from India.

Clinical parameters	**Laul et al (79)**	**Daniel et al(78)**	**Deshwal et al(80)**	**AVID study**
State/centre	Delhi	Kerala	Uttar Pradesh	Tamil Nadu
Year	Jun-Aug 2015	Jun-Dec 2003	Aug-Dec 2013	May16-Aug 17
Study number	115	250	515	62
Median age (yrs)	30	42.6	21-40	22
M: F (N)	64/51	130/120	376/139	40/22
Duration of symptoms (days)	Not known	6	Not known	5
Symptoms: %				
Headache	100	77.2	94.8	100
Myalgia	86	-	90.7	100
Bleeding (any)	21	15.2	5.4	23.6
Rash	21	13.2	37.9	88.7
Cough	-	-	-	21
Breathlessness	19	-	5.2	11.3
Nausea/vomit	68	-	5.4	53.2
Abd pain	57	62.4	24.5	25.8
Loose stools	27	15.2	2.5	21
CNS symptoms	2	0.8	1.6	1.6
Clinical/radiological findings: (%)				
Pleural effusion	15	33	20	14.5
Ascites	1	30	16.3	11.3
Dengue severity: (%)				
Non severe dengue	73	66.4	Not mentioned	82.2
Severe dengue (includes DHF/DSS)	27	33.6		17.8

Table 11: Comparison of clinical and laboratory parameters in dengue subjects of prior published study from India

Laboratory parameters:

Our study looked at multiple laboratory parameters at presentation and subsequent stay. Certain parameters that were found significant included the platelet count at presentation, lowest platelet counts, highest transaminitis, bilirubin levels at presentation, abnormal urinanalysis and presence of any form of kidney injury. On multivariate analysis total bilirubin on the day of presentation, and proteinuria were significantly associated with dengue severity. The analysis of platelet counts at presentation and lowest platelet counts had a confidence interval of 1 on multivariate analysis, rendering it statistically insignificant.

Though thrombocytopenia is a disease defining manifestation in dengue the absolute values often do not direct correlate with disease severity. Rapidly dropping platelet counts have been shown to herald the onset of severe dengue. *In this study initial and lowest platelet values showed significant association with disease severity on univariate analysis.* Though the initial platelet value was without any prior transfusion, some patients had been supported with platelet rich concentrates post presentation, rendering the lowest platelet counts not a thoroughly transfusion unsupported value.

Median (Range)	**Non severe dengue**	**Severe forms of dengue**	**P value**
Platelet on day of presentation (/ cumm)	58,000 (19000-95000)	27,000 (4,000-93000)	**0.008**
Lowest platelet counts (/cumm)	53,000 (6000-78000)	14000 (4000-77000)	**0.0001**

Table 12: Median platelet values at presentation and lowest platelet values in the two groups of dengue patient stratified according to severity and univariate analysis

ADAMTS 13:

Our data failed to show any significant difference in the ADAMTS 13 levels between the two groups of dengue patients as described before. The possible explanations include:

1) Difference in the day of presentation after symptom onset
2) Lack of biological association
3) Confounding factors (level of liver dysfunction, acute kidney injury etc.)
4) Small numbers

Further analysis showed a significant association between ADAMTS 13 levels and transfusion requirement. Larger studies will be needed to assess gender specific difference, variations based on the day of presentation and sample collection and other confounding factors to comment on the role and association of ADAMTS 13 in dengue severity.

SOFA score:

We used SOFA score in our study to assess the disease severity at presentation and subsequently for daily follow up. We calculated the highest SOFA score attained by each subject. SOFA score has been studied extensively in dengue as has been described before (73,74). In the present study SOFA score on Day 1 and the highest SOFA scores showed significant association with dengue severity on univariate analysis, while the SOFA score on Day 1 showed significance on multivariate analysis. SOFA can thus be used as a good tool for early assessment of disease severity, planning of inpatient versus outpatient care or referral in resource poor settings. As mentioned before, the presence of thrombocytopenia as

one of its variables, when it is a disease defining manifestation in dengue might overestimate risk. The second caveat with SOFA score is the need for monitoring of platelets and bilirubin, which is often not possible on a daily basis. Even in our study, while calculating the daily SOFA scores the latest laboratory parameters (platelet and bilirubin) were used on those days when these tests were not conducted.

qSOFA is a point of care clinical tool that has been studied and validated in patients with suspected sepsis outside the ICU setting (81). The role of qSOFA in dengue has not been adequate looked at. It eliminates the need for laboratory tests and can be used even on field settings and emergency wards. However incorporation of dengue specific parameters might make it more prudent for its use in assessing dengue severity and large studies will be need to establish and validate such a score. As of now SOFA score appears as a valuable tool to assess for dengue severity at presentation while W.H.O. dengue severity scales can be used for a more dynamic assessment.

Pregnancy and dengue:

We intended to study the effect of pregnancy on dengue and differences in ADAMTS 13 levels in pregnant dengue patients compared to non-pregnant women. Our data did not show any significant any difference in dengue severity in the pregnant population and also in the ADAMTS 13 values. However gestation specific analysis of ADAMTS 13 values might throw more light on its levels in pregnancy and role in dengue severity, which could not be done in the present study due to small numbers.

Study limitations

Our study had few significant limitations. We were unable to reach the desired number of study subjects in view of significant variation in dengue incidence after study initiation. In the limited time period we recruited 62 subjects who fulfilled the inclusion criteria. Thus several parameters described before which were not statistically significant might actually be so when done in large numbers. We had wide confidence intervals of odds ratio (OR) on multivariate analysis in view of our small numbers.

We had initially conceptualized a serial monitoring of ADAMTS 13 and vWF:CBA levels on all patients recruited for the study. However due to logistic and funding issues we could perform the above tests only on day 1. A serial monitoring of ADMATS 13 levels might provide insight about the ongoing dynamic changes and may help to mirror better the thrombocytopenia and disease severity.

The number of pregnant subjects in our study was very small which precluded significant statistical analysis. Larger numbers of subjects need to be studied to assess the differences in ADAMTS 13 levels in this subgroup of patients who usually have a higher propensity for severe disease.

Conclusion

The AVID study aimed to look at the role of ADAMTS 13 and its association with severity of dengue infection. We also aimed to look at the association of ADAMTS 13 and transfusion requirement; the difference in their levels in pregnant women with dengue amongst other previously studied parameters. It is the first study in adult dengue patients measuring ADAMTS 13 levels and analyzing its association with disease severity. We also aimed to analyse SOFA score and its correlation to ADAMTS 13 levels in dengue.

In our study low levels of ADAMTS 13 were associated with increased transfusion requirement, but not to dengue severity either by W.H.O. grading or by SOFA score. SOFA score on Day 1 showed significant association to W.H.O. grading of dengue severity on multivariate analysis. We used dichotomous outcome variables by clubbing non severe dengue with warning signs and severe dengue into one pool of "more severe dengue" and non sever dengue with no warning sign as "less severe dengue.'

Though we had only 6 pregnant patients, ADAMTS 13 levels were not significant different in them compared to the non-pregnant women. Pregnancy per se did not negatively affect dengue severity.

Deficiency of ADAMTS 13 is only one of the many mechanisms implicated in thrombocytopenia of dengue and its possible role in propagating dengue pathogenesis. Its role though appears biologically plausible, needs to be studied in further details to implicate it strongly in dengue. Treatment decisions and transfusion practices will change once a causal relationship between the two is strongly established.

Future direction:

Large population and hospital-based studies are warranted to study appropriately the variation of ADAMTS 13 levels in dengue and its association with disease severity, level of thrombocytopenia and other organ system injury. Factors that can confound ADAMTS 13 levels like age, blood group, ethnicity should be considered to derive algorithms that accurately mimic its role in dengue. Normative data from population should be derived to assess trimester-based changes of ADAMTS 13 levels in pregnant women. These should then be used to analyse the differences in the levels of ADAMTS 13 in pregnant women with dengue and non-pregnant women.

As dengue continues to be a global threat and even more so for India, studies aiming to find new disease severity markers are important as are scoring systems that can help in early disease assessment and modulate treatment decisions.

