Management of Dengue Shock Syndrome:

Evaluating Fluid Resuscitation Strategies and Novel Monitoring Systems to Improve Outcomes

by

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Abstract

Dengue is the most significant mosquito-borne viral infection in humans. Dengue shock syndrome (DSS) is one of the dengue complications and can be fatal if not recognized and treated promptly. The management of DSS relies mainly on judicious fluid replacement and frequent haemodynamic assessments. Optimal fluid therapy is yet to be defined due to limited evidence. Current methods for monitoring patients with DSS are also unreliable and less sensitive. My thesis aimed to review different fluid resuscitation strategies currently used for paediatric DSS across dengue-endemic countries in Asia, and assess the clinical utility of several novel non-invasive monitoring devices in children with DSS.

First, I performed a systematic review of randomised controlled trials on fluid resuscitation for children with DSS or septic shock. I collected retrospective data on fluid management in 7 hospitals in Asia anonymously and compared the outcomes of different fluid strategies. Second, I used prospective data from 103 severe dengue patients admitted to ICU and investigated the ability of the Compensatory Reserve Index (CRI) to predict recurrent shock (reshock). Finally, I conducted a prospective observational study on 92 children with DSS to describe the fluid response and trajectories of intravascular volume changes during fluid resuscitation using point of care ultrasound and a bioimpedance device (NICaS).

I demonstrated the high heterogeneity of published studies on fluid resuscitation in children could not provide strong recommendations on fluid types and fluid rates for both DSS and septic shock. There was a wide variation of fluid management for DSS across the Asian regions. The use of colloid or crystalloid in the first 2-hour of resuscitation provided similar outcomes; the prolonged use of synthetic colloids for more than 4 hours reduced the reshock occurrence but increased significantly respiratory compromise. I have demonstrated the utility of CRI in predicting reshock, as well as showing the reliability of continuous measurements derived from NICaS in intravascular volume monitoring compared to echocardiography.

Overall, my work has added to the overall body of evidence of fluid management for DSS patients and the utility of different monitoring tools. This may help pave the way for personalised fluid trials for DSS management in the future.

Declaration

The work presented in this thesis is my own. However, it would not have been done without the statistical support of Dr Nguyen Lam Vuong and Mr Trinh Manh Hung (Chapter 4), Dr Phung Khanh Lam (Chapter 5), Ms Nguyen Thi Minh Nguyet (Chapter 6). For the systematic review, Dr Ho Quang Chanh contributed to the study as an independent assessor for selecting articles, assessing the risk of bias and collecting data. For all studies described in this thesis, I am responsible for the design, protocol writing under the supervision of my supervisors, obtaining ethical approvals, collecting all clinical data (with assistance of study nurses), enrolling patients, performing full ultrasound assessments (with assistance of Dr Ho Quang Chanh for measuring echo parameters of 37 cases, Dr Tran Kim Hung for echo assessments of two cases), data management, statistical analyses (with assistance) and writing of the thesis. This thesis has not been submitted for a degree or other qualification to this or any other university.

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Abbreviations

ACCM	American College of Critical Care Medicine
AICU	Adult Intensive Care Unit
AKI	Acute Kidney Injury
ALT	Alanin Aminotransferase
AST	Aspartate Aminotransferase
aPTT	Activated partial thromboplastin time
ARDS	Acute Respiratory Distress Syndrome
ADE	Antibody-Dependent Enhancement
AUC	Area under the curve
BP	Blood Pressure
BIA	Bio-impedance analysis
BMI	Body Mass Index
BS	Balanced Solution
BSA	Body Surface Area
CO	Cardiac Output
CI	Cardiac Index
CRF	Case Report Form
CRI	Compensatory Reserve Index
CVP	Central Venous Pressure
DBP	Diastolic Blood Pressure
DOI	Day of Illness
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DSS	Dengue shock syndrome
EF	Eject Fraction
EFIC	Exception from Informed Consent
ES	Effect size
etCO2	end-tidal CO2
FDP	Fibrinogen-Degradation Products
FEAST	Fluid Expansion as Supportive Therapy
GBWT	Gallbladder Wall Thickness

HCMC	Ho Chi Minh City
HDU	High Dependency Unit
HSD/5D	Half-strength Darrows/5% dextrose
Hct	Haematocrit
HTD	Hospital for Tropical Diseases
HLA	Human Leukocyte Antigen
HES	Hydroxyethyl starch
HR	Heart Rate
HS	Hypertonic Saline
HSL	Hypertonic Sodium Lactate
ICU	Intensive Care Units
ICAM-1	Intercellular Adhesion Molecule 1
IPW	Inverse Probability Weighting
IQR	Interquartile Range
IVC	Inferior Vena Cava
IVCCI	IVC collapsibility index
LMICs	Low Middle-Income Countries
LBNP	Lower Body Negative Pressure
LVEF	Left Ventricular Eject Fraction
LVEDD	Left Ventricular End-Diastolic Dimension
LVEDV	Left Ventricular End-Diastolic Volume
LVESD	Left Ventricular End-Sustolic Dimension
LVOT	Left Ventricular Outflow Tract
MW	Molecular Weight
NCPAP	Nasal Continuous Positive Airway Pressure
VO2	Oxygen consumption
DO2	Oxygen delivery
OxTREC	Oxford Tropical Research Ethics Committee
OR	Odd Ratio
PALS	Paediatric Advanced Life Support
PICU	Paediatric Intensive Care Unit
PICU	Paediatric Intensive Care Unit
POC	Point of Care

PT	Prothrombin Time
PTT	Partial Thromboplastin Times
PPG	Photoplethysmography
POC	Point-of-Care
POCUS	Point-of-Care Ultrasound
PP	Pulse Pressure
RCT	Randomised Controlled Trial
RIC	Regional Impedance Cardiography
RL	Ringer's Lactate
RoB	Risk of Bias
RRT	Renal Replacement Therapy
SBP	Systolic Blood Pressure
SSC	Surviving Sepsis Campaign
SV	Stroke Volume
SVI	Stroke Volume Index
SVR	Systemic Vascular Resistance
StO2	Tissue Oxygen Saturation
TIC	Thoracic Impedance Cardiography
TLR4	Toll-like Receptor 4
TOE	Transoesophageal Echocardiography
TTE	Transthoracic Echocardiography
VCAM-1	Vascular Cell Adhesion protein 1
VTI	Velocity-Time Integral
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Epidemiology of dengue

1.1.1 History

There are many theories and discussions on the origins of dengue. A dengue-like illness was first recorded in a Chinese medical encyclopedia from the Jin Dynasty (265-420 AD), edited in the Tang Dynasty (610 AD) and also in 992 during the Northern Sung Dynasty (Gubler D. J. 2006). The disease was described as a "water poison" and was said to be associated with flying insects. Probable dengue outbreaks were later described in the West Indies in 1635 and in Panama in 1699 (Gubler D. J. 2006). A dengue-like illness was given the name "breakbone fever" in 1789 by Benjamin Rush, describing an outbreak in Philadelphia in the 1780s, and soon afterwards similar epidemics were reported across Asia, Africa, and North America.

The name dengue is thought to derive from the Swahili phrase "Ka-dinga pepo" which means "cramp-like seizure caused by an evil spirit" – the word "dinga" describes the gait of a person with bone pain. Later the disease was known as "Dandy Fever", taken from a description of slaves in the West Indies who contracted dengue and had the posture of a dandy during the St Thomas epidemic in 1827 (Christie J. 1881). The first use of the name "dengue" was in Spain in 1802. Subsequently, during an 1828 epidemic of dunga in Cuba, the Spanish recognized the similarity to dengue in Spain, and changed the name *dunga* to *dengue* (Gubler D. J. 2006).

Considerable research done in the early 20th century by Graham (1903), Bancroft (1906) and Cleland (1918) identified *Aedes* mosquitoes to be the vectors responsible for transmission of dengue. *Aedes* mosquitoes like warm humid environments and preferentially feed on humans when breeding. In the 1940s, during World War II when dengue was the main cause of fever among soldiers in Asian and Pacific countries, the virus responsible was isolated (Hotta S. 1952,

Sabin A. B. 1952). Sabin and his colleagues found antigenically distinct strains (i.e. different serotypes) of the dengue virus (DENV) in different geographic locations, DENV-1 in Hawaii and DENV-2 in New Guinea. Two other serotypes, DENV-3 and DENV-4 were found later in Manila in 1953, isolated from paediatric patients with haemorrhagic disease with a 10% fatality rate (Hammon W. M. et al. 1960). In 1955, DENVs 2, 3 and 4 were isolated from a significant outbreak in Bangkok, Thailand with 2158 confirmed cases and 300 deaths (Rojanapithayakorn Wiwat 1998). Over the next 30 years, dengue was reported in many countries in the region, including Cambodia, China, Indonesia, Laos, Malaysia, Maldives, Myanmar, Singapore, Sri Lanka, Vietnam and several Pacific Island groups (World Health Organization 2009).

1.1.2 Burden of dengue

Estimation of the global burden of dengue is difficult because around 80% of those infected are thought to be asymptomatic, while amongst the remainder of patients the range of clinical manifestations is broad and differentiation from other febrile illnesses is challenging. It has been estimated that 3.9 billion people across 129 countries are at risk of dengue infection (Brady O. J. et al. 2012). One modelling study estimated that 390 million dengue infections occur annually, of which 96 million manifest clinical symptoms (Bhatt S. et al. 2013). Another group has suggested an average of 105 million infections all over the world per year, with an estimated 4 million symptomatic cases requiring hospital admission (Cattarino L. et al. 2020)

According to recent WHO figures, the number of reported dengue cases went up more than eightfold over two decades, from 505430 in 2010 to 4.2 million in 2019. The mortality rate also increased, from 960 reported deaths in 2000 to 4032 in 2015. Among the WHO's 6 geographical regions it is estimated that more than 70% of the world's at-risk population for dengue live in the Western Pacific and South-East Asia Regions. India alone accounts for 34% of the total, while the Americas and Africa contribute 14% and 16% of the total respectively (Bhatt S. et al. 2013).

Not only is the incidence of dengue infection high in these regions, dengue is also spreading rapidly to new regions. Before 1970, dengue epidemics were only been seen in 9 countries in South-East Asia and the Western Pacific. Dengue is now reported from more than 100 countries worldwide, including several European nations. Imported cases and local transmission have been described in France, Croatia, Portugal and other 10 European countries (World Health Organization 2022). In 2019, alongside the highest reported number of dengue infections ever from a global perspective, dengue cases were reported from Afghanistan for the first time (World Health Organization 2022). Thus the geographical reach of dengue continues to expand globally, with the overall footprint now encompassing much of the tropics and subtropics. Further continuing expansion is predicted going forward into the 21st century (Messina J. P. et al. 2019), especially in light of changing climate/environmental factors.

A number of circumstances are thought to have contributed to the progressive spread of dengue over time. Human migration (initially from forest dwellings to rural villages and later from villages to towns and cities in tropical countries), alongside progressive deforestation, facilitated the spread of dengue vectors such as *Aedes aegypti* and other *Stegomyia* mosquito species to new urban habitats. Increasing commerce and trade between West Africa and the Americas via sailing ships during the 17th, 18th and 19th centuries then resulted in widespread distribution of *Aedes* species across the New World. The most common vector, *Aedes aegypti*, breeds in small collections of standing water, such as in flower pots, plastic jars, bottles, cans, discarded tires and so on. The rise in uncontrolled urban development in the 20th century, often combined with poor sanitation and inadequate waste disposal services, resulted in the expansion of these mosquito populations and facilitated major disease outbreaks from the 1950s onwards, especially in huge Asian megacities such as Bangkok, Manila, Jakarta and Ho Chi Minh City.

Global warming may be another factor contributing to the spread of dengue. Higher environmental temperatures serve to lengthen the dengue season in endemic regions and also promote dengue infection in new areas such as southern Europe. In addition, the effects of

flooding in rural areas can result in significant migration into urban areas, increasing overcrowding and exerting pressure on the often-limited public health resources (Whitehorn J. and Yacoub S. 2019). Finally, air travel has been associated with the rapid spread of many diseases in the modern era, and recent data has shown that the spread of dengue is associated with air transportation (Quam M. B. et al. 2015, Tian H. et al. 2017)

In addition to the massive burden on public health services, the economic impact of dengue is substantial. A study merging healthcare cost data from 108 countries with information on 95 million symptomatic cases found that 60% of dengue cases occurred in countries with GDP per capita below 6000 USD. The global cost for reduced productivity, premature death and health care utilization related to dengue was 39.3 billion USD or about 414 USD per case (18.5 billion USD for productivity loss, 11.1 billion USD for premature death and 9.8 billion USD for health care utilization). About 40% of the total costs were incurred in South East Asia, 24.7% in the Americas, 21.6 % in the Western Pacific Region, 5.9% in Africa and 6.1% in the Eastern Mediterranean Region (Castro M. C. et al. 2017).

Shepard and colleagues used data from 141 dengue-endemic countries and estimated that the global cost was 70.10 USD per admitted dengue case, 52.16 USD for an ambulatory case, and 84,730 USD for a fatal case. The authors estimated that in 2013 58.40 million dengue cases occurred across 141 countries, costing a total of 8.9 billion USD or 1.56 USD per capita (Shepard D. S. et al. 2016). This cost was higher than for several other notable infectious diseases: cholera, 2 billion USD (WHO 2020); rotavirus gastroenteritis, 900 million USD (Rheingans R. D. et al. 2009); and rabies, 8.6 billion USD (Hampson K. et al. 2015).

1.1.3 Dengue in Vietnam

Vietnam is situated on the eastern border of the Indochinese Peninsula between latitudes 8^{o} and 24^{o}N and longitudes 102^{o} and 110^{o}E. Its total area is approximately 331,212 km^2 with a population of 96.2 million inhabitants in 2019; it is the 15^{th} most populous country in the world. The Vietnamese climate varies during the year, typically with a dry season extending from

November to April and a rainy season from May to October. Temperatures vary between 15-33ºC in northern areas and 21-35ºC in the south. With 40% of the total land mass being mountainous, considerable temperature differences are also apparent between the mountains and low-lying plateaus. The rainy season lasts for almost half of the year with average rainfall from 1500 to 2000 mm in the rainy season, often leading to extensive flooding.

Given the tropical climate, high annual rainfall and high density of inhabitants, Vietnam is vulnerable to many tropical diseases, particularly mosquito-borne infections such as dengue. In 1958 Mihov and his colleagues reported an outbreak of hemorrhagic fever in Hanoi, which seems to have been the earliest report of dengue in the Indochina Peninsula. The report described the clinical characteristics observed among 68 children admitted to the Paediatric Clinic of the University of Hanoi; gastrointestinal haemorrhage and collapse were common and the mortality rate was 7%. Thrombocytopenia, leukocytosis and hepatomegaly were seen in many patients (Mihov C. 1959). Halstead and colleagues reported another outbreak of a dengue-like illness that occurred in Cai Be, Tien Giang, southern Vietnam in 1963 led to 50 deaths. The virus identified was DENV-2 and serological tests were performed to confirm the diagnosis of dengue (Halstead S. B. et al. 1965).

Since that time dengue has become recognised as a major public health issue in Vietnam. The majority of the reported cases are from the southern part of the country. There were 1,518,808 DHF cases and 14,133 deaths reported during the period from 1963 to 1995 (Anders K. L. et al. 2011). However, the number of dengue cases reported each year changes over time without a regular cycle. For example, in 1998 there was a significant increase in dengue admissions across 19 provinces in southern Vietnam with 119,429 DHF cases and 342 deaths, which was 150% higher than the number of DHF cases reported in 1997. One possible reason for the fluctuating case numbers might be a change in the proportion of the different dengue serotypes that are circulating each year. All four dengue serotypes circulate in Vietnam but one serotype tends to dominate for several dengue seasons. DENV-3 predominated between 1997-1999,

responsible for more than 70% of cases in 1998 (Ha D. Q. et al. 2000), however DENV-4 was the most prevalent from 2000 to 2002, followed by DENV-2 during 2002-2006 (Recker M. et al. 2009). Currently, DENV-2 is the most common serotype circulating since 2019 (Vietnam General Department of Preventive Medicine 2020).

In the past, dengue has affected mainly children and young adults in Vietnam, but in recent years the number of dengue admissions among adults has increased (Anders K. L. et al. 2011, Huy B. V. et al. 2019). Data from the Hospital for Tropical Diseases in Ho Chi Minh City (HCMC) shows that the proportion of adults admitted to the hospital with dengue increased from 18% in 1996 to 66% in 2009. Among children with dengue admitted to Children's Hospital 1, Children's Hospital 2 and Hospital for Tropical Diseases, the median age decreased from 10 years old in 2002-2004 to 8 years old in 2007-2009, while the overall median age increased from 20 years old in 2001 to 23 years old in 2009 (Anders K. L. et al. 2011). More recent data indicate that dengue is now seen at all ages from 18 to 85 years old, but with the majority of people affected (74.6%) below 40 years old (Huy B. V. et al. 2019).

In experienced hands the fatality rate for severe dengue is generally low in Vietnam, below 1% according to a 10 year-prospective study conducted at the Hospital for Tropical Diseases in Ho Chi Minh City (Lam P. K. et al. 2013). The mortality for severe dengue in children is still unchanged at Hospital for Tropical Diseases (unpublished data), however, the mortality of severe dengue in adults at this hospital and the National Hospital for Tropical Diseases in 2017 was 7.2% (Huy B. V. et al. 2019).

1.2 The virus and the human immune response

Dengue virus is a single-stranded RNA virus belonging to the family Flaviviridae and includes four serotypes DENV-1 to DENV-4 which are distinguished by serological techniques. The virus has similar characteristics to other flaviviruses, being comprised of an icosahedral nucleocapsid covered by a lipid envelope. The virion has a diameter of 50nm and includes a genome of 11kb in length. This genome is constituted of three structural protein genes

(encoding a core protein C, a membrane-associated protein prM/M, and the envelope protein E) and seven non-structural (NS) protein genes (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) (Perera R. and Kuhn R. J. 2008).

After inoculation into the skin by an infected mosquito, the virus first infects innate immune cells in the dermis layer, including keratinocytes, Langerhans cells, and dendritic cells (Noisakran S. et al. 2010). Later the virus infects monocytes and macrophages in the lymph nodes, liver and spleen and these infected cells travel through the lymphatic system and bloodstream generating an adaptive immune response that is specific to the infecting virus.

All four viral serotypes can cause both symptomatic infection and severe disease. A large cohort study on 3,185 Thai children showed that 58.3% of children were susceptible to primary dengue infection; 39.4% had secondary infection and 1.8% of secondary infection children developed shock (Sangkawibha N. et al. 1984). A 12-year-cohort study on 8002 children in Nicaragua reported higher antibody levels in DHF/DSS cases. The probability of DHF/DSS for children with pre-existing dengue antibodies was 7.64 fold higher than those without pre-existing antibodies (Katzelnick L. C. et al. 2017). These observational results confirmed the strong association between secondary infection with severe dengue progression. The high antibody level did not bring better protection but in reverse, it can induce more severe disease. It is important to understand the mechanism underlying this phenomenon not only for disease prognosis but also for the development and testing of a dengue vaccine.

During the first (primary) infection with any dengue serotype, long-lived immunity develops against that particular serotype, but protection against other serotypes is short-lived, estimated at 2-3 months by Sabin in the 1950s (Sabin A. B. 1952). More recent evidence suggests the length of cross-protection can be up to 2.6 years (Montoya M. et al. 2013, Anderson K. B. et al. 2014). During a subsequent (secondary) infection with a different serotype, a phenomenon known as antibody-dependent enhancement (ADE) is thought to induce higher viremia. Halstead originally proposed the ADE theory, by which low levels of non-neutralising heterotypic antibodies present following the first infection bind to the new virus, increasing the

efficiency of uptake by Fc receptors on monocytes and macrophages and amplifying viral replication. This results in higher viremia during the early phase of a secondary infection (Halstead S. B. 1989). As a consequence, the host immune response to the infection is more aggressive, which then results in increased clinical disease severity. A number of in-vitro and in-vivo studies provide support for the occurrence of ADE in secondary dengue infections (Gromowski G. D. et al. 2008, Katzelnick L. C. et al. 2017, Muhammad Azami N. A. et al. 2020). Other factors that may influence the severity of secondary infections include rapid mobilisation of serotype cross-reactive memory T cells (Screaton G. and Mongkolsapaya J. 2006) and effects of pre-existing antibodies that may trigger the release of cytokines and cytotoxic granules by direct lysis of natural killer cells and/or mast cells (Figure 1-1) (St John A. L. and Rathore A. P. S. 2019)

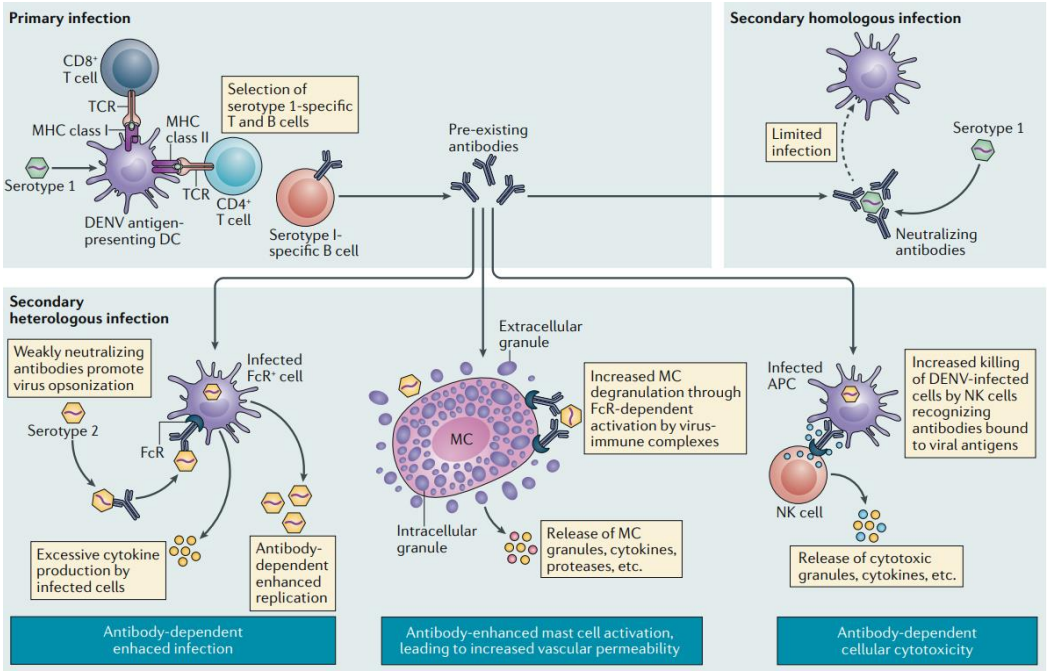


Figure 1-1. Adaptive immune responses to primary and secondary dengue virus infections (Rathore A. P. et al. 2019)

1.3 Pathogenesis

Among symptomatic dengue infections, there is a wide range of clinical manifestations from an uncomplicated flu-like illness to severe disease with two major pathological characteristics:

vascular leakage and haemorrhage. The mechanisms responsible for severe disease are poorly understood but are likely to relate to differences in immune response in different individuals as well as to characteristics of the infecting virus. Potential factors contributing to the two major features are summarised below.

1.3.1 Vascular leakage

The underlying mechanisms of vascular leakage are thought to be associated with endothelial dysfunction rather than endothelial cell infection or degradation. The structure of vascular endothelium includes endothelial cells with intercellular junctions and a glycocalyx layer This glycocalyx layer has a negative charge and consists of proteoglycan, glycosaminoglycans, plasma and endothelium-derived soluble proteins (hyaluronic acid, soluble proteoglycans, antithrombin III) (Reitsma S. et al. 2007) and plays important roles in capillary permeability which influences the movement of water and other molecules through capillary walls. During dengue infection, endothelial activation occurs, which is evidenced by the increase in plasma levels of adhesion markers including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion protein 1 (VCAM-1), E-selectin and vascular endothelial cadherin (VE-cadherin) (Sharma S. et al. 2016, Vitoria W. O. et al. 2019). Another important product of excessive endothelium activation is nitric oxide which can modulate vascular permeability (Kubes P. and Granger D. N. 1992). A large clinical prospective study on 324 dengue patients has shown that endothelial dysfunction occurs at the early phase of illness and is associated with plasma leakage through endothelial nictric oxide pathways, evidenced by hypoargininemia and high arginase-1 (Yacoub S. et al. 2017). Regarding viral factors, NS1 antigen activates macrophages and monocytes through the Toll-like receptor 4(TLR4), which produces inflammatory cytokines associated with endothelial dysfunction (Modhiran N. et al. 2015). In addition, cross-reactive memory T cells trigger the inflammatory cascade in secondary infection that also results in endothelial dysfunction (Screaton G. et al. 2015).