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Annexure 1: IRB approval / Page 1

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

August 05, 2016

Dr. Jayastu Senapati,
Post Graduate Registrar,
Department of Medicine - 3,
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
ADAMTS 13 levels and von Wille brand Factor (vWF) collagen activity in Dengue fever
Dr. Jayastu Senapati, Emp. No: 29640, PG Registrar, Dr. Sowmya Sathyendra, Emp. No: 28181, Medicine 3, Dr. Peter John Victor, Professor, Emp. No: 13328, Department of Medical I.C.U. Dr. Sukesh Chandran, Professor, Emp. No: 13758, Department of Transfusion Medicine and Immuno Haematology, Dr. Biju George, Professor, Emp. No: 30156, Department of Clinical Haematology, Dr. Annie Regi, Professor, Emp. No: 11190, Department of Obstetrics and Gynaecology, Unit III, Dr. Reeta Vijaya Selvi Associate professor, Emp. No: 50600, Department of Obstetrics and Gynaecology Unit IV, Dr. Santosh Joseph, Benjamin, Associate Professor, Emp. No: 31318, Obstetrics and Gynaecology Unit V, Dr. Alice Joan Mathuram, Associate Professor, Emp. No: 28529, Department of Medicine Unit I, Dr. Vignesh Kumar C. Assistant Professor, Emp. No: 33782 Department of Medicine Unit II, Dr. Cijoy K. Kuriakose, Assistant Physician, Emp. No: 33909, Department of Medicine Unit IV, Dr. Ramya I.Professor, Emp. No: 31571 Department of Medicine Unit V Dr Visalakshi Jeyaseelan, 31093,Biostatistics

Ref: IRB Min No: 10025 [OBSERVE] dated 04.04.2016

Dear Dr. Jayastu Senapati,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Sowmya Sathyendra, Department of Medicine - 3, CMC, Vellore. 1 of 4

IRB approval / Page 2

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

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Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on April 04th 2016 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore - 632002.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist &Epidemiologist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil,BL	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. B. J. Prashantham	MA(Counseling Psych MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Chri Counseling Centre, Vellore	External, Social Scientist
Dr. Rajesh Kannangai	MD, PhD.	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MD, DNB(Endo), Phd(Endo)	Professor, Endocrinology, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse

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Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Sathish	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of with drawals for the study entitled: "ADAMTS 13 levels and von Wille brand Factor (vWF) collagen activity in Dengue fever" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 99,700/- INR (Rupees Ninety nine thousand seven hundred Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 49,700/- INR (Rupees Forty nine Thousand seven hundred only) will be released at the end of the first year as 2 nd Installment

Yours sincerely

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10025 [OBSERVE] dated 04.04.2016 4 of 4

Annexure 2: Patient information sheet

1) What is Dengue?

Dengue is a common mosquito borne illness that is spread by the bite of Aedes aegypti mosquito. The mosquito stays in marshy and water clogged areas and usually bites in the daytime.

2) What are the symptoms of Dengue?

Dengue presents as an undifferentiated febrile illness, which ranges from only mild fever with fatiguability requiring an outpatient basis treatment to a severe illness with multi-organ dysfunction requiring hospital admission and organ support.

Usually dengue presents with high grade fever, a rash over the chest and abdomen, muscle pains, headache, eye pain and severe fatiguability. There may be bleeding from the nose or oral cavity. Severe forms can present as above with cough, breathlessness, abdominal pain, decreased urine output and altered consciousness.

3) What is ADAMTS 13 and von Willebrand factor collagen activity

ADAMTS 13 is a protein present in blood, which together with Von Willebrand factor takes role in blood coagulation. Blood coagulation is a process by which excessive bleeding is prevented in case of any injury to the blood vessels and requires interplay of many proteins, blood cells and the blood vessels.

4) What does this study aim to do?

The present study aims to find out an association between the levels of the action of the above 2 proteins and the severity of Dengue fever

5) In which way will this study affect my treatment at CMC Vellore?

The study will not affect your treatment in CMC Vellore Hospital in any way. In the event

that you wish to participate in the study, your blood samples will be taken at your presentation to CMC at the time of your other routine sampling with no additional poke .The above sample will be stored and if you test positive for Dengue, these samples will be analysed for ADAMTS13 levels and vWF collagen activity. In the event you need admission you will be followed up the doctor (principal investigator) in the ward to check your progress. Your treatment will not be influenced in this study.

6) **How will this study benefit the treatment of Dengue?** The study will help to understand the underlying mechanism of disease severity in Dengue fever and also aims to understand the reason why platelets decrease in dengue fever. This might help to positively change treatment for Dengue fever in the future.

For any further clarification please feel free to contact the principal investigator at: Dr. Jayastu Senapati Email- jsalwayswins@gmail.com

Mobile number- 07639831089 MD Resident, Department of Medicine, Unit III Christian Medical College and Hospital, Vellore.

Annexure 3: Patient consent form

AVID Study

Informed Consent form to participate in a research study

Study Title: ADAMTS 13 levels and von Willebrand Factor (vWF) collagen activity in Dengue fever

Study Number: _____

Subject's Initials: _____ Subject's Name:

_____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

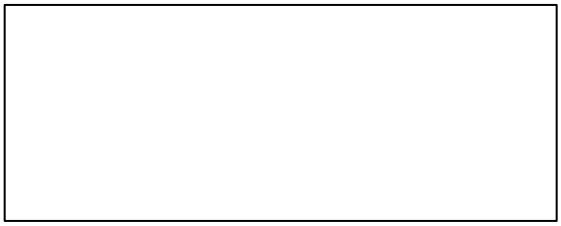
(iv) I agree not to restrict the use of any data or results that arise from this study provided such a useis only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or (Thumb sign)



Representative: _____ Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____ Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

Annexure 4: Data abstraction sheet

Clinical proforma for AVID Study
All patients

Study title: ADAMTS 13 levels and von Willebrand Factor (vWF) collagen activity in Dengue fever

Study number-

Demographics:

Name- Gender- M/F

Age-

Address: City- District- State-

Occupation- 1) Professional 2) Skilled 3) Semi skilled 4)Labourer/Unskilled

: Define-

Medical past history:

Comorbidities:

1) Diabetes- [1] Type 1 [2] Type 2 [3] Pancreatic [4] LADA [5] Drug induced [6]Type not defined
2) Hypertension- [1] Essential [2] Secondary
3) Cardiovascular disease-
   [1]Old ACS [2] Diagnosed IHD [3] Congestive cardiac failure [4] Valvular heart disease [5] Others
4) Cerebrovascular disease-
   [1] Old CVA [2] Old TIA [3] Neurodegenerative disease [4] Others
5) Chronic kidney disease- [1] On RRT [2] Not on RRT
6) Endocrinopathy- [1] Thyroid disorders (Hypo/Hyper) [2] Cushing's [3] Acromegaly [4]Others
7) Infections- [1] TB [2] Other requiring hospitalization
   If [1]: a) Cured b) On treatment c) Defaulted
   If [1]: a) Pulmonary b) Other
8) Prior surgery- Y/N
9) Prior Dengue infection: Y/N: If Y how was it diagnosed- [1] clinical [2] serological [3] unknown
10) Prior transfusion: Y/N: If Y what was the indication: Define-

Personal history:

1) Smoker- Y/N: If Y [1] Present [2] Ex

2) Alcohol consumption: Y/N: If Y [1] Dependence pattern [2] Non dependence pattern
3) Significant family history: Y/N: If Y: Define-

Present Medical History:

1) Fever: Duration at day of presentation: (Number)
   Temp at presentation-
2) Rash : Y/N/ Not noticed
3) Bleeding manifestation: Y/N: If Y- [1] major [2] minor
   (Major- Hematemesis, hemoptysis, hematuria, hematochezia, maleana)
   (Minor- Epistaxis, Oral bleed, Sub conjunctival bleed, skin purpura/petechiae)
4) Headache: Y/N
5) Retro-orbital pain: Y/N
6) Myalgia : Y/N
7) Fatiguability: Y/N
8) Respiratory and cardiovascular:
   Cough : Y/N **Breathlessness** : Y/N **Chest pain**: Y/N **Palpitations** : Y/N
   Facial puffiness: Y/N **Pedal edema** : Y/N **Subjective decrease in urine output**: Y/N
9) Gastrointestinal:
   Abdominal pain: Y/N **Abdominal distension** (subjective) Y/N
   Vomiting: Y/N **Nausea**: Y/N **Loose stools**: Y/N **Constipation**: Y/N **Tensemus**: Y/N
10) Neurological:
   Altered sensorium: Y/N **Seizures**: Y/N **Focal deficits**: Y/N
   Others: YN: If Y Define-
11) Outside evaluation and treatment:
   Platelet count: Index presentation day from onset of fever:
   Serology: [1] NS1Ag : +/- [2] Dengue IgM : +/-
   Outside transfusion support: Y/N: If Y: Platelets- ____ Packed cells- _____ FFP- _____ WB- ____

Clinical Examination:

At index presentation:
Casualty/ OPD/ Ward

1) Pulse-
   Character- [1] Normal volume [2] Bounding [3] Thready
2) BP- RUL:
3) Respiratory rate:

Saturation (room air) –

4) Skin: [1] Blanching rash -Y/N
[2] Petechiae - Y/N

5) Bleeding manifestation: [1] Major- Define-
[2] Minor- Define-

6) GCS- /15

7) Respiratory system:
Crackles: Y/N: If Y: [1] Basal [2] up to middle 2/3 [3] Diffuse
Decreased air entry: Y/N: If Y, define side- R/L/ Both
Bronchial breath sounds: Y/N
Wheeze: Y/N

8) Abdominal:
Tenderness: Y/N: If Y: Guarding: Y/N
Free fluid: Y/N

9) CNS examination:
In hospital seizure: Y/N
Focal deficits: Y/N
Other: Y/N: If Y, define-

10) SOFA score at admission:
Respiratory: Coagulation: Liver: CNS: Renal: ***Cumulative:***

SOFA score	0	1	2	3	4
Respirationa PaO_2/FIO_2 (mm Hg) SaO_2/FIO_2	>400	<400 221–301	<300 142–220	<200 67–141	<100 <67
Coagulation Platelets $10^3/mm^3$	>150	<150	<100	<50	<20
Liver Bilirubin (mg/dL)	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Cardiovascular[b] Hypotension	No hypotension	MAP <70	Dopamine </=5 or dobutamine (any)	Dopamine >5 or norepinephrine </=0.1	Dopamine >15 or norepinephrine >0.1
CNS Glasgow Coma Score	15	13–14	10–12	6–9	<6
Renal Creatinine (mg/dL) or urine output (mL/d)	<1.2	1.2–1.9	2.0–3.4	3.5–4.9 or <500	>5.0 or <200

Investigations: On day of presentation

1) Hb- gm/dl 2) WBC- /cu mm :- N- L- M- E- B- My- Pro- BF-

3) Platelet (Coulter) - /cu mm (Manual- /cu mm)

4) LFT- TB: DB: TP: Alb: AST: ALT: ALP

5) Creatinine: /mg dl Urea- /mg dl

6) Na- meq K- meq

7) PT- INR- aPTT-

8) Urinanlysis:
[1] Bland [2] hematuria [3] Pyuria [4] Both 2 and 3

9) ABG: If done
pO2- BE= Lac=

10) C/S Blood-

11) Dengue serology: [1] NS1Ag +/- [2] Dengue IgM +/- [3] Dengue IgG +/-
12) Other serology: Y/N : If Y, define-

13) Miscellaneous:
ECG-
Chest Xray-
Troponin T- CKMB- Malarial Parasites-

USG abdomen-
14)

Serial monitoring of in patients										
Days →	1	2	3	4	5	6	7	8	9	10
Modality										
SOFA										
Platelet										
PT/INR										
aPTT										
Creat										
AST/ALT										
Organ support: Ventilation Y/N If Y - [1]NIV/[2] INV										

RRT Y/N										
Transfusion:Y/ N If Y Platelet= FFP= Cryo= Packed cells=										
ADAMTS 13										
vWF collagen activity										

14) Overall outcome: [1] Discharged cured [2] died [3] Discharged against advice
Duration of stay:

Additional data for pregnant patients

1) Gestational score:

2) Gestational age on day of presentation:

3) Pregnancy complications: Y/N: If Y, [1] PIH [2] GDM [3] Pre-ecclampsia/Ecclampisa [4] HELLP [5] Prior complicated pregnancies [6] Twin pregnancy [7] Pregnancy related liver disease [8] Infections [9] Artificial conception

Annexure 5: Data sheet/ Page 1

	Name	HospNum	sex	POCare	Age	State	Occupation
1	HEMANTH KUMAR	527431G	1	2	22	TAMIL NADU	STUDENT
2	MD MUSFIKUR RAHMAN BALY	664178G	1	2	44	BANGLADESH	BUSINESSMAN
3	RAGHUL M	392696B	1	2	20	TAMIL NADU	STUDENT
4	SHAVNIK SINGH	532423G	1	1	20	DELHI/VELLORE	STUDENT
5	GOKUL P	541913G	1	2	19	TAMIL NADU	STUDENT
6	KOUSHICK T	561970G	1	2	19	TAMIL NADU	STUDENT
7	BANU PRIYA	549244G	2	2	32	TAMIL NADU	HOUSEWIFE
8	MAHABOOB BEE SHAIKH	548991G	2	2	44	ANDHRA	HOUSEWIFE
9	SHUMAILA NOORAIN	634592G	2	2	26	TAMILNADU	HOUSEWIFE
10	BANDI SAHITHI	549868G	1	2	18	ANDHRA	STUDENT
11	NIKIL B	558555G	1	2	18	ANDHRA	STUDENT
12	SHAIK MUNNIJA BEGUM	534760G	2	2	21	ANDHRA	HOUSEWIFE
13	PALANI M	541914G	1	2	51	TAMILNADU	LABOURER
14	ELUMALAI	548373G	1	2	35	TAMILNADU	DRIVER
15	SARASWATI SHARMA	561631G	2	2	25	BIHAR/TAMILNADU	HOUSEWIFE
16	SHANTHI	520550G	2	2	48	TAMILNADU	STUDENT
17	VIJI	386977B	2	2	21	TAMILNADU	STUDENT
18	MEIGNANAM	562934G	1	2	19	TAMILNADU	STUDENT
19	LUMINA EVANGELIN	691586D	2	2	29	TAMILNADU	NURSE
20	SATHYA D	562476G	2	2	22	TAMILNADU	HOUSEWIFE
21	SANTHA KUMAR	564217G	1	2	32	TAMILNADU	ARMYMAN
22	SHAMSHUR RAHMAN	564134G	1	2	52	TAMILNADU	PROFESSIONAL
23	RANJITHA	564287G	2	2	19	TAMILNADU	STUDENT
24	CHRISTOPHER RAJASEKHARAN	564572G	1	2	41	TAMILNADU	PROFESSIONAL
25	PAVITHRA K	566054G	2	2	25	ANDHRA	HOUSWEIFE
26	DHEEPAN GSR	863419G	1	2	17	TAMILNADU	STUDENT
27	SHANKAR E	566157G	1	2	27	TAMILNADU	TECHNICIAN
28	TARUGU PRAVEEN KUMAR	562064G	1	2	26	ANDHRA	STUDENT
29	GOPALA KRISHNAN	260624F	1	2	21	TAMILNADU	STUDENT
30	ABDUL ROPE G	566473G	1	2	84	TAMILNADU	RETIRED
31	PRATIBHA	425949D	2	2	31	TAMILNADU	HOUSEWIFE
32	ARUL J	548009G	1	1	25	TAMILNADU	LABOURER
33	SANTOSH KUMAR	566973G	1	2	20	ANDHRA	STUDENT
34	JOYDEB MONDAL	906170G	1	1	34	WEST BENGAL	SHOPKEEPER
35	NASEEMA ALAM	567775G	2	2	25	BIHAR	HOUSEWIFE
36	MUTHAMIZH SELVAM	562048G	1	2	42	TAMILNADU	MECHANIC
37	JAI KUMAR Y	567910G	1	2	34	TAMILNADU	TEACHER
38	TANVEER AHAMED	986810F	1	2	26	TAMILNADU	BUSINESS
39	NAVEEN KUMAR	930245G	1	1	27	TAMILNADU	LABOURER
40	RAJASEKAR P	078186G	1	2	54	TAMILNADU	SERVICEMAN
41	JAYALAKSHMI	930290G	2	2	41	TANILNADU	HOUSEWIFE
42	ESWARAMMA	942742G	2	2	42	ANDHRA	HOUSEWIFE
43	AASIMA TABASSUM	930413G	2	2	30	TAMILNADU	HOUSEWIFE
44	PALANISAMY K	930548G	1	2	38	TAMILNADU	CLERK
45	PUNNET KUMAR REDDY	930694G	1	2	16	ANDHRA	STUDENT
46	JASON JOHN DEVARAJ	930656G	1	2	20	KARNATAKA	STUDENT
47	RAJA RAJAN	930680G	1	2	32	TAMILNADU	LABOURER
48	VIJAY RAGHAVAN	930745G	1	2	30	TAMILNADU	BUSINESS
49	SANGEETHA LAKSHMI	930579G	2	2	35	TAMILNADU	HOUSEWIFE
50	MOHAMMED ARAFATH	930746G	1	2	21	TAMILNADU	STUDENT
51	SHEELA	930835G	2	2	35	TAMILNADU	HOUSEWIFE
52	CHRISTY P	545766G	2	2	26	TAMILNADU	DOCTOR
53	NAVIYARASAN	931111G	2	1	18	TAMILNADU	STUDENT
54	SHANKAR K	931126G	1	1	23	TAMILNADU	LABOURER
55	SIKANDER BARWA	954571G	1	1	28	JHARKHAND	SERVICEMAN
56	SATHYARAJ	931864G	1	1	29	TAMILNADU	BUSINESSMAN
57	MOHAMMAD RAFI	867152G	1	2	21	TAMILNADU	STUDENT
58	VENKATESAN	932811G	1	2	37	TAMILNADU	BUSINESSMAN
59	DHANIYA SAJIDA	577983G	2	2	40	TAMILNADU	HOUSEWIFE
60	MURUGAN A	933263G	1	2	28	KARNATAKA	SERVICEMAN
61	ZEENATH BEGUM	933282G	2	2	19	TAMILNADU	STUDENT
62	Y SUNDAR REDDY	933278G	1	2	28	ANDHRA	PROFESSIONAL