Besides evidence of over-activated endothelium, several recent studies showed evidence of glycocalyx alteration demonstrated by elevated plasma levels of proteoglycans, syndecan 1 and endocan during the critical phase, associated with the severity of plasma leakage (Lam P. K. et al. 2020). The consequence of this is that many proteins with different molecular sizes may be lost through glycocalyx degradation, including anticoagulant proteins and albumin (Wills B. A. et al. 2004). Dengue NS1 can also bind to glycocalyx, causing salicylic loss and heparin sulfate cleavage (Puerta-Guardo H. et al. 2016). Endothelial infection was observed in *in-vitro* studies on a dengue mouse model, where the apoptosis of endothelial cells was observed. Autopsy research however did not show infected endothelial cells during the dengue infection (Aye K. S. et al. 2014).

In summary, multiple factors affect the vascular endothelium causing plasma leakage in dengue with more evidence in regards to malfunction than its disruption.

1.3.2 Haemorrhage

Bleeding is another important clinical manifestation of dengue and varies from mild to severe, but thrombocytopenia and coagulopathy are almost invariably present.

Thrombocytopenia occurs commonly in dengue and there are several hypotheses regarding mechanisms that may explain this. First, there is increased destruction of platelets during dengue infection. This is associated with immune complexes containing antigens found on platelet cell surfaces (Boonpucknavig S. et al. 1979); due to activities of complements and anti-platelet antibodies (Lin C. F. et al. 2006, Hottz E. D. et al. 2013). Secondly, bone marrow function is significantly depressed, resulting in decreased platelet production (Mitrakul C. 1987). In addition, platelet function was also abnormal with hypoaggregation (Srichaikul T. et al. 1989)

Regarding coagulopathy, early studies showed that dengue patients typically have minor increases in prothrombin time (PT), significantly prolonged partial thromboplastin times (PTT), moderate to severe reduction in fibrinogen levels and slight to moderate increases in fibrinogen-

degradation products (FDPs) (Mitrakul C. et al. 1977, Funahara Y. et al. 1987). Concentrations of various coagulation factors, including factors II, V, VII, VIII, IX, X and XII, have also been found to be slightly decreased (Mitrakul C. et al. 1977). Many subsequent studies have confirmed the typical pattern of an increase in PTT, sometimes with reduced fibrinogen levels but without much increase in PT, with the degree of derangement of these parameters generally correlating with the severity of leakage. Several elements of the glycocalyx layer are important in haemostatic control – it is possible that disruption of this layer results in the release of factors such as heparan sulfate (which may function as an anticoagulant similar to heparin), and/or that many proteins including fibrinogen and coagulation factors leak to the interstitial space when the selective barrier function is disturbed (Wills B. A. et al. 2002, Wills B. A. et al. 2004). Another possible contributory factor is molecular mimicry, as the core, prM, E and NS1 proteins of the dengue virus have a similar amino acid sequence to certain coagulation molecules (Lin Y. S. et al. 2011). For example, antibodies produced during acute dengue have been shown to cross-react with plasminogen and might interfere with the fibrinolytic pathway (Markoff L. J. et al. 1991)

1.4 Clinical manifestations and diagnosis

Clinical manifestations of dengue can vary from a mild fever to life-threatening hypovolemic shock, severe bleeding and multi-organ impairment. The WHO classification for dengue was established firstly in 1997 when dengue was classified into an undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF). DHF was sub-classified into 4 grades with grade III and IV incorporating compensated shock and decompensated shock (World Health Organization 1997). This classification could not fit many severe dengue cases with the criteria of DHF (Bandyopadhyay S. et al. 2006), therefore the WHO guideline released in 2009 recommended a new classification in which dengue was classified into dengue with/without warning signs and severe dengue (Figure 1-2). Although the new classification has higher

sensitivity and specificity compared to the 1997 WHO classification (Horstick O. et al. 2014), the old classification is still widely used in many countries.

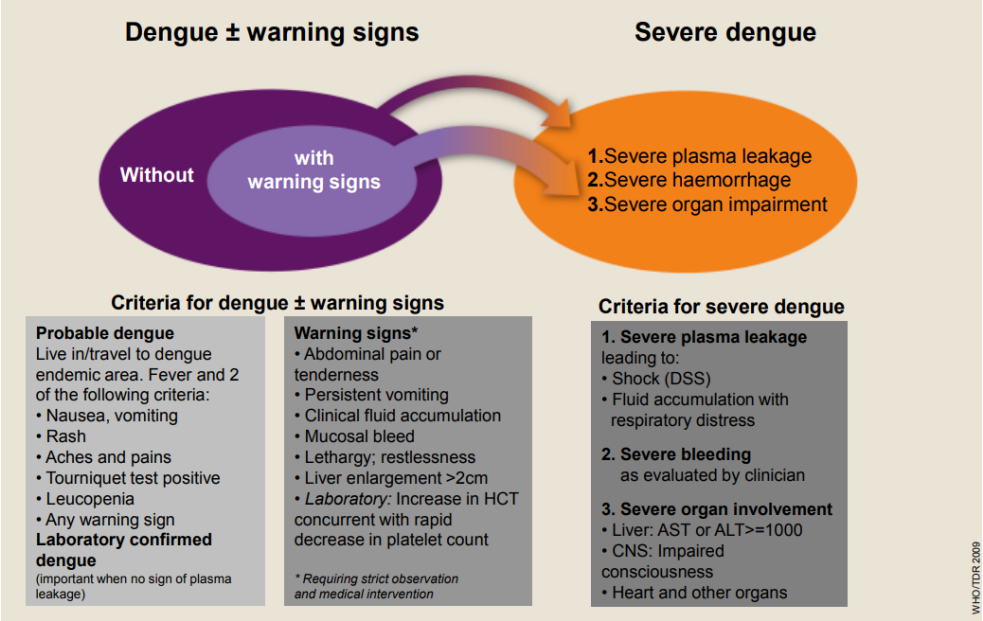


Figure 1-2. Dengue cases classification by severity (retrieved from WHO hand book of dengue management 2012, page 7) (World Health Organization 2009)

The dengue manifestations are can be divided into 3 phases: febrile, critical and recovery phase (Figure 1-3). The critical phase of the disease often occurs around defeveresence

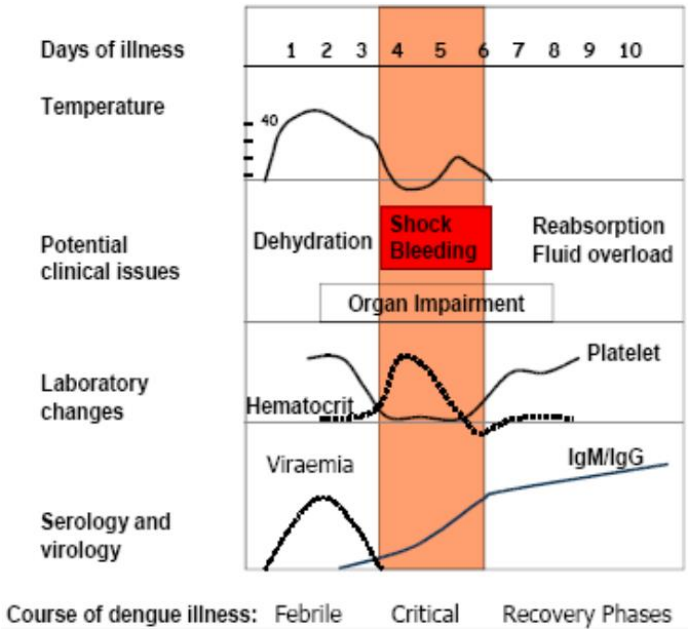


Figure 1-3. The course of dengue illness retrieved from the WHO hand book of dengue management 2012 (World Health Organization 2009)

1.4.1 Febrile phase

This phase often occurs from day 1-3 of the onset of illness. Dengue patients develop a sudden high-grade fever which usually lasts 2 -7 days accompanied by generalised body ache, myalgia, arthralgia, facial flushing, skin erythema, retro-orbital eye pain, rubeliform exanthema and headache. Other non-specific symptoms are sore throat, anorexia, nausea and vomiting. Petechiae and mild mucosal bleeding may be seen in some cases but massive bleeding is not common. Hepatomegaly is often detected after a few days of fever (World Health Organization 2012).

Due to non-specific clinical symptoms, it is difficult to distinguish dengue from other viral infections during this phase. A tourniquet test can be performed during this phase to increase the probability of dengue diagnosis. Haematology changes are commonly seen including

decreased total white cell count and a slightly decreased platelet count. NS1 antigen and PCR tests are positive and often used to give a confirmed diagnosis during this phase.

1.4.2 Critical phase

This phase is often seen from day 3-7 of illness and is marked by an abrupt fever. Some patients with increased vascular permeability may develop many warning signs (abdominal pain or tenderness, persistent vomiting, mucosal bleed, restlessness, hepatomegaly) and severe complications including shock, bleeding and/or multi-organ impairment. Dengue shock syndrome (DSS) is more often seen in children than in adults due to higher vascular permeability (Gamble J. et al. 2000) and weaker compensative ability with intravascular volume depletion. During the initial stage of shock, systolic blood pressure is normal and diastolic blood pressure increases resulting in a narrow pulse pressure $\leq$ 20 mmHg in children, due to peripheral vasoconstriction and tachycardia as the compensatory mechanism responses to a hypovolemic status. In adults, tachycardia and narrow pulse pressure are often manifestations of decompensated shock. Therefore other signs of poor capillary perfusion (delayed capillary refill time, cold extremities) should be recognised as early as possible to give an appropriate diagnosis and management. This is in contrast with septic shock, where the patient with dengue shock syndrome is often alert and lucid.

If shock is not managed, a few hours later it will progress to decompensated shock and multi-organ failure with hypotension, weak or peripheral pulseless, mottled, cold and clammy skin, decreased urine output and bleeding. Metabolic acidosis occurs manifested by more rapid and deep breathing. Children and young adults with profound shock still have clear mental status until the late phase of hypotensive shock. Major bleeding may occur with/without prolonged shock due to coagulation abnormalities in combination with thrombocytopenia, acidosis and hypoxemia alongside disseminated intravascular coagulation (World Health Organization 2012).

In terms of changes in laboratory tests, the presence of plasma leak varies in different patients and ultrasound can help to detect early plasma leak from day 2 of illness with images showing thickening of the gallbladder, small pleural effusion (more dominant on the right than the left) and ascites (Colbert J. A. et al. 2007). Haematological abnormalities can be seen with progressive leukopenia, rapidly decreased platelets and increased haematocrit. Thrombocytopenia is more severe in adults than in children (Trung D. T. et al. 2012) and platelets below 20,000/mm3 are common in severe cases. Coagulation tests are abnormal with prolonged PT, prolonged aPTT, decreased fibrinogen and coagulation factors but full blown diffuse intravascular coagulation is rare (Wills B. A. et al. 2002). As albumin concentration decreases, liver enzymes increase. Renal failure and metabolic acidosis may occur in those with prolonged shock. NS1 antigen or PCR test may be still positive during this phase. IgM is often negative on the first five days of illness; IgG is positive in secondary dengue cases at an early day and negative in primary infection cases.

1.4.3 Recovery phase

This phase is often after day 6 of illness. The plasma leakage stops and there is gradual reabsorption resulting in a high intravascular volume status lasting for 2-3 days (Yacoub S. et al. 2017). Clinical symptoms improve with stable haemodynamic status, appetite, and increased urine output. Recovery rash appears on the extremities, often accompanied with pruritus. Bradycardia is commonly seen during this phase. Respiratory distress will occur in some patients due to a large amount of pleural effusion, ascites or pulmonary oedema.