Data sheet/ Page 2

	Diabetes	Hypertension	CVD	CerebroVD	CKD	PriorTB	PriorDengue	PriorTrans	OtherComorb
1	N	N	N	N	N	N	N	N	NIL
2	N	N	N	N	N	N	N	N	NIL
3	N	N	N	N	N	N	N	N	NIL
4	N	N	N	N	N	N	N	N	NIL
5	N	N	N	N	N	N	N	N	NIL
6	N	N	N	N	N	N	N	N	NIL
7	N	N	N	N	N	N	N	N	NIL
8	N	N	N	N	N	N	N	N	NIL
9	N	N	N	N	N	N	N	N	NIL
10	N	N	N	N	N	N	N	N	NIL
11	N	N	N	N	N	N	N	N	NIL
12	N	N	N	N	N	N	N	N	NIL
13	N	N	N	N	N	N	N	N	NIL
14	N	N	N	N	N	N	N	N	NIL
15	N	N	N	N	N	N	N	N	NIL
16	N	N	N	N	N	N	N	N	NIL
17	N	N	N	N	N	N	N	N	NIL
18	N	N	N	N	N	N	N	N	NIL
19	N	N	N	N	N	N	N	N	NIL
20	N	N	N	N	N	N	N	N	NIL
21	N	N	N	N	N	N	N	N	NIL
22	Y	Y	N	N	N	N	N	N	NIL
23	N	N	N	N	N	N	N	N	NIL
24	Y	Y	N	N	N	N	N	N	NIL
25	N	N	N	N	N	N	N	N	NIL
26	N	N	N	N	N	N	N	N	NIL
27	N	N	N	N	N	N	N	N	NIL
28	N	N	N	N	N	N	N	N	NIL
29	N	N	N	N	N	N	N	N	NIL
30	N	N	N	N	N	N	N	N	NIL
31	N	N	N	N	N	N	N	N	NIL
32	N	N	N	N	N	N	N	N	NIL
33	N	N	N	N	N	N	N	N	NIL
34	N	N	N	N	N	N	N	N	NIL
35	N	N	N	N	N	N	N	N	NIL
36	Y	N	N	N	N	N	N	N	NIL
37	N	N	N	N	N	N	N	N	NIL
38	N	N	N	N	N	N	N	N	NIL
39	N	N	N	N	N	N	N	N	NIL
40	Y	N	N	N	N	N	N	N	NIL
41	N	N	N	N	N	N	N	N	NIL
42	N	N	N	N	N	N	N	N	NIL
43	N	N	N	N	N	N	N	N	NIL
44	N	N	N	N	N	N	N	N	NIL
45	N	N	N	N	N	N	N	N	NIL
46	N	N	N	N	N	N	N	N	NIL
47	N	N	N	N	N	N	N	N	NIL
48	N	N	N	N	N	N	N	N	NIL
49	N	N	N	N	N	N	N	N	NIL
50	N	N	N	N	N	N	N	N	NIL
51	N	N	N	N	N	N	N	N	NIL
52	N	N	N	N	N	N	N	N	NIL
53	N	N	N	N	N	N	N	N	NIL
54	N	N	N	N	N	N	N	N	NIL
55	N	N	N	N	N	N	N	N	NIL
56	N	N	N	N	N	N	N	N	NIL
57	N	N	N	N	N	N	N	N	NIL
58	N	N	N	N	N	N	N	N	NIL
59	N	N	N	N	N	N	N	N	NIL
60	N	Y	N	N	N	N	N	N	NIL
61	N	N	N	N	N	N	N	N	NIL
62	N	N	N	N	N	N	N	N	NIL

Data sheet/ Page 3

	Smoker	Alcohol	DOP	Temp	Rash	Bleeding	Headache	Myalgia	Fatiguability	Cough
1	N	N	7	98	Y	1	Y	Y	Y	N
2	N	N	5	101	Y	1	Y	Y	Y	N
3	N	N	6	101	Y	1	Y	Y	Y	N
4	N	N	4	100	Y	1	Y	Y	Y	N
5	N	N	6	98	Y	1	Y	Y	Y	N
6	N	N	4	100.8	N	1	Y	Y	Y	N
7	N	N	4	101	Y	1	Y	Y	Y	Y
8	N	N	10	102	Y	3	Y	Y	Y	N
9	N	N	4	100	Y	1	Y	Y	Y	Y
10	N	N	5	98	Y	2	Y	Y	Y	N
11	N	N	4	100	Y	1	Y	Y	Y	N
12	N	N	7	100	Y	1	Y	Y	Y	N
13	Y	N	10	101	Y	1	Y	Y	Y	Y
14	N	N	6	101	Y	1	Y	Y	Y	N
15	N	N	7	100	N	1	Y	Y	Y	N
16	N	N	10	98	N	1	Y	Y	Y	N
17	N	N	6	98	N	1	N	Y	Y	N
18	N	N	7	98	Y	2	Y	Y	Y	N
19	N	N	5	98	N	1	N	Y	Y	N
20	N	N	3	101	Y	1	Y	Y	Y	N
21	N	N	5	98	Y	1	Y	Y	Y	N
22	N	N	5	98	Y	1	Y	Y	Y	N
23	N	N	14	98	Y	1	Y	Y	Y	N
24	N	N	3	100	N	1	Y	Y	Y	N
25	N	N	5	98	Y	1	Y	Y	Y	N
26	N	N	4	100	Y	1	Y	Y	Y	N
27	N	N	6	98	Y	1	Y	Y	Y	Y
28	N	N	15	100.2	Y	3	Y	Y	Y	Y
29	N	N	4	100.6	Y	2	Y	Y	Y	Y
30	N	N	7	98	Y	3	Y	Y	Y	Y
31	N	N	5	98	Y	1	Y	Y	Y	N
32	N	N	5	98	Y	1	Y	Y	Y	N
33	N	N	5	100	N	1	Y	Y	Y	N
34	N	N	4	99.8	N	1	Y	Y	Y	N
35	N	N	9	98	Y	2	Y	Y	Y	N
36	N	N	5	98	Y	3	Y	Y	Y	N
37	N	N	5	98	Y	1	Y	Y	Y	N
38	N	N	3	101	Y	1	Y	Y	Y	N
39	N	N	7	100	Y	2	Y	Y	Y	Y
40	N	N	3	102	Y	1	Y	Y	Y	N
41	N	N	5	100	Y	1	Y	Y	Y	N
42	N	N	7	100	Y	1	Y	Y	Y	N
43	N	N	5	98	Y	2	Y	Y	Y	N
44	N	N	6	98	Y	1	Y	Y	Y	N
45	N	N	4	98	Y	2	Y	Y	Y	N
46	N	N	5	100	Y	1	Y	Y	Y	N
47	N	N	4	98	Y	1	Y	Y	Y	N
48	N	N	5	98	Y	3	Y	Y	Y	N
49	N	N	5	98	Y	1	Y	Y	Y	N
50	N	N	6	98	Y	1	Y	Y	Y	Y
51	N	N	5	100.4	Y	1	Y	Y	Y	N
52	N	N	2	101	Y	1	Y	Y	Y	Y
53	N	N	4	100	Y	1	Y	Y	Y	N
54	N	N	5	98	Y	1	Y	Y	Y	N
55	N	N	4	100	Y	1	Y	Y	Y	N
56	N	N	6	98	Y	1	Y	Y	Y	N
57	N	N	7	98	Y	1	Y	Y	Y	N
58	N	N	7	98	Y	3	Y	Y	Y	Y
59	N	N	5	98	Y	1	Y	Y	Y	Y
60	N	N	5	101	Y	2	Y	Y	Y	Y
61	N	N	4	98	Y	1	Y	Y	Y	N
62	N	N	3	101	Y	1	Y	Y	Y	N

Data sheet/ Page 4

	Breathlessness	ChestPain	DecreaseUO	AbdPain	NauseaVomit	LooseStools	AltSens	Seizures
1	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N
3	N	N	N	N	Y	N	N	N
4	N	N	N	N	N	N	N	N
5	N	N	N	N	Y	N	N	N
6	N	N	N	N	Y	N	N	N
7	Y	N	N	N	Y	N	N	N
8	Y	N	N	Y	Y	N	N	N
9	N	N	N	N	N	N	N	N
10	N	N	N	N	Y	N	N	N
11	N	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N
13	N	N	N	N	Y	N	N	N
14	N	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	N
16	N	N	N	N	Y	N	N	N
17	N	N	N	N	N	Y	N	N
18	N	N	N	N	Y	N	N	N
19	N	N	N	N	Y	Y	N	N
20	N	N	N	N	N	N	N	N
21	N	N	N	N	Y	N	N	N
22	N	N	N	N	N	N	N	N
23	Y	N	N	N	N	N	Y	N
24	N	N	N	N	Y	N	N	N
25	N	N	N	Y	Y	N	N	N
26	N	N	N	N	N	N	N	N
27	N	N	N	Y	Y	N	N	N
28	Y	N	N	Y	Y	Y	N	N
29	Y	N	N	N	N	N	N	N
30	N	N	N	N	N	Y	N	N
31	N	N	N	N	Y	N	N	N
32	N	N	N	N	N	N	N	N
33	N	N	N	N	Y	N	N	N
34	N	N	N	N	N	N	N	N
35	N	N	N	Y	Y	N	N	N
36	N	N	N	N	N	N	N	N
37	N	N	N	N	N	N	N	N
38	N	N	N	Y	Y	Y	N	N
39	N	N	N	N	N	N	N	N
40	N	N	N	N	N	N	N	N
41	N	N	N	N	N	N	N	N
42	N	N	N	Y	Y	Y	N	N
43	N	N	N	Y	N	N	N	N
44	N	N	N	N	N	N	N	N
45	N	N	N	Y	Y	Y	N	N
46	N	N	N	N	Y	Y	N	N
47	N	N	N	N	N	N	N	N
48	N	N	N	N	N	Y	N	N
49	N	N	N	Y	Y	N	N	N
50	N	N	N	N	N	N	N	N
51	N	N	N	Y	Y	N	N	N
52	N	Y	N	Y	N	N	N	N
53	N	N	N	N	Y	N	N	N
54	N	N	N	N	Y	N	N	N
55	N	N	N	N	Y	N	N	N
56	N	N	N	N	N	N	N	N
57	N	N	N	Y	Y	N	N	N
58	Y	N	N	Y	Y	Y	N	N
59	N	N	N	N	Y	Y	N	N
60	Y	N	N	Y	Y	Y	N	N
61	N	N	N	N	Y	N	N	N
62	N	N	N	Y	Y	Y	N	N

Data sheet/ Page 5

	Misc	Pulse	PulseCharacter	SBP	DBP	RR	SPO2	Blanchingrash	Petechi
1	NIL	88	1	90	60	20	99	Y	N
2	NIL	80	1	110	80	20	98	Y	N
3	NIL	128	1	90	60	20	98	Y	N
4	NIL	110	1	100	70	20	100	Y	N
5	NIL	80	1	110	70	18	98	Y	Y
6	NIL	120	1	100	60	22	99	N	N
7	NIL	110	2	100	60	26	93	Y	N
8	NIL	126	1	110	60	40	88	Y	N
9	PREGNANT	98	1	100	60	18	98	Y	N
10	NIL	100	1	120	80	34	97	Y	N
11	NIL	95	1	110	60	20	99	Y	N
12	PREGNANT	130	1	90	60	26	98	Y	N
13	NIL	110	1	90	60	18	99	Y	N
14	NIL	80	1	90	60	18	94	Y	N
15	PREGNANT	96	1	100	60	18	100	N	N
16	NIL	112	1	100	60	30	96	N	N
17	NIL	90	1	110	60	18	100	N	N
18	NIL	68	2	110	70	14	97	Y	N
19	NIL	88	1	130	70	16	98	N	N
20	NIL	100	1	100	60	18	99	Y	N
21	NIL	120	1	100	60	16	99	Y	N
22	NIL	94	1	110	70	18	98	Y	N
23	NIL	119	2	94	60	34	90	Y	N
24	NIL	84	1	120	80	20	99	N	N
25	NIL	96	1	90	60	18	99	Y	N
26	NIL	90	1	110	60	20	100	Y	N
27	NIL	100	1	120	70	24	97	Y	N
28	NIL	102	1	120	70	26	96	Y	N
29	NIL	110	1	110	90	20	96	Y	N
30	NIL	92	1	120	60	24	96	Y	N
31	NIL	80	1	100	90	16	99	Y	N
32	NIL	94	1	100	70	20	99	Y	N
33	NIL	84	1	120	80	22	99	Y	N
34	NIL	100	1	130	70	20	100	N	N
35	PREGNANT	76	1	110	70	16	97	Y	N
36	NIL	80	1	110	70	19	99	Y	N
37	NIL	92	1	100	60	22	100	Y	N
38	NIL	98	1	100	60	20	98	Y	N
39	NIL	84	1	100	60	22	98	Y	N
40	NIL	106	2	90	50	22	99	Y	N
41	NIL	80	1	100	60	16	99	Y	N
42	NIL	84	1	90	60	22	99	Y	N
43	PREGNANT	88	1	110	70	18	98	Y	N
44	NIL	76	1	110	80	14	100	Y	N
45	NIL	72	1	100	60	20	97	Y	N
46	NIL	120	1	100	70	20	98	Y	N
47	NIL	88	1	100	70	16	100	Y	N
48	NIL	90	1	110	70	24	99	Y	N
49	NIL	126	1	120	90	22	98	Y	N
50	NIL	76	1	100	60	16	99	Y	N
51	NIL	84	1	70	50	22	96	Y	N
52	PREGNANT	100	1	100	60	20	97	Y	N
53	NIL	100	1	100	60	20	99	Y	N
54	NIL	84	1	110	70	20	100	Y	N
55	NIL	80	1	110	60	22	100	Y	N
56	NIL	90	1	120	70	22	98	Y	N
57	NIL	100	1	110	60	20	98	Y	N
58	NIL	120	1	110	60	24	98	Y	Y
59	NIL	68	1	100	70	21	98	Y	N
60	NIL	112	1	120	70	24	98	Y	N
61	NIL	92	1	100	70	22	100	Y	N
62	NIL	90	1	100	70	24	99	Y	N

Data sheet/ Page 6

	GCS	AbdNormal	AbdFreefluid	AbdGuarding	AbdTender	RespNormal	RSredairentry	RSBBS
1	15	Y	N	N	N	Y	N	N
2	15	Y	N	N	N	Y	N	N
3	15	Y	N	N	N	Y	N	N
4	15	Y	N	N	N	Y	N	N
5	15	N	Y	N	Y	Y	N	N
6	15	N	N	N	Y	Y	N	N
7	15	Y	N	N	N	Y	N	N
8	15	Y	N	N	N	Y	N	N
9	15	Y	N	N	N	Y	N	N
10	15	Y	N	N	N	Y	N	N
11	15	Y	N	N	N	Y	N	N
12	15	N	N	N	Y	Y	N	N
13	15	Y	N	N	N	Y	N	N
14	15	N	N	N	Y	Y	N	N
15	15	Y	N	N	N	Y	N	N
16	15	Y	N	N	N	Y	N	N
17	15	Y	N	N	N	Y	N	N
18	15	N	Y	N	Y	N	Y	N
19	15	Y	N	N	N	Y	N	N
20	15	Y	N	N	N	Y	N	N
21	15	Y	N	N	N	Y	N	N
22	15	Y	N	N	N	Y	N	N
23	15	Y	N	N	N	Y	N	N
24	15	Y	N	N	N	Y	N	N
25	15	N	N	N	Y	Y	N	N
26	15	Y	N	N	N	N	Y	N
27	15	Y	N	N	N	Y	N	N
28	15	Y	N	N	N	Y	N	N
29	15	Y	N	N	N	Y	N	N
30	15	Y	N	N	N	Y	N	N
31	15	Y	N	N	N	Y	N	
32	15	Y	N	N	N	Y	N	N
33	15	Y	N	N	N	Y	N	N
34	15	Y	N	N	N	Y	N	
35	15	N	Y	N	Y	N	Y	N
36	15	N	Y	N	Y	N	Y	N
37	15	Y	N	N	N	Y	N	N
38	15	Y	N	N	N	Y	N	N
39	15	Y	N	N	N	Y	N	N
40	15	Y	N	N	N	N	Y	N
41	15	Y	N	N	N	Y	N	N
42	15	Y	N	N	N	N	Y	N
43	15	Y	N	N	N	Y	N	N
44	15	Y	N	N	N	Y	N	N
45	15	Y	N	N	N	Y	N	N
46	15	Y	N	N	N	Y	N	N
47	15	Y	N	N	N	Y	N	N
48	15	Y	N	N	N	Y	N	N
49	15	N	Y	N	N	N	Y	N
50	15	Y	N	N	N	Y	N	N
51	15	Y	N	N	N	Y	N	N
52	15	Y	N	N	N	Y	N	N
53	15	Y	N	N	N	Y	N	N
54	15	Y	N	N	N	Y	N	N
55	15	N	Y	Y	Y	N	Y	N
56	15	N	Y	N	N	N	Y	N
57	15	Y	N	N	N	Y	N	N
58	15	Y	N	N	N	Y	N	N
59	15	Y	N	N	N	Y	N	N
60	15	Y	N	N	N	Y	N	N
61	15	Y	N	N	N	Y	N	N
62	15	Y	N	N	N	Y	N	N