With regard to haematological changes, the haematocrit returns to the normal range or even lower due to the reabsorption. White blood cells begin to return to normal levels, often after defervescence and earlier than platelets. Coagulation abnormalities are improved. Ultrasound can detect pleural effusion, ascites and increased cardiac output (World Health Organization 2012, Yacoub S. et al. 2017).

1.5 Predictors of severe dengue

There are multiple factors associated with severe dengue including both viral and host factors.

NS1 level is correlated with viremia and DHF cases have higher NS1 levels compared to those with DF. One study showed that a higher level of NS1 within the first three days of illness is a predictor of developing DHF (Libraty D. H. et al. 2002) While viremia was associated with more severe disease (Vuong N. L. et al. 2020), the duration of viremia seems to not influence progression; in reverse, a rapid viral clearance due to adaptive immune response during secondary infection is more commonly seen in DHF cases (Vaughn D. W. et al. 2000). With regard to dengue serotype, it may play a role in contributing to a severe prognosis of dengue infection.

A cross-sectional study in Brazil from 2009 to 2013 showed that patients infected with DENV-2 had seven times higher risk of severe dengue compared to those infected with other dengue serotypes (Vicente C. R. et al. 2016). Another large prospective study in Thailand from 1994 to 2006 showed that DENV-2 and DENV-3 were associated with more severe dengue (Fried J. R. et al. 2010). However, there were also reports of a severe dengue epidemic related to DENV-4 (Rocha B. A. M. et al. 2017), hence serotype may be just one of multiple factors associated with severe dengue.

The most common immune-related factor associated with severe dengue is secondary infection due to ADE. The immune complex includes the heterotypic antibodies binding to the Fc receptor of monocyte or macrophages, facilitating more viral uptake resulting in higher viremia during the early phase of dengue infection. The consequence of this is a stronger immune response through T-cell stimulation, followed by a cytokine storm and the complement cascade. The endothelial layer is damaged directly by NS1 or considered to be in a state of malfunction due to immune-related factors. Of note, ADE can happen in infants who have a primary infection causing a high proportion of DHF/DSS among infants infected with dengue (Chau T. N. et al. 2010).

Other host factors related to dengue severity are age, nutritional status, underlying disease (diabetes, hypertension) and genetic determinants. Dengue studies have shown that vascular leak varies by age, with younger children having higher vascular permeability (Gamble J. et al. 2000), resulting in younger children having a higher risk of dengue shock syndrome. Although the proportion of DSS among children with dengue is higher than in adults, the case fatality rate in adult DSS is much higher than in paediatric DSS (5,5% in adults versus 1.4 % in children) (Anders K. L. et al. 2011). Recent studies have shown that elderly people above 60 years old with dengue have a 1.56 times higher risk of having severe dengue (Vicente C. R. et al. 2017). The reason for this could be the higher prevalence of secondary infection among the elderly and comorbidities, especially diabetes, hypertension, chronic renal disease, chronic obstructive lung disease and ischemic heart disease (Toledo J. et al. 2016). Besides age, nutrition status is also an important risk factor for severe dengue. Several systematic reviews and meta-analyses focusing on children showed that obesity increases the risk of severe dengue while undernourishment may be the protective factor (Zulkipli M. S. et al. 2018, Sangkaew S. et al. 2021). The mechanisms are related to the increase in viral replication when there is higher lipid accumulation in the endoplasmic reticulum and a decrease in immune response and endothelial dysfunction (Gallagher P. et al. 2020). The admission rate of males and females with dengue are similar but more females develop severe dengue (Anders K. L. et al. 2011, Sangkaew S. et al. 2021). Comorbidities have shown a strong correlation with severe dengue, among them, diabetes and renal failure increased the risk of severe progression more than 4 times (Sangkaew S. et al. 2021).

Results of a recent meta-analysis study suggested vomiting, abdominal pain, fluid accumulation within the first 4 days of illness, low platelets, low albumin, high AST and ALT are important predictors of developing severe dengue (Sangkaew S. et al. 2021). In addition, genetic factors may contribute to the severity of dengue infection. A case-controlled study in children from 5 to 15 years of age in the Philippines found lower levels of human leukocyte antigen (HLA) HLA-A*33:01 in severe dengue, suggesting HLA could be a protective factor (Mercado E. S.

et al. 2015). Other studies reported HLA-A11 is related to a higher risk of DHF (Chen Y. et al. 2019, Chaisri S. et al. 2020). A meta-analysis on genetic factors found that HLA-A*0203, A*24 are also related to race and dengue severity (Chen Y. et al. 2019).

The mortality of severe dengue varies from less than 1% to 23% (Lam P. K. et al. 2013, Chen C. M. et al. 2016) depending on the severity of patients at admission as well as the supportive management. There are several independent predictors for poorer outcomes among paediatric DSS patients including developing shock earlier, ecchymosis, low platelet counts, narrow pulse pressure at first shock (Huy N. T. et al. 2013), younger age, higher temperature, higher haematocrit, female and worse hemodynamic status at shock (Lam P. K. et al. 2015). For adult DSS patients, a prospective study on 369 Indian cases showed that age >= 24 years, dyspnea at rest and decreased sensorium were independent factors of mortality (Jain S. et al. 2017). A large retrospective study in Taiwan on 4787 severe dengue cases in ICU found that the mortality was related to lower Glasgow Coma Score, lower platelet counts and more organ failure (Chen C. M. et al. 2016). In addition, venous lactate above 4 mmol/l was another independent marker that can be used practically to predict recurrent shock in patients with DSS (Yacoub S. et al. 2017).

1.6 Prevention

The prevention of dengue infection relies on mosquito control and interruption of human-vector contact. There are different methods to control Aedes aegypti including modifying vector environment (e.g. installation of a piped water supply, discarding of containers and tires…), using a mosquito net or applying mosquito repellent. Insecticides are also used to treat mosquitoes with precaution as they are toxic to humans, however, mosquitos develop resistance to the chemical insecticides (World Health Organization 2009). The biological method that has been applied in recent years seems to be promising in that genetically modified mosquitos are used to displace wild mosquitos. The method introduces *Wolbachia*, an intracellular bacteria, to infect *Aedes* mosquitos, which reduces dengue replication in mosquitos and subsequently

decreases the mosquitos' life span (McMeniman C. J. et al. 2009, Moreira L. A. et al. 2009). Field trials held in northern Australia and Indonesia demonstrating primarily good results (73% reduction in dengue incidence during a 24-month intervention) bring new hope for a vector control strategy (Ryan P. A. et al. 2019, Indriani C. et al. 2020).

For long-term prevention, different dengue vaccines have been developed. Four types currently include: live attenuated viruses, chimeric live attenuated viruses, inactivated or subunit vaccines and nucleic-acid-based vaccines ((World Health Organization 2009). The ultimate goal is effective against all four co-circulating dengue serotypes and to avoid inducing antibody-dependent enhancement (ADE). Dengvaxia was the first dengue vaccine available commercially in 2015. It is a live attenuated tetravalent chimeric vaccine with was tested in over 31,000 children from 2-14 years old. The efficacy was 56.5 % in the Asian population and 64.7% in American people (Capeding M. R. et al. 2014, Villar L. et al. 2015). This vaccine however may cause ADE and may not be indicated for dengue-seronegative individuals. The most recent dengue vaccine approved by the European Medicines Agency is TAK-003, a recombinant chimeric vaccine. This vaccine showed good efficacy at 74.9% for the seronegative population and 80.2% overall to protect against symptomatic dengue (Biswal S. et al. 2019). Other types of vaccines are being developed with the aim to achieve broader efficacy (Dejnirattisai W. et al. 2015).

1.7 Clinical management

There are currently no effective antiviral agents or immune-modulatory therapy for dengue infection; therefore management relies on supportive care depending on the severity of clinical manifestations. A patient with dengue will be classified as dengue with or without warning signs or severe dengue and assigned appropriate care. With specific pathology related to plasma leak and coagulopathy, the principle of dengue management is fluid replacement and diminishing the risk of bleeding. Of note, only paracetamol is recommended as an antipyretic for dengue patients. Aspirin should not be used in concern of Reye's syndrome and liver

involvement. Other non-steroidal anti-inflammatory drugs may induce thrombocytopenia and carry the risk of gastrointestinal bleeding (Russell R. I. 2001). They are also not recommended to be used in dengue cases.

Dengue patients without warning signs can stay at home for self-care. After 3 days of illness, all dengue patients without warning signs need to revisit clinicians daily for checking disease progress until the recovery phase.

Dengue patients with warning signs or those with comorbidities (pregnancy, infancy, old age, obesity, diabetes, renal failure, liver failure, haematological abnormalities, cardiac abnormalities) or those with social problems (living alone, living far from health care centers) need to admit to the hospital for closer complication monitoring. Oral fluid intake is recommended for all patients with warning signs. The use of parenteral fluid is only indicated for those who have persistent vomiting or diarrhoea. The fluid choice is an isotonic crystalloid such as 0.9% saline, Ringer Lactate, or Hartmann solution. These patients have a higher risk of developing shock, therefore Hct should be monitored before and during fluid infusion in combination with closer vital signs monitoring and clinical assessment every 2-4 hours (World Health Organization 2009).

Severe dengue cases will be managed at high dependency units or intensive care units for critical support which will be discussed in detail below

1.7.1 Fluid management for dengue shock syndrome

Fluid management algorithms for DSS are different between compensated shock and profound shock; between children and adults in terms of fluid types and dose at initial resuscitation and maintenance (Figure 1-4 and Figure 1-5).

For paediatric patients with compensated shock, isotonic crystalloid is used at the beginning with a rate of 10-20ml/kg for 1 hour. Hct measurements are checked before and after a fluid bolus, which helps to interpret the fluid response. Improvement signs include the improvement in heart rate, normal blood pressure, improved peripheral perfusion and decreased Hct.

- If the patient improves after the first fluid bolus, intravenous fluids will be reduced to 10 ml/kg/h for 1-2 hours; 7ml/kg/hour for 2 hours; 5 ml/kg/h for 4 hours and maintained at 3 ml/kg//h for up to 24- 48 hours.
- If the patient does not improve after the first fluid bolus and Hct is still high, this suggests the need for greater fluid replacement. A colloid solution is used at a rate of 10-20 ml/kg for 1 hour then reduced to 10ml/kg/hour for 1 hour; 7ml/kg/h for 1 hour. The colloid solution should be changed to crystalloid when the patient improves.
- If the patient does not improve after the first fluid bolus and Hct decreases rapidly and severe bleeding is a concern, then it is considered urgent to give a blood transfusion for any overt bleeding. In some situations, the bleeding may be occult and require a careful assessment combined with the use of imaging diagnosis tools to look for bleeding sites.

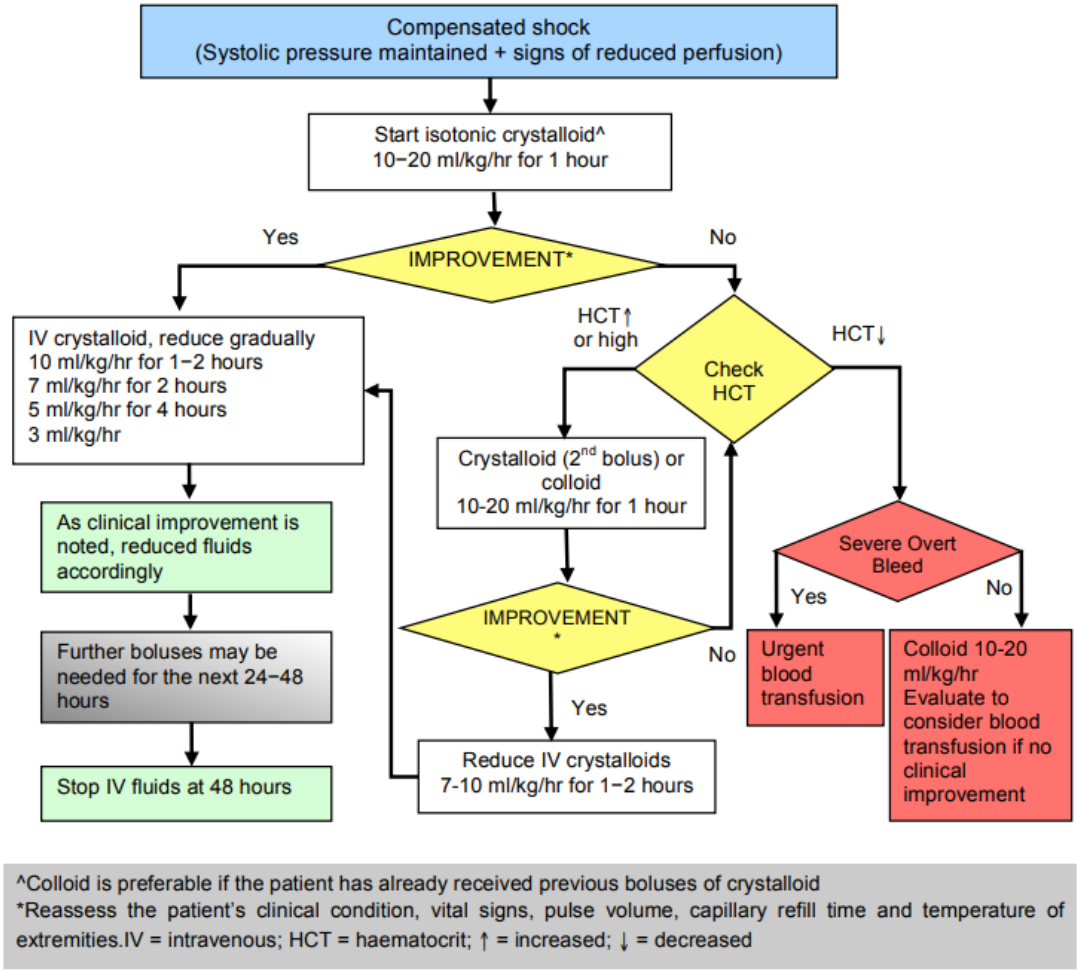


Figure 1-4. Fluid management for infants and children with compensated shock (retrieved from WHO handbook of dengue management 2012, page 30) (World Health Organization 2012)

For profound shock cases (hypotensive or unmeasurable blood pressure and pulse), a bolus of crystalloid or colloid fluid at 20ml/kg over 15-30 minutes should be given to quickly restore intravascular volume. Colloids are preferred as they can increase cardiac index faster than crystalloids (Dung N. M. et al. 1999, Ngo N. T. et al. 2001, Wills B. A. et al. 2005) .

- If the patient improves, the colloid is continually given at 10ml/kg/h for one hour then switched to crystalloid 10 ml/kg/h; 7.5 ml/kg/h for 2 hours; 5 ml/kg/h for 4 hours and maintained at 3ml/kg/h for up to 24-48 hours but this should not exceed 48 hours.

- If the patient is still unstable and Hct is still high after the first bolus, the patient will require a second bolus with colloid fluid at 10-20 ml/kg over 30 minutes to 1 hour. If the patient's status improves, the fluid rate is reduced to 7-10ml/kg for 1-2 hours, then switched back to crystalloid at the rate mentioned above. If the patient is still unstable after the second bolus and Hct is still high, a third bolus of colloid should be given at 10-20 ml/kg over 1 hour, then reduced to 7-10 ml/kg/h for 1-2 hours.
- If Hct decreases rapidly accompanied with no improvement, this indicates severe bleeding and requires a blood transfusion.

During 24-48 hours of fluid management, several episodes of recurrent shock may occur (Hct increases, narrow pulse pressure, weak pulse, poor peripheral perfusion), further colloid boluses may be needed. The rate should be judiciously titrated depending on the Hct and clinical response. Of note, infants have a low baseline Hct of 30%, therefore, a Hct of 40% is high and more concentrated than in older children.

In adults with decompensated shock or profound shock, the choice of initial fluid is often crystalloids. The rates of fluid decrease more rapidly from 10 ml/kg/h to 5-7 ml/kg/h for 1-2 hours; 3-5 ml/kg/h for 2-4 hours and 2-3 ml/kg/h in maintenance. If the shock status does not improve, a second bolus with the colloid solution at 10-20 ml/kg for one hour is preferred then changed to crystalloids. Compared to fluid management strategy for children, the use of colloid for adults is less and only for a 1-hour bolus; also the rates of fluid infusion decrease quicker.

During fluid management, patients with DSS should be monitored closely every 30 minutes or hourly during the danger period and then at 1-2 hourly intervals when the clinical status improves. Vital signs, comfort level, peripheral perfusion and urine output should be monitored. Hct should be checked before and after a bolus and repeated if necessary.

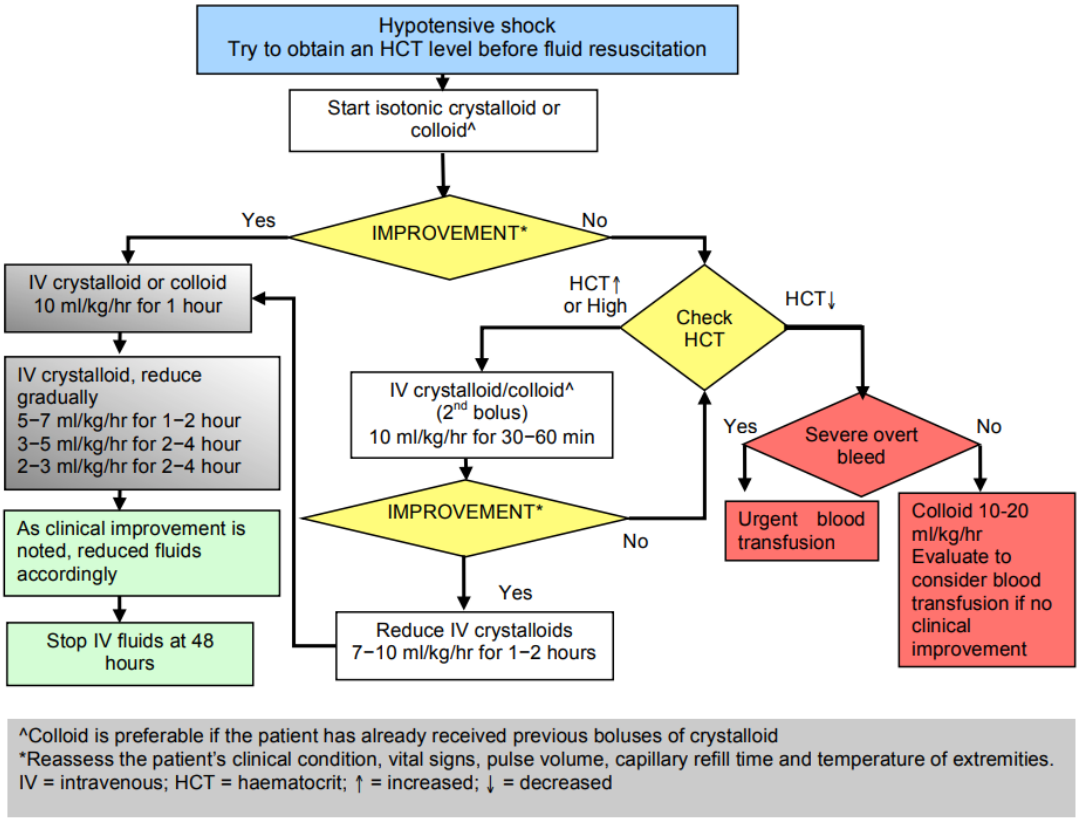


Figure 1-5. Fluid management for profound shock (retrieved from WHO hand book of dengue management 2012, page 32) (World Health Organization 2012)

There are different types of crystalloids and colloids used for DSS patients, which depends on their availability in different countries as well as the preferences of clinicians. The summary of different fluid types is shown in the below table (Table 1-1). Crystalloids often used include 0.9% saline, Ringer's Lactate, or Hartmann solution. Colloids used are synthetic colloids including dextran, hydroxyethyl starch and gelatin. Despite the remarkable amount of evidence in regards to synthetic colloids' harmful effects in septic shock patients (Lewis S. R. et al. 2018), hydroxyethyl starch, dextran and gelatin are being used across many dengue epidemic countries for profound shock or recurrent shock cases. There is a lack of evidence or reports about the benefits as well as side effects of these colloids in DSS management. On the other hand, albumin, a natural colloid with fewer side effects, is too expensive and has limited availability, therefore it seems to be impossible to use albumin to replace synthetic colloids. In

addition, results of studies about colloids were mainly in the adult population that may not be generalised to children. There is not enough evidence both in literature and in clinical practice about which fluid types and fluid resuscitation strategies improve outcomes of children with DSS.

Table 1-1. Different types of fluid used in dengue shock syndrome (Mitra S. and Khandelwal P. 2009, Myburgh J. A. and Mythen M. G. 2013, Trung D. T. et al. 2020)

Types		**Products**	**Source**	**MW (kDa)**	**Osmolality (mOsm/L)**	**Oncotic pressure (mmHg)**	**Volume expansion (%)**	**Maximal dose/kg**	**Main Concerns**
Human Plasma		Plasma	Human		275-295	-	-	-	Anaphylactic reaction
Colloids	Albumin	4% Albumin	Human	69	250	20-29	80	-	High cost
	Hydroxyethyl starch	6% HES 200/0.5	Maize starch	200	304	30-37	100	33 ml	Renal impairment, coagulation abnormality
		6% HES 130/0.42	Potato starch	130	296	36	130	50 ml	
	Gelatin	4% Gelatin	Bovine Gelatin	35	274	26-29	70	-	Anaphylactic reaction
	Dextran	10%Dextran 40	Bacteria	40	280-324	168-191	200	1.5 g	Renal failure Coagulation abnormalities Allergic reaction
		6%Dextran 70	Bacteria	70	280	56-68	120	1.5 g	
Isotonic Crystalloids		0.9 %saline	-	-	308	-	20-25	-	Hyperchloremic acidosis
	Compounded Sodium Lactate	Hartmann or Ringer Lactate	-	-	281	-	20-25	-	Accumulated lactate in the case with liver failure
	Balanced Salt Solution	PlasmaLyte	-	-	294	-	-	-	-

1.7.2 Management for bleeding

Patients with dengue carry a high risk of bleeding, therefore invasive procedures should be avoided as much as possible. Minor mucous bleeding may occur in dengue patients (nasal bleeding or gum bleeding) and is often self-limited, improving rapidly during the recovery period. In children, severe bleeding rarely happens and is usually associated with prolonged shock, meanwhile, in adults, major mucosal bleeding (gastrointestinal, vaginal) is more common. Prophylactic platelet transfusion for thrombocytopenia <20, 000/mm3 without severe bleeding is not helpful and may result in serious adverse events (Lye D. C. et al. 2009, Lye D. C. et al. 2017). If severely overt bleeding occurs, fresh whole blood or a combination of packed red cells and other blood products should be transfused urgently but with careful monitoring for signs of fluid overload. At the same time, any possible interventions to stop bleeding should be undertaken (nasal packing with adrenaline for example). Platelet transfusion with or without fresh frozen plasma and cryoprecipitate should be considered for obviously severe bleeding. Proton pump inhibitors and H2-antagonists can be used though there is a lack of evidence for their efficacy (World Health Organization 2012).

1.7.3 Management for other complications

Respiratory distress often occurs in patients with DSS during fluid management due to massive pleural effusion, ascites, acute respiratory distress syndrome and/or pulmonary oedema. The majority of these cases respond well to bed rest, nasal positive pressure support and diuretic therapy. However, some cases require mechanical ventilation with high-pressure support in combination with inotropes (Lam P. K. et al. 2013). Drainage of ascites and pleural effusion are considered if hypoxemia gets worse and the patient still requires fluid infusion. An intravenous diuretic should be started as soon as the reabsorption phase starts, which effectively improves the respiratory status.

The management for other complications including hepatitis, acute kidney injury, myocarditis, encephalopathy, electrolyte and acid-base imbalances is based upon standard guidelines (World Health Organization 2012).

1.8 Hemodynamic monitoring

Hemodynamic monitoring gives continuous information and a series of data on vital signs as well as intravascular volume status including heart rate, blood pressure, intravascular pressures (arterial blood pressure, central venous pressure, pulmonary artery occlusion pressure), stroke volume, cardiac output, and systemic peripheral resistance. This information is routinely used in hospitals to assist clinicians to evaluate critically ill patients with different disorders and monitor disease progression, as well as to guide appropriate therapeutic interventions, for example, fluid resuscitation and inotropic support.

1.8.1 Principles of hemodynamic monitoring

Hemodynamic monitoring refers to two key entities of the circulatory system: oxygen delivery (DO2) and oxygen consumption (VO2)

DO2 (ml O2/min) = CO x Hgb x 1.34 x SaO2

VO2 (ml O2/min) = CO x Hgb x 1.34 x (SaO2 –SvO2)

Where CO is cardiac output and CO = SV x HR (SV is stroke volume and HR is heart rate), Hgb is haemoglobin in g/dl, SaO2 is the percentage of arterial oxygen saturation, SvO2 is the percentage mix of venous oxygen saturation (Hamzaoui O. et al. 2015). Intensive care resuscitation aims to improve DO2 and decrease VO2 which would improve outcomes.

In addition, the dynamics of human circulation can be explained by Ohm's law (Figure 1-6), which states that current (I) equals to the voltage difference (ΔV) divided by resistance (R). Translating this into the human circulation, ΔV is the pressure difference between arterial pressure and venous pressure or blood pressure (BP), R is the systemic vascular resistance (SVR) and I is the blood flow or cardiac output. From there, we have SVR = BP/ CO. This formula allows us to calculate SVR which decreases in septic shock or increases in cardiogenic

shock or hypovolemic shock to guide the resuscitation. It is also used to calculate SVR in different types of hemodynamic monitors as a derived parameter from CO and BP.

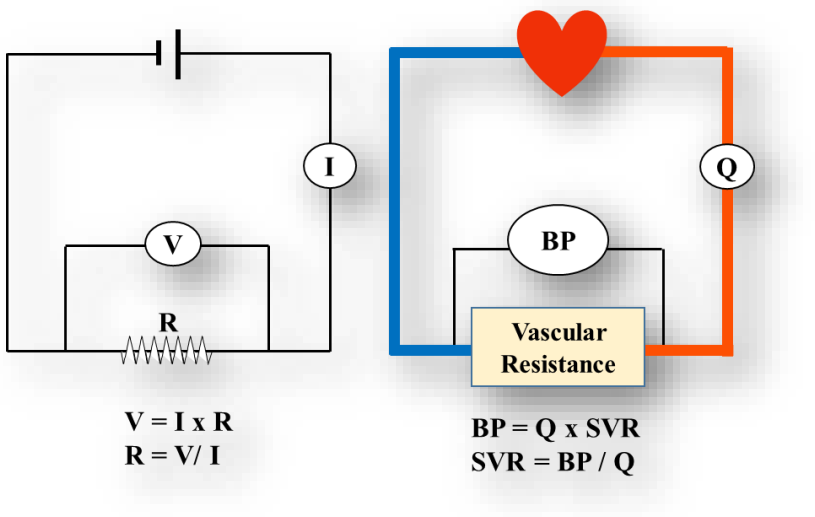


Figure 1-6. Diagram demonstrating translating Ohm's Law (left) and human circulation (right) (reproduced from http://www.learnhemodynamics.com/).

1.8.2 Shock

The most common and life-threatening condition of patients admitted to intensive care units is shock. Shock is defined as "an acute circulatory failure associated with inadequate cellular oxygen utilization" resulting in cellular dysfunction. Shock can be recognised at the early phase (compensated shock) or late phase (decompensated shock). Early manifestations of shock are related to signs of tachycardia and impaired tissue perfusion (skin mottling skin, acrocyanosis, delayed capillary refill time due to vasoconstriction, and decreased urine output). Blood pressure can be normal or narrow and mental status often remains normal. Blood lactate, a product of anaerobic glycolysis due to tissue hypoxemia or hepatic/renal failure, increases as shock evolves and is associated with higher mortality. Of note, hypotension is an indicator of decompensated shock, which occurs in the later phase of shock (Bonanno F. G. 2011). Therefore, patients presenting with any signs of impaired tissue perfusion or hyperlactatemia should be monitored carefully and timely therapeutic interventions like fluid replacement and/or inotropic support initiated.

Causes of shock can be classified into 4 main categories (Table 1-2) including cardiogenic shock, hypovolemic shock, distributive shock and obstructive shock, which determines the principles of management and type of supportive care. A 12-year-cohort study in Denmark comprising 1,553 shock patients found that hypovolaemic shock and distributive septic shock were the most common shock etiologies with 30.8% and 27.2%, followed by distributive non-septic shock, cardiogenic shock, and obstructive shock (23.4%, 14%, 0.9% respectively) (Gitz Holler J. et al. 2019)

There are 3 important components to be considered in regards to shock, intravascular volume, cardiac contractibility and systemic vascular resistance (Figure 1-6). Accordingly, clinical management of shock involves addressing three questions: 1/ is the patient intravascularly volume depleted? 2/ does the patient need inotropic or cardiac support? 3/ does the patient require vasopressors? These questions can be derived from a combination of careful medical history, clinical examination, and imaging. If possible bedside point-of-care ultrasound (POCUS) should also be utilised. POCUS can be used to answer those three questions giving a rapid diagnosis in under 5 minutes in the hands of trained staff. (Volpicelli G. et al. 2013). However, for more complex cases when we need additional information regarding tissue perfusion for advanced support, other techniques of continuous hemodynamic monitoring are required to optimize DO2 and VO2.

Cardiac output decreases in the majority of shock cases and therefore is the most common parameter to monitor as a surrogate for DO2. CO is closely monitored during fluid resuscitation of shock and an increase in CO can be used as an indicator of fluid responsiveness.

Table 1-2. Description of the four types of shock

Categories	Causes	CO	LVEF	SVR
Cardiogenic shock	Dysrhythmias Myocarditis Cardiomyopathies Coronary disease Valvular heart disease	decreased	decreased	increased
Hypovolemic shock	Hemorrhagic Diarrhea Vomiting Heat stroke	decreased	increased	increased
Distributive shock	Neurogenic shock Anaphylactic shock Septic shock	normal /decreased	increased	decreased
Obstructive shock	Tension pneumothorax Pericardial tamponade Pulmonary embolism	decreased	decreased	increased

CO Cardiac Output, **LVEF** Left Ventricular Eject Fraction, **SVR** Systemic Vascular Resistance

1.8.3 Fluid responsiveness

Fluid balance and giving the right amount of fluid to patients admitted to intensive care units (ICUs) is critical for two main reasons: 1/ Volume depletion, if not corrected promptly will result in reduced oxygen delivery to vital organs leading to multi-organ impairment. 2/ Fluid overload is associated with higher mortality of patients in ICU, both pediatric and adults due to respiratory compromise (Messmer A. S. et al. 2020). Assessing which patients need fluid and how much to give is extremely challenging even with the best monitoring systems in the ICU. Fluid responsiveness, which is defined as an increase of at least 10% of CO after a bolus 300-500ml of fluid administration, helps to guide fluid resuscitation. One study found that 50% of ICU patients did not respond to fluid resuscitation (Michard F. and Teboul J. L. 2002), therefore highlighting the need to carefully assess whether fluid therapy is necessary for a patient with shock.

The fluid response depends on changes in preload and cardiac contractibility. Preload is the volume of blood in the ventricle prior to systole and is measured as left ventricular end-

diastolic volume (LVEDV). From the Frank-Starling diagram in Figure 1-7, it can be seen that there is a positive correlation between stroke volume and preload taking into account different levels of cardiac contractibility. An increase of LVEDV on the upward steep portion of the curve will increase stroke volume. However, increasing the LVEDV once the flat portion of the curve has been reached will not augment the SV and possibly have a detrimental effect. Based on this model, increasing the preload (either by a bolus of intravenous fluid or passive leg raise maneuver) can be used to assess fluid responsiveness. The stroke volume variation is often below 10% but will be higher in volume-depleted cases. An increase in stroke volume of more than 10% indicates that the patient has volume depletion and is fluid responsive (Monnet X. et al. 2016).

In ventilated patients in the ICU, the effect of increased intrathoracic pressure on the heart-lung interaction needs to be considered. An increase in intrathoracic pressure would diminish venous return and therefore reduce stroke volume. Based on this, a tidal volume challenge is another method used for ventilated patients to access the fluid response, whereby end-expiratory occlusion can be performed to assess stroke volume variation before and after an increase of tidal volume from 6 ml/kg to 8 ml/kg. Measuring changes in end-tidal CO2 (etCO2) after a bolus can also be rapidly adopted to assess fluid response. An increase in etCO2 of more than 2 mmHg equates to fluid responsiveness with AUROC of 0.82 (Oberg E. B. et al. 2019). Overall fluid responsiveness is a useful indicator of fluid depletion in consideration of cardiac function, which guides appropriate fluid resuscitation in intensive care.

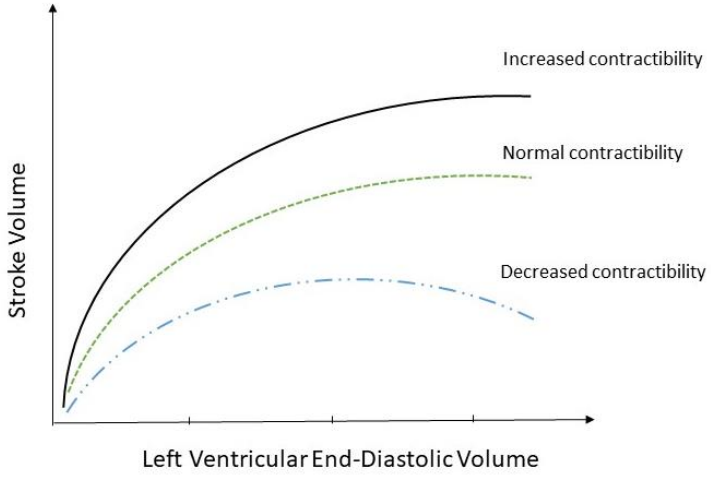


Figure 1-7: The Frank-Starling curve

Fluid response with a bolus of fluid depends on the heart contractibility and actual volume status. Fluid challenge when stroke volume at the steep portion will increase stroke volume significantly. Fluid bolus when stroke volume at the flat portion will not increase stroke volume.

1.8.4 Hemodynamic monitoring techniques for critically ill patients in intensive care units

a/ Invasive monitoring

a.1 Thermodilution (pulmonary artery catheter)

This technique uses a bolus of ice-cold fluid (often dextrose 5% solution or normal saline at 0^0C) injected to the right atrium via the proximal port of a pulmonary artery catheter. The change of the blood temperature is detected by a thermistor placed at the tip of the catheter. Cardiac output is calculated as the area under the thermodilution curve. This method measures cardiac output intermittently and its accuracy is influenced by several factors including tricuspid regurgitation, atrial/ventricular septal defects or very low cardiac output states. The main drawback of this method is the invasive nature of this technique, which can result in serious complications including pulmonary infarction, haemorrhage, rupture of the catheter's balloon tip and cardiac arrhythmias (Evans D. C. et al. 2009). New techniques have been developed based on similar principles, including using a thermal filament or thermal coil to

warm blood flow in the superior vena cava, followed by measuring changes in blood temperature at the thermistor, thus providing continuous cardiac output monitoring. Although thermodilution using a pulmonary artery catheter is still the standard method of cardiac output measurement in ICU, many studies showed it did not improve outcomes and is being replaced by other less-invasive techniques (Rajaram S. S. et al. 2013).

a.2 Transpulmonary thermodilution

This technique uses a bolus of cold saline injected into the superior vena cava through a central venous catheter. A thermistor positioned at the tip of the femoral catheter senses the decrease in temperature of the blood and a thermodilution curve is used to calculate the CO. This method does not require a pulmonary artery catheter but a central line is needed as well as a femoral artery catheter. It also measures global end-diastolic volume, cardiac function index and extravascular lung water which are important haemodynamic variables, hence it is often used in patients with refractory shock and ARDS. Hypothermia therapy does not affect the cardiac output measurement by this method. However, this method cannot be used for patients on extracorporeal membrane oxygenation (Monnet X. and Teboul J. L. 2017).

a.3. Central venous pressure

For patients being treated for shock in ICUs, measuring central venous pressure (CVP) is the most common technique used to assess volume status. A catheter is inserted into the jugular vein and the tip positioned prior to the right atrium. The CVP reflects the right atrial pressure, which can be an indicator of the left ventricular preload as well as right ventricular function and may therefore be beneficial to guide fluid management. However, CVP can be influenced by many factors, including vascular tone, thoracic pressure, and right heart failure, so CVP readings must be interpreted with caution and the clinical circumstances taken into account. Osman et al. showed that CVP >8 mmHg could predict fluid responsiveness with a positive predictive value of 47% in a retrospective study investigating fluid challenges in 96 septic patients on mechanical ventilation (Osman D. et al. 2007). A meta-analysis of 24 studies including 803 patients, showed that the area under the curve of CVP in predicting fluid

responsiveness was 0.56 (95% CI, 0.54-0.58). The authors concluded that CVP has a poor predictive role in evaluating haemodynamic response and should not be used in making clinical decisions for fluid therapy (Marik P. E. and Cavallazzi R. 2013) (Marik P. E. et al. 2008). Additionally, in special patients with serious coagulopathy, severe dengue for example, the procedure of inserting a central catheter for the CVP measurement can cause severe bleeding, which should be taken into account. However, CVP could still play a role in haemodynamic monitoring when used in conjunction with other parameters and in certain clinical scenarios (De Backer D. and Vincent J. L. 2018)

b/ Minimally-invasive monitoring

b.1 Arterial waveform analysis

Analysis of the arterial pressure waveform can be used to estimate the cardiac output using either devices with external calibration eg PiCCO2^{R}, LiDCOplus^{TM} or without external calibration eg LidCOrapid^{TM}, Vigileo^{TM}, MostCare^{TM}. These devices apply a specific algorithm for converting pressure-based signals into flow values. The algorithms used by the different monitors include individual characteristics (age, gender, height, weight). These devices have the benefit of being less invasive compared to thermodilution or transpulmonary dilution requiring only an arterial line, however, their accuracy depends on the clinical circumstances. The reliability of measurements from these devices decrease in cases with considerable changes in vascular compliance (for example, vascular leak syndromes cause vascular wall oedema leading to decreased arterial compliance) or changes in arterial pressure (for example, aortic regurgitation, aortic stenosis) (Romagnoli S. et al. 2009, Scolletta S. et al. 2011, Esper S. A. and Pinsky M. R. 2014).

b.2 Transesophageal echocardiography

Transesophageal echocardiography (TOE) can be used to assess the intravascular volume and cardiac function of patients. The cardiac transducer is passed into the oesophagus, which allows better image acquisition compared to transthoracic echocardiography, to define any cardiac abnormalities. The transducer can be a small Doppler probe attached to a flexible cable. When this is introduced into the oesophagus it provides the continuous velocity of blood flow at the descending thoracic aorta, from which cardiac output can be calculated. This method is usually reserved for critical care patients who failed transthoracic ultrasound when cardiogenic shock, pulmonary emboli, aortic desertion or infective endocarditis is suspected. However, this technique is expensive, only appropriate for sedated patients and requires expert training. It also carries the risk of oropharyngeal bleeding and dislocation of the endotracheal tube, thus, it is not recommended for use in patients with oesophagal abnormality or coagulopathy (Cecconi M. et al. 2014, Vieillard-Baron A. et al. 2019).

c/ Non-Invasive monitoring

c.1 Transthoracic echocardiography

Transthoracic echocardiography (TTE) is more commonly used than TOE due to its non-invasive nature. TTE can measure stroke volume intermittently with pulse wave Doppler put at the left ventricular outflow tract. This method not only assesses cardiac output but also provides information about cardiac function, valve function, pericardium, and fluid status. Technicians with experience in echocardiography can rapidly judge the volume status of a patient, even without formal measurements of CO or SV, eg the so-called “kissing ventricles” indicating hypovolemia. Other pathologies can be diagnosed too, including the “D-shape” of the left ventricle or dilated right ventricle suggesting an acute pulmonary embolism (McLean A. S. 2016). Cardiac ultrasound is a very helpful tool particularly for severe patients with shock to differentiate the aetiology of shock. Limitations include the measurements being operator-dependent and good images can be difficult to obtain in obese cases or ventilated patients (Cecconi M. et al. 2014).

c.2 Ultrasonic cardiac output monitoring (USCOM):

This technique uses a Doppler transducer of 2.0-3.3MH, positioned over the parasternal or suprasternal notch, to measure the aortic flow. Combined with pre-calculated valve diameters this then estimates cardiac output (Knobloch K. et al. 2005). Limitations of this method are operator variability as well as the estimation of the value diameter which is based on height, weight and age, but can differ a lot from the true values of the patients. Studies also demonstrate that there is a low agreement between this device and the standard hemodynamic monitor in cases with low and high cardiac output states (Tan H. L. et al. 2005).

c.3 PPG-based monitor devices

There are different devices using photoplethysmography (PPG) in combination with/without other physiological parameters, which can provide information about hemodynamic status. PPG utilizes infrared light with one or many different wavelengths allowing it to penetrate the tissue at different depths where a photodetector measures the reflected light from that tissue (Han S. et al. 2019). The blood volume changes are represented by the amount of reflected light, hence, the blood volume can be estimated. The advantages of these methods are that they are totally non-invasive, convenient, and can be assessed at peripheral location (finger, toe or earlobe). However, their limitations are related to the accuracy of readings in severely ill patients with low peripheral perfusion (shock, hypothermia, on vasoconstrictive agents, oedema) and motion artefacts (Castaneda D. et al. 2018).

- **Estimated continuous cardiac output monitoring (esCCO)** uses pulse wave transit time (duration for blood flow from the heart to reach the fingertip) and other standard parameters (ECG, SpO2, blood pressure) to provide estimated cardiac output continuously. Pulse wave transit time includes three components and depends on cardiac contractibility, arterial compliance and vascular resistance, therefore, this technique can provide some important hemodynamic parameters. This is one of the more recent technologies, so the evidence is lacking, however, several recent studies have shown cardiac output measured from this monitor correlates with those from the

thermodilution technique with a high percentage error (47%) (Ehlers U. et al. 2020, Schneck E. et al. 2020).

- **ClearSight/Nexfin/CNAP/T-line** uses PPG and a pressure cuff wrapped around the fingers to calculate estimated cardiac output based on auto-calibrating algorithms. These devices are new and require more validation for use in critically ill patients (Pour-Ghaz I. et al. 2019).
- **Compensatory reserve index (CRI)** The compensatory reserve index (CRI) is a novel parameter that uses feature extraction and machine learning of the pulse arterial waveform to reflect real-time changes in central intravascular volume. The pulse arterial waveform is processed by an algorithm to calculate the CRI with a value between 0 and 1, where 1 indicates normovolaemia and 0 represents the point of decompensated shock [7]. The CRI has been shown to correlate with a simulated model of hypovolaemia and to detect changes that occur during the compensatory phase of shock, preceding changes in conventional haemodynamic parameters. The value of CRI also takes into account individual variability and ability to compensate for volume loss (Suresh M. R. et al. 2019). This device was been approved for use in hemorrhagic shock and is undergoing evaluation for monitoring critically ill patients with other forms of shock (Moulton S. L. et al. 2016, Johnson M. C. et al. 2018, Benov A. et al. 2020).

c.4 Bio-impedance

This technique is based on the delivery of a low-amplitude and high–frequency electrical current passed across the body. Changes in impedance with each cardiac cycle can be measured using skin electrodes placed at the extremities. These changes are then used to generate waveforms to calculate the cardiac output. This method is safe, totally non-invasive and easy to use. In paediatric surgical patients, there was a good correlation in the cardiac index measured with a bio-impedance device (NICaS^{R}) compared to the transesophageal doppler technique (CardioQ ^{R}) (Beck R. et al. 2018). However, this method has some limitations, as the

impedance is influenced by changes in fluid composition, and changes in systemic vascular resistance as well as the electrical interference commonly seen in ICUs, hence, its reliability still needs further evaluation in critically ill patients (Sanders M. et al. 2020).

d/ Microcirculation monitoring

Microcirculation is crucial in shock resuscitation as microcirculatory dysfunction leads to impaired tissue perfusion and is associated with organ failure and poor outcome (Sakr Y. et al. 2004, den Uil C. A. et al. 2010). Microcirculation does not always reflect macrohaemodynamic improvement and monitoring microcirculation through various methods can provide additional information on hemodynamics at the tissue level. There are several methods to assess microcirculatory perfusion, some are widely used and some are currently only used in research settings.

- **Videomicroscopy of sublingual microvessels** using sidestream dark-field and orthogonal polarization spectral imaging provides high-resolution videos of the microcirculation to estimate capillary density, the magnitude of blood flow and heterogeneity of perfusion. However, understanding the meaning of the microvascular parameters and their utility as resuscitation targets remains unclear.(Cecconi M. et al. 2014, Edul V. S. et al. 2016)
- **Near-infrared spectroscopy** (NIRs) uses the principles of light transmission and absorption to identify tissue oxygen saturations. This technique is mainly applied to brain and peripheral tissues. For critically ill patients, this technique is often combined with an occlusion test and provides changes in absolute tissue oxygen saturation StO2, StO2 –deoxygenation rate and StO2- reoxygenation rate, which is associated with ICU stay and organ failure (Mesquida J. et al. 2012). The values of near-infrared spectroscopy are influenced by adipose tissue thickness, myoglobin, and skin pigmentation. Despite these limitations, this technique can be useful in emergency settings to rapidly assess the peripheral perfusion or continuously monitor the risk for

lower limb ischemia in patients during extracorporeal membrane oxygenation (Jones S. et al. 2016, Kim D. J. et al. 2017, Nardi O. et al. 2018).

- **Tonometry**

  Gastric tonometry has been used in ICU for patients undergoing cardiac surgery, as well as patients with sepsis and other forms of acute circulatory failure, based on the principle that increasing PCO2 is produced from the stomach wall during states of hypoperfusion to the gastrointestinal tract. The benefit of this technique in decreasing mortality of critical care patients is in question because of the inconsistent results from different studies (Palizas F. et al. 2009, Zhang X. et al. 2015). Limitations of this method are that pH and PCO2 can be influenced by temperature, gastric reflux, enteral feeding and so on (Kipnis E. et al. 2012). Therefore the interpretation of the result needs to take those factors into account.

- **Metabolic parameters** including blood lactate and mixed venous oxygen saturation has been routinely used in ICUs as a predictor of disease severity, mortality and to guide resuscitation therapy. For example, arterial lactate above 4 mmol/l is used as a trigger for initiating fluid resuscitation(Rhodes A. et al. 2017). It is also a predictor of disease severity and is associated with worse outcomes for ICU patients. Patients with lactate levels above 10mmol/L had a mortality of 65% and 12h lactate clearance was able to predict mortality with an AUC of 0.78 (%CI 0.74-0.83) (Baysan M. et al. 2020). Lactate clearance has been used in septic shock resuscitation as a targeted endpoint for fluid resuscitation. Similarly, targeting a mixed venous oxygen saturation of > 70% could improve outcomes. Management of critically ill patients in the ICU always requires an individualized approach taking into consideration the patient and disease-specific response.

1.8.5 Hemodynamic monitoring methods for patients with dengue shock syndrome

Dengue shock syndrome is the most common form of shock presenting in children and young people in endemic countries. As this type of shock is often managed at local health care facilities in low-middle income countries, the hemodynamic monitoring parameters used are often just traditional vital signs including pulse, blood pressure, urine output, O2 saturation and haematocrit to assess haemoconcentration from the vascular leak. For severe cases admitted to an ICU with multiple episodes of shock or severe bleeding or organ failure, CVP monitoring is still used, although this carries significant bleeding risks, as well as continuous arterial pressure monitoring. Due to the coagulopathy associated with severe dengue, invasive procedures should be kept to a minimum. Therefore, in clinical practice, central lines are often inserted using the brachial vein through a venous cut down to control the bleeding instead of using a Seldinger technique. In contrast with septic shock patients, individuals with DSS are monitored with fewer parameters.

- **Pulse pressure**

  The increase in vascular permeability cause characteristic dengue plasma leak syndrome and hypovolemic shock in dengue. At an early phase of plasma leak, peripheral vasoconstriction helps to maintain normal systolic blood pressure. As the plasma leak continues, the compensation mechanisms start to be overwhelmed resulting in increased diastolic blood pressure causing a narrow pulse pressure, which is a unique phenomenon of dengue shock syndrome. In addition to the narrowing of the PP, other compensatory mechanisms manifest clinically including an increase in heart rate, cold extremities, delayed capillary refill time and reduced urine output. If the hypovolemia continues to deteriorate, both systolic and diastolic pressure eventually drops. A dengue patient is diagnosed in shock if there is a narrow pulse pressure (pulse pressure is equal/ below 20 mmHg) or hypotension in combination with signs of poor capillary perfusion. In older children or adult patients with dengue, the narrow pulse pressure may be a sign of more severe shock (World Health Organization 2009). In the elderly and patients with hypertension, blood pressure can be higher

during acute dengue infection (Yeung W. et al. 2020). In these cases, normal blood pressure may indicate intravascular volume depletion and imminent hypotensive shock. With the differing normal ranges by age and underlying diseases, although blood pressure monitoring is a standard haemodynamic parameter, it is an inaccurate measure of intravascular volume and changes late during the decompensation phase of shock. It is, therefore, necessary to combine blood pressure monitoring with other vital signs, clinical manifestations, and advanced haemodynamic monitoring techniques to give appropriate intervention and guide fluid management for severe dengue patients.

- **Heart rate**

  This often increases when circulating volume starts to decrease. However this parameter is sensitive to body temperature, stress, and respiratory distress, hence it can only be used in conjunction with other parameters. In patients with DSS during the compensation period, the heart rate is often not as high as those with other infectious diseases (Senanayake S. N. 2008, Yacoub S. et al. 2014). The mechanism of this phenomenon is unclear, possibly due to myocardial interstitial oedema, decreased coronary microcirculation, subclinical myocarditis, calcium derangement or altered autonomic tone (Yacoub S. et al. 2014). In complicated cases with prolonged shock, bleeding and/or multi-organ failure, heart rate often increases significantly and can predict a poor outcome.

- **Haematocrit (Hct)**

  This reflects the proportion of packed red cell volume in the blood. In dengue, haematocrit increases as the plasma leakage progress causing relative haemoconcentration. This parameter is checked daily during the febrile phase and more frequently during the critical phase to assess plasma loss. For dengue patients with shock, Hct is checked every 2 hours in the first 6 hours to guide resuscitation. A decrease in Hct combined with improved peripheral perfusion is an indicator of fluid responsiveness. High Hct or a persistently high Hct after fluid boluses is a predictor of

recurrent shock and poorer outcomes (Lam P. K. et al. 2013, Lam P. K. et al. 2015). Of note, the baseline Hct of each patient varies depending on age, gender, and therefore the percentage change over time is monitored during the illness. Percentage change of Hct is also used as an endpoint for clinical trials, eg a randomised controlled trial of different fluid regimens for children with DSS showed that after a 20ml/kg bolus of normal saline, ringer lactate, gelatin and dextran 70, Hct decreased by 6.5%, 5.7%, 9.7% and 11.5% respectively (Ngo N. T. et al. 2001). It is important to investigate serial Hct measurements in combination with other vital signs to support the management of severe dengue patients.

- **Central venous pressure**

  CVP is not routinely measured for patients with severe dengue because of the invasiveness and high risk of bleeding associated with this technique in patients with abnormal coagulation and thrombocytopenia. CVP may be used in patients with multiple episodes of shock or in those not responding to initial fluid of resuscitation, exhibiting severe bleeding or renal failure. A study on 43 Vietnamese children with DSS, a mean value of CVP after 30 minutes, 1 hour, 2hour, 3 hours at initial fluid resuscitation was 9.5 ± 2.6, 10.2 ± 2.3, 10.4 ± 2.3 and 10.5 ± 2.5 cmH2O, respectively. CVP increased after 24 hours and 36 hours to 12.0 ± 2.8 and 12.4 ± 4.1 cmH2O respectively (published in Vietnamese). CVP measurement has also been used in dengue complicated with kidney injury and receiving continuous hemofiltration (Dalugama C. and Gawarammana I. B. 2018). However, CVP is influenced by many factors which are commonly seen in dengue including pleural effusion, ascites, right heart dysfunction. The majority of patients with dengue shock syndrome are alert and breathing spontaneously, which can also affect the CVP. This reason, in addition to the bleeding risk, means a more non-invasive measure of volume status is preferred for dengue patients.

1.9 Knowledge gaps and objective of this thesis

Although the mortality rate of patients with severe dengue is below 1% in some dengue-experienced medical centres, in some centres with limited experience or facilities of supportive care, the mortality can be up to 23% (Chen C. M. et al. 2016). Regarding the fluid management of severe dengue to improve outcomes, there are still many knowledge gaps that we need to address.

Firstly, there is a lack of evidence on fluid management strategies for DSS patients in terms of their safety and benefits to improve final outcomes (not only fatality rate but also respiratory failure, organ impairment and economic health). Fluid resuscitation, the cornerstone of DSS management, is based mainly on expert opinions and three single-centre-randomised control trials in Vietnam. Different types of fluid are used for DSS management including artificial colloids (hydroxyethyl starch, dextran, and gelatin) which are evidently harmful to critically ill patients with sepsis (Lewis S. R. et al. 2018). It is unclear whether for a severe dengue population who are young and with no underlying diseases, if this resuscitation fluid would induce similar complications or would improve outcomes. The WHO guideline for dengue management has not changed or been updated since 2009 while there were many updates on fluid resuscitation for septic shock in line with evidence from multiple-centre RCTs. With different pathogenesis between dengue and sepsis and different population characteristics, the results of studies on septic shock cannot be generalized to dengue shock. Therefore, the effects of resuscitation fluid used for patients with DSS are still questionable. Regarding this issue, I completed a systematic review of fluid management strategies in children with DSS or with septic shock, to have evidence of which fluid types/strategies would provide better outcomes (chapter 3). I also reviewed a large-scale retrospective study on DSS management in different countries to find which fluid strategies in clinical practice provide better outcomes for patients with DSS (chapter 4).

Secondly, there are few dynamic markers/tools to predict who is not responding to fluid management and who has poorer outcomes among severe dengue cases. For hypovolemic

shock management, cardiac output monitoring is the most important and the most common parameter to assess fluid response as well as the need for further volume replacement. In developed countries, there are various techniques invasively or non-invasively used to monitor cardiac output for intensive care patients. Meanwhile for patients with DSS in developing countries only traditional vital signs are monitored hourly (pulse, blood pressure) alongside a series of haematocrit readings during the early phase of resuscitation, which may clearly change at the late phase of shock. In addition, due to coagulopathy-related dengue, some invasive procedures may be harmful and carry the risk of severe bleeding complications. In this specific circumstance, non-invasive techniques seem to be the appropriate choice and could be helpful for severe dengue patients. However, there is no data for their use in severe dengue. It is necessary to assess the use of different haemodynamic monitoring methods for severe dengue cases to provide useful tools to assess fluid response and intravascular volume to guide fluid therapy. I carried out a number of studies to assess the use of different non-invasive monitors for child patients with DSS including monitoring of compensatory reserve index (chapter 5), bioimpedance device, arterial pulse waveform analysis and point of care ultrasound (chapter 6).

In summary, my PhD research project has the following objectives:

1/ Investigate which fluid type or fluid resuscitation strategies for children with DSS or septic shock have better outcomes from a systematic review

2/ Describe the different fluid strategies for DSS management across many countries in Asia and further explore the association of different fluid strategies as compared to several important outcomes in DSS patients (recurrent shock, respiratory distress, severe bleeding, mortality, length of hospital stay, organ impairment)

3/ Describe the use of different non-invasive techniques (CRI, bioimpedance, arterial waveform analysis and echocardiography) in monitoring DSS patients during fluid resuscitation; their abilities in assessing fluid response and in predicting reshock.

CHAPTER 2

METHODS

In this chapter, I describe the protocols of two prospective clinical studies including the design, patient recruitment, data collection and an overview of the non-invasive haemodynamic monitoring techniques used. The first study is a prospective study that was conducted between 2014 -2015 and is where the data for the compensatory reserve index (CRI) was collected (chapter 5). The second study was performed from 2018 to 2020, and is where the echocardiography and bioimpedance data were collected (Chapter 6). More details on the specific methodology will be described in the relevant chapters.

2.1 Clinical study protocol 1

2.1.1 Study design

This was a prospective observational study conducted in 2014-2015 at Paediatric Intensive Care Unit and adult Intensive Care Unit of Hospital for Tropical Diseases (HTD), Ho Chi Minh City, Vietnam.

2.1.2 Inclusion criteria

All patients admitted to adult intensive care unit (AICU) or paediatric intensive care unit (PICU)

- Clinically diagnosed dengue with warning signs or established severe dengue
- Age > 5 years
- Written inform consent

2.1.3 Exclusion criteria

- Obvious local infection suggesting an alternative diagnosis
- Patients lived too far and could not return for follow up

2.1.4 Subject recruitment and informed consent process

Patients admitted to ICUs at HTD with suspected severe dengue were approached by study staff. The study staff described the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to adult patients or parents of paediatric patients at enrolment. If the patient was not able to make decisions and sign the informed consent form, the study was discussed with their legal representative to consent on their behalf. For patients who are 12 years old or above, when they recovered, study doctors explained about the study and got the signatures from the patients.

2.1.5 Ethical consideration

Ethical approval was obtained from the Oxford Tropical Research Ethics Committee (OxTREC) in the UK (Reference number 1030-13), and the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam (number 90/HDDD-QD)

2.1.6 Data collection instrument

We used a structured clinical questionnaire to record daily data from enrollment to ICU discharge. The case report form (CRF) for this study included hemodynamic and clinical parameters as well as detailed information on fluid balance. The CRF was filled out daily by the study staff. In addition, an electronic CRF for peripheral waveform record was integrated with hourly clinical data on vital signs and fluid management.

2.1.7 Clinical evaluation

Patients were reviewed daily in ICU until discharge or for up to 5 days. Standardized clinical information was recorded in the CRF, ideally with the clinical examination carried out by the same member of the study staff daily. Vital signs were performed hourly for a minimum of 48 hours as part of routine care in PICU by ward staff, and these figures were transcribed to the CRF later by study staff.

2.1.8 Compensatory reserve index monitoring

The compensatory reserve Index (CRI) is a new physiological parameter that tracks real-time changes in central volume. It is derived from feature analysis of the pulse arterial waveform. Arterial waveform data is processed by an algorithm to calculate the CRI, giving a value between 0 and 1, where 1 represents normovolaemia and 0 represents the point of decompensated shock.

CRI was measured by fingertip pulse oximetry with waveform data continuously measured for a maximum of 5 days. Data was wirelessly transmitted to a bedside laptop, and synchronized with the patient's clinical data. The raw pulse waveform data was converted to CRI values by research data scientists at Flashback Technologies, Inc. in the USA, after the data collection period was completed; this individual was blinded to all clinical data. Real-time CRI values were not available to the doctors in the ICU so as not to influence patient management. All clinical data (including clinical status, vital signs and detailed intravenous fluid volumes, organ support and hourly urine output) were entered into a Vietnamese electronic medical record (MEDICS, Sierra Nevada Corp), which was specifically designed for the project. Data on fluid management, daily clinical assessment, summary of important outcomes (respiratory support, severe bleeding, organ impairment, duration of hospital stay, mortality) and laboratory tests were recorded using paper case report forms (CRF). This data was subsequently integrated with digital data in each patient's electronic medical record. All the CRI values were cross-checked after converting waveform data into numerical data for statistical analysis.

2.2 Clinical study protocol 2

2.2.1 Study design

2.2.2 Inclusion criteria

Any patients admitted to the PICU at HTD (between 2018-2020) with the following criteria:

- Clinically suspected dengue with signs of DSS – narrowing of the pulse pressure $\leq$ 20 mmHg or hypotension for age, together with signs of impaired end-organ perfusion (eg cool peripheries, sweating, tachycardia)

- Age 5-15 years
- Written informed consent
- Staff and equipment available for the hemodynamic studies

2.2.3 Exclusion criteria

- Referral from another hospital after initiation of fluid management for DSS
- Fluid resuscitation at HTD before obtaining the initial consent.
- Patients with any pre-existing serious underlying disease.
- Bio-impedance devices cannot be used in individuals with indwelling electronic devices (such as a pacemaker) or individuals with missing limbs, so patients for whom there are practical