Data sheet/ Page 7

	RSCrackles	RSWheeze	RScondsounds	CNSNormal	CNSAltSens	CNSSeizures
1	N	N	N	Y	N	N
2	N	N	N	Y	N	N
3	N	N	N	Y	N	N
4	N	N	N	Y	N	N
5	N	N	N	Y	N	N
6	N	N	N	Y	N	N
7	N	N	N	Y	N	N
8	N	N	N	Y	N	N
9	N	N	N	Y	N	N
10	N	N	N	Y	N	N
11	N	N	N	Y	N	N
12	N	N	N	Y	N	N
13	N	N	N	Y	N	N
14	N	N	N	Y	N	N
15	N	N	N	Y	N	N
16	N	N	N	Y	N	N
17	N	N	N	Y	N	N
18	N	N	N	Y	N	N
19	N	N	N	Y	N	N
20	N	N	N	Y	N	N
21	N	N	N	Y	N	N
22	N	N	N	Y	N	N
23	N	N	N	Y	N	N
24	N	N	N	Y	N	N
25	N	N	N	Y	N	N
26	N	N	N	Y	N	N
27	N	N	N	Y	N	N
28	N	N	N	Y	N	N
29	N	N	N	Y	N	N
30	N	N	N	Y	N	N
31	N	N	N	Y	N	N
32	N	N	N	Y	N	N
33	N	N	N	Y	N	N
34	N	N	N	Y	N	N
35	Y	N	Y	Y	N	N
36	N	N	N	Y	N	N
37	N	N	N	Y	N	N
38	N	N	N	Y	N	N
39	N	N	N	Y	N	N
40	Y	N	N	N	Y	N
41	N	N	N	Y	N	N
42	N	N	N	Y	N	N
43	N	N	N	Y	N	N
44	N	N	N	Y	N	N
45	N	N	N	Y	N	N
46	N	N	N	Y	N	N
47	N	N	N	Y	N	N
48	N	N	N	Y	N	N
49	N	N	N	Y	N	N
50	N	N	N	Y	N	N
51	N	N	N	Y	N	N
52	N	N	N	Y	N	N
53	N	N	N	Y	N	N
54	N	N	N	Y	N	N
55	N	N	N	Y	N	N
56	N	N	Y	Y	N	N
57	N	N	N	Y	N	N
58	N	N	N	Y	N	N
59	N	N	N	Y	N	N
60	N	N	N	Y	N	N
61	N	N	N	Y	N	N
62	N	N	N	Y	N	N

Data sheet/ Page 8

	SOFARespi	SOFACoag	SOFALiver	SOFACVS	SOFACNS	SOFARenal	SOFADay1	PlateletCoulter
1	0	3	0	0	0	1	4	33000
2	0	3	0	0	0	0	3	20000
3	0	2	0	0	0	0	2	67000
4	0	2	0	0	0	0	2	50000
5	0	3	0	0	0	0	3	42000
6	0	3	0	0	0	0	3	21000
7	0	2	0	0	0	0	2	51000
8	0	2	0	0	0	0	2	72000
9	0	2	0	0	0	0	2	78000
10	0	2	1	0	0	0	3	55000
11	0	3	0	0	0	0	3	23000
12	0	4	0	1	0	0	5	11000
13	0	2	0	0	0	0	2	58000
14	0	2	0	0	0	0	2	76000
15	0	2	0	0	0	0	2	66000
16	0	4	0	0	0	0	4	18000
17	0	3	0	0	0	2	5	40000
18	0	3	0	0	0	0	3	29000
19	0	4	0	0	0	0	4	12000
20	0	2	0	0	0	0	2	69000
21	0	4	0	0	0	0	4	8000
22	0	3	0	0	0	0	3	35000
23	0	2	0	1	0	1	4	77000
24	0	3	0	0	0	0	3	30000
25	0	2	0	1	0	0	3	53000
26	0	2	0	0	0	0	2	57000
27	0	3	0	0	0	0	3	22000
28	0	3	0	0	0	0	3	29000
29	0	2	0	0	0	0	2	53000
30	0	3	0	0	0	0	3	25000
31	0	3	0	0	0	0	3	31000
32	0	4	0	0	0	1	5	12000
33	0	4	2	0	0	1	7	16000
34	1	3	1	0	0	1	6	27000
35	1	4	2	0	0	1	8	14000
36	0	4	0	0	0	0	4	14000
37	0	3	0	0	0	0	3	20000
38	0	4	0	0	0	0	4	11000
39	0	2	1	0	0	1	4	54000
40	1	3	3	0	0	1	8	47000
41	0	4	0	0	0	1	5	15000
42	0	4	0	0	0	0	4	12000
43	0	2	0	0	0	0	2	58000
44	0	2	0	0	0	0	2	71000
45	0	4	0	0	0	0	4	19000
46	0	2	0	0	0	0	2	58000
47	1	4	0	0	0	1	6	17000
48	0	2	0	0	0	0	2	55000
49	1	2	0	0	0	1	4	93000
50	0	4	0	0	0	0	4	4000
51	0	4	0	0	0	0	4	19000
52	0	4	0	0	0	0	4	19000
53	0	4	2	0	0	0	6	14000
54	0	2	0	0	0	0	2	65000
55	3	2	0	0	0	0	5	75000
56	1	4	0	0	0	0	5	17000
57	0	1	0	0	0	0	2	95000
58	0	4	1	0	0	0	5	16000
59	0	3	0	0	0	0	3	41000
60	0	3	0	0	0	0	3	43000
61	0	4	0	0	0	0	4	15000
62	0	3	0	0	0	0	3	31000

Data sheet/ Page 9

	Hb	TWBC	Neutrophil	Lymph	Monocyte	Creat	Urea	Sodium	Potassium	Bicarb
1	16.8	4700	72	20	6	1.3	21	132	4	16
2	13.2	2700	26	64	10	0.63	15	139	3.8	17
3	14.1	2200	70	24	6	1		136	3.9	14
4	10.4	6100	91	4	5	1.08	15	132	3	24
5	16	9800	44	48	6	0.87	18	137	3.3	20
6	17.3	3800	56	34	10	0.89	12	138	4.3	21
7	19.2	3400	65	23	8	0.87	13	130	3.8	23
8	13.6	2900	66	20	9	0.79	25	135	4.1	24
9	14.1	2000	49	29	19	0.7	17	140	4.2	24
10	17.3	3400	46	38	8	1.03	36	127	3.8	20
11	11	1800	80	12	4	0.47		133	3.6	
12	15.5	4400	60	30	10	1.02	21	136	3.8	21
13	14.2	8200	44	46	10	0.71	12	134	3.9	22
14	9.7	3300	63	27	10	0.63	25	130	3.6	14
15	13.3	3100	85	10	5	0.93	20	135	4.1	23
16	17	6300	61	18	21	1.11	19	133	4.2	19
17	14.9	4700	71	19	8	2.18	35	133	3.5	24
18	14.4	3300	65	28	5	0.99	15	129	4.4	18
19	14.6	3100	60	25	15	0.94	11	133	4.7	
20	8.7	4500	70	20	8	0.46	8	128	3.1	22
21	13.7	9900	28	55	15	0.67	21	138	4	16
22	12.1	1700	60	24	16	0.68	13	134	2.8	19
23	14.5	2600	50	26	24	1.59	70	130	3.7	17
24	17.6	4500	50	30	8	0.94		135	4.1	15
25	16.1	2900	68	22	10	1.1	30	135	3.8	19
26	5.2	3300	79	14	7	1.06	16	131	3.7	18
27	14.6	3200	60	26	14	0.83	20	132	3.6	
28	10.6	5100	84	11	5	0.52	13	132	3.7	14
29	14.5	15000	48	50	2	0.92	20	135	3.6	
30	15.8	6500	50	33	14	1.07	19	133	3.6	19
31	17	3300	71	26	3	1.16	15	136	4.1	
32	19	6500	92	2	2	1.33		129	5.7	15
33	10.1	10300	83	5	9	1.21	65	134	3.2	15
34	19.2	3400	78	13	8	1.44	50	135	3.6	15
35	16.7	13700	68	15	7	1.35	71	128	4.4	14
36	13.4	5400	48	37	13	0.72		135	3.6	22
37	14	1700	72	24	4	0.62		135	3.3	23
38	16	5000	38	40	22	0.86	16	134	4.3	19
39	16.1	4900	52	30	18	1.28	25	134	3.7	13
40	10.8	37600	73	5	3	1.7	136	125	4.2	6
41	14.8	4500	42	42	15	1.36	36	132	4.5	
42	17.3	4100	37	38	21	0.85	10	130	4	17
43	10.9	2300	64	26	8	0.5	10	130	3.3	19
44	12.2	3300	59	33	8	0.53		136	3.5	17
45	10.9	4300	48	24	18	0.58		135	3.6	20
46	11.4	3000	23	64	4	0.64		134	3.5	23
47	17.2	7400	74	19	4	1.94	64	139	4.5	12
48	11.4	2400	48	27	24	0.51	11	132	3	
49	15.7	4000	76	17	17	1.21	27	138	4.2	
50	13.5	9000	89	8	3	1.09	62	125	3.7	
51	15.6	7900	61	24	12	0.47	12	136	3.4	
52	16.1	2900	59	33	8	1.19	24	135	3.7	
53	11.6	3800	43	40	17	0.46	18	141	3.8	
54	13.4	5200	52	3	11	0.59		132	3.9	
55	3.5	29800	78	9	2	0.96		142	4.3	
56	13.4	6700	36	53	11	0.67	15	136	3.9	
57	14.3	3100	66	24	10	0.89		138	3.7	
58	16.8	6800	30	24	29	0.82	34	134	3.8	
59	15.4	3300	79	16	5	0.97	15	134	4.6	
60	13.5	4500	67	18	15	1		136	3.5	
61	16.8	9400	42	49	9	0.82		132	3.5	
62	17.2	4800	50	41	9	0.75	14	135	4.5	

Data sheet/ Page 10

	DirectBil	TotalProt	Albumin	SGOT	SGPT	ALP	PT	INR	aPTT	Urinanlysis	Hematuria
1	0.2	7.7	4.7	1372	508	79				Y	N
2	0.1	6.9	4	515	430	38	10.1	0.93	32.8	Y	N
3	0.2	7.3	3.6	129	60	49				Y	N
4	0.3	7	4.5	23	12	52				N	N
5	0.4	6.8	3.5	446	271	51	10.3	0.95	33.6	Y	N
6	0.2	6.2	3.2	228	149	70				Y	N
7	0.7	7.4	3.9	1480	605	72	10.3	0.95	26.5	Y	N
8	0.1	7.4	4.4	47	37	51	9.4	0.86	32.8	Y	N
9	0.2	6.9	4.2	54	45	83				N	N
10	0.4	7.9	4.3	126	52	71				N	N
11	0.1	5.5	3.4	118	59	112	9.4	0.87	73.4	Y	N
12	0.1	6.6	3.4	70	31	74	8.9	0.82	48	Y	N
13	0.2	8.3	4	56	34	34	9.7	0.9	33.1	N	N
14	0.3	7.3	4.2	165	123	68				Y	N
15	0.9	6.6	3.8	514	363	82				Y	Y
16	0.3	7	4	123	81	107				N	N
17	0.2	7.5	4.6	385	219	75				Y	N
18	0.4	6.7	3.6	1119	428	187	11.5	1.05	56.4	Y	N
19	0.3	6.7	3.9	59	36	50				Y	N
20	0.3	6.7	3.3	214	74	69				Y	N
21	0.5	6.4	3.2	168	127	361				Y	N
22	0.4	6.1	3.3	871	253	67				N	N
23	0.1	6.9	4	170	79	52	10.4	0.96	30.9	Y	N
24	0.5	7.7	4	599	550	106				N	N
25	0.5	6.2	3.8	69	21	61				N	N
26	0.4	8	4.3	495	304	82	13.2	1.2	48.6	N	N
27	0.5	5.8	3.6	190	84	77	9.3	0.86	39.2	Y	N
28	0.1	6.9	3	132	54	59				Y	N
29	0.2	7.4	4	40	41	90				N	N
30	0.2	6.6	3.6	82	33	41				N	N
31	0.2	6.6	3.8	100	38	70				N	N
32	0.5	6.1	3.3	434	189	130	10.9	1	48.4	Y	N
33	3.5	4.8	1.8	1112	704	335	12.5	1.15	37.9	Y	N
34	1.2	7	3.8	264	113	101				Y	N
35	2.2	4.8	3	6860	2958	91	24.3	2.16	45.8	Y	N
36	0.6	5.2	2.8	583	342	57	10.8	0.99	40.3	Y	N
37	0.2	5.8	4.1	257	84	66				N	N
38	0.1	6.7	3.7	128	63	39	9.6	0.88	45.4	Y	N
39	0.2	7.3	4.1	76	73	75	10	0.92	34.2	Y	N
40	7.8	4.2	2.1	1284	400	246	32.3	2.83	53.6	Y	N
41	0.2	7.6	4.1	101	134	96	9.4	0.86	40.1	Y	N
42	0.1	6.1	3.5	363	182	68	10.3	0.95	62.9	Y	N
43	0.5	5	2.7	744	337	66	10.1	0.93	40.2	N	N
44	0.1	6	3.2	44	27	62				Y	N
45	0.4	6.1	2.9	315	190	83	9.6	0.89	38.9	N	N
46	0.2	7.1	4	102	63	54				Y	N
47	0.4	6.6	2.9	2800	920	85	18.9	1.74	170	Y	N
48	0.2	6.6	3.5	70	18	44	11.4	1.07	31.6	Y	N
49	0.2	6.5	3.8	71	60	63	12.4	1.16	33.3	N	N
50	0.6	5.9	2.5	111	74	189				Y	N
51	0.2	5.4	2.8	349	114	161	10.7	0.99	58.1	Y	Y
52	0.2	5.3	3.4	173	85	50	13.1	1.23	39.3	N	N
53	1	6.1	2.9	235	144	184				Y	Y
54	0.2	6.7	3.3	78	23	204				Y	N
55	0.3	4.9	2.3	327	186	101	12.7	1.19	26.8	Y	N
56	0.3	5	2.5	693	230	63	14.9	1.39	42.5	Y	N
57	0.2	6.6	4.3	40	25	126				N	N
58	0.8	6.6	3.8	197	101	63				Y	N
59	0.2	6.4	3.6	294	113	78				N	N
60	0.3	6.8	3.7	40	30	66	11.3	0.07	35.6	Y	N
61	0.5	6	3.5	259	199	64				Y	N
62	0.5	7	3.5	284	25	76	9.8	0.91	34.3	N	N

Data sheet/ Page 11

	Proteinuria	CSBlood	Culturegrowth	NS1Ag	DenIgM	DenIgG	Malaria
1	Y	N		Y	Y	Y	N
2	Y	N		N	Y	Y	N
3	Y	N		Y	Y	Y	N
4	N	N		Y	Y	Y	N
5	Y	Y	ESBL ENTEROBACTER	Y	Y	Y	N
6	Y	N		Y	Y	Y	N
7		N		Y	Y	N	N
8	Y	N		N	Y	Y	N
9	N	N		N	Y	Y	N
10	N	N		Y	Y	Y	N
11	N	N		Y	Y	Y	N
12	Y	N		Y	Y	Y	N
13	N	Y	NFGNB	N	Y	N	N
14	Y	N		Y	N	Y	N
15	Y	N		Y	N	Y	N
16	N	N		N	Y	Y	N
17	Y	N		N	Y	Y	N
18	Y	N		Y	N	N	N
19	Y	N		Y	Y	Y	N
20	Y	N		Y	N	Y	N
21	Y	N		N	Y	Y	N
22	N	N		Y	Y	Y	N
23	Y	N		Y	Y	Y	N
24	N	N		Y	Y	Y	N
25	Y	N		Y	N	N	N
26	N	N		Y	N	N	N
27	Y	N		Y	Y	Y	N
28	Y	N		Y	N	Y	N
29	N	N		N	Y	Y	N
30	N	N		Y	Y	Y	N
31	N	N		Y	N	N	N
32	Y	N		Y	Y	Y	N
33	Y	N		Y	Y	Y	N
34	Y	N		Y	N	N	N
35	Y	N		Y	Y	Y	N
36	Y	N		N	Y	Y	N
37	N	N		Y	N	Y	N
38	Y	N		Y	Y	Y	N
39	Y	N		N	Y	Y	N
40	Y	N		N	Y	Y	N
41	Y	N		Y	Y	Y	N
42	Y	N		Y	Y	Y	N
43	N	N		Y	Y	Y	N
44	Y	N		Y	N	N	N
45	N	N		N	Y	Y	N
46	Y	N		N	Y	Y	N
47	Y	N		Y	Y	Y	N
48	Y	N		Y	Y	Y	N
49	N	N		Y	Y	Y	N
50	Y	N		N	Y	N	N
51	Y	Y	CONS	Y	Y	Y	N
52	N	N		Y	Y	Y	N
53	Y	N		N	Y	Y	N
54	Y	N		Y	Y	N	N
55	Y	N		Y	Y	Y	N
56	Y	N		Y	Y	Y	N
57	N	N		Y	Y	N	N
58	Y	N		N	Y	Y	N
59	N	N		Y	Y	Y	N
60	Y	N		Y	N	N	N
61	Y	N		Y	Y	Y	N
62	Y	N		N	Y	Y	N

Data sheet/ Page 12

	VWFCBA	ECG	CXR	USGABd	DenSeverity	StayDuration	SOFAHighest	PltLowest
1	147.5	1	1	2	3	6	4	17000
2	304	4	1		2	3	3	20000
3	259.5	1	1		2	6	3	39000
4	309	1	3	2	2	5	4	15000
5	134.5	1	1	2	2	4	3	42000
6	180.5	4	1		2	4	4	13000
7	172	4	1		2	0	2	51000
8	227.5	4	1		1	0	2	57000
9	240.2	4	1		1	0	2	78000
10	121.8	4	1		2	0	4	17000
11	190	4	5		2	12	4	12000
12	117.5	4	1		3	5	5	11000
13	175.5	1	2		1	3	2	58000
14	76.7	1	1		3	5	4	12000
15	135	1	1	1	2	6	4	12000
16	108	4	1		2	4	4	10000
17	90.5	1	1		2	4	5	21000
18	71.9	1	3	2	3	8	5	11000
19	100.7	1	1		2	5	4	7000
20	128	1	5	1	1	6	3	30000
21	182.5	1	1		2	4	4	8000
22	94.5	1	1		2	5	4	16000
23	132.5	1	1		2	12	4	77000
24	143	1	1	1	2	0	3	30000
25	60.6	1	1	2	3	5	4	20000
26	60.2	4	1		2	4	4	14000
27	61.1	1	1	1	2	4	4	12000
28	139	1	5	2	2	7	4	7000
29	112.1	4	1		1	0	2	53000
30	121	1	1		1	4	4	19000
31	148	4	1		1	0	3	31000
32	79.3	1	1		2	6	5	12000
33	256	1	1		2	10	7	16000
34	95.7	1	3	2	2	5	6	6000
35	97.7	1	3	2	3	10	8	14000
36	109.5	4	3	2	2	4	4	14000
37	74.2	4	1		2	5	4	9000
38	91.8	1	1		2	4	4	11000
39	117	1	1		2	4	4	19000
40	297.5	2	2	2	3	6	21	15000
41	101.1	1	1		2	4	5	11000
42	39.7	1	3		2	3	4	6000
43	86.3	1	1		1	6	2	58000
44	171.5	1	1		1	3	2	71000
45	159.5	4	1		2	5	4	19000
46	287	1	1		1	5	2	58000
47	180.5	3	2		3	3	7	17000
48	251	1	5	1	1	4	2	53000
49	205.5	1	1	2	2	6	4	19000
50	136	1	1		2	9	5	4000
51	152.5	4	1		3	30	7	19000
52	101	1	1		1	5	4	6000
53	207	1	1		2	4	6	6000
54	246.5	4	5		2	6	4	19000
55	99.7	1	2	2	3	10	5	47000
56	175	1	3		3	4	5	13000
57	38.3	4	1		1	5	3	38000
58	105	1	1		2	4	5	16000
59	157	4	1		1	0	3	41000
60	295	1	1		2	6	3	20000
61	159	4	1		2	3	4	15000
62	161	1	1		1	3	3	25000

Data sheet/ Page 13

	SGPTHighest	Outcome	IcuCare	RRT	Transfusion	VentSupport	AKI	VasoReq	PltTX
1	508	1	N	N	N	N	Y	N	0
2	430	1	N	N	N	N	N	N	0
3	60	1	N	N	N	N	N	N	0
4	47	1	N	N	N	N	N	N	0
5	271	1	N	N	Y	N	N	N	4
6	149	1	N	N	N	N	N	N	0
7	605	1	N	N	N	N	N	N	0
8	43	1	N	N	N	N	N	N	0
9	106	1	N	N	N	N	N	N	0
10	52	1	N	N	N	N	N	N	0
11	97	1	Y	N	Y	N	N	N	6
12	31	1	N	N	Y	N	N	N	4
13	34	1	N	N	N	N	N	N	0
14	1125	1	N	N	N	N	N	N	0
15	363	1	N	N	N	N	N	N	0
16	81	1	N	N	N	N	N	N	0
17	219	1	N	N	N	N	Y	N	0
18	428	1	N	N	N	N	N	N	0
19	38	1	N	N	N	N	N	N	0
20	74	1	N	N	N	N	N	N	0
21	127	1	N	N	N	N	N	N	0
22	523	1	N	N	N	N	N	N	0
23	79	1	N	N	N	N	Y	N	0
24	550	1	N	N	N	N	N	N	0
25	21	1	N	N	N	N	N	N	0
26	304	1	N	N	N	N	N	N	0
27	84	1	N	N	Y	N	N	N	4
28	96	1	N	N	Y	N	N	N	4
29	41	1	N	N	N	N	N	N	0
30	130	1	N	N	N	N	N	N	0
31	38	1	N	N	N	N	N	N	0
32	189	1	N	N	Y	N	Y	N	2
33	704	1	N	N	N	N	N	N	0
34	143	1	N	N	Y	N	Y	N	2
35	2958	1	N	N	N	N	Y	N	0
36	342	1	N	N	N	N	N	N	0
37	84	1	N	N	N	N	N	N	0
38	63	1	N	N	N	N	N	N	0
39	151	1	N	N	N	N	Y	N	0
40	400	2	Y	Y	Y	Y	Y	Y	12
41	134	1	N	N	N	N	Y	N	0
42	200	1	N	N	Y	N	N	N	2
43	337	1	N	N	N	N	N	N	0
44	27	1	N	N	N	N	N	N	0
45	190	1	N	N	N	N	N	N	0
46	63	1	N	N	N	N	N	N	0
47	920	2	Y	N	Y	Y	Y	Y	2
48	18	1	N	N	N	N	N	N	0
49	85	1	N	N	N	N	N	N	0
50	100	1	N	N	Y	N	N	N	2
51	1487	1	Y	N	Y	Y	Y	N	17
52	180	1	N	N	Y	N	N	N	4
53	144	1	N	N	N	N	N	N	0
54	23	1	N	N	Y	N	N	N	2
55	186	1	N	N	Y	N	N	N	0
56	230	1	N	N	N	N	N	N	0
57	118	1	N	N	N	N	N	N	0
58	101	1	N	N	Y	N	N	N	4
59	113	1	N	N	N	N	N	N	0
60	167	1	N	N	N	N	N	N	0
61	159	1	N	N	Y	N	N	N	1
62	262	1	N	N	N	N	N	N	0

Data sheet/ Page 14

	FFPTX	CryoTx	PregComplex	GestAge	Gravida
1	0	0			
2	0	0			
3	0	0			
4	0	0			
5	4	0			
6	0	0			
7	0	0			
8	0	0			
9	0	0			
10	0	0			
11	5	0			
12	0	0			
13	0	0			
14	0	0			
15	0	0			
16	0	0			
17	0	0			
18	0	0			
19	0	0			
20	0	0	N	32	3
21	0	0			
22	0	0			
23	0	0			
24	0	0			
25	0	0			
26	0	0			
27	2	0			
28	0	0	N	16	1
29	0	0			
30	0	0			
31	0	0			
32	0	0			
33	0	0			
34	8	0			
35	0	0			
36	0	0			
37	0	0			
38	0	0			
39	0	0			
40	11	33			
41	0	0			
42	2	0			
43	0	0	N	18	1
44	0	0			
45	0	0			
46	0	0			
47	4	0			
48	0	0	N	8	2
49	0	0			
50	0	0			
51	20	57	Y	38	1
52	8	0			
53	0	0			
54	2	0	N	34	1
55	0	0			
56	0	0			
57	0	0			
58	0	0			
59	0	0			
60	0	0			
61	0	0			
62	0	0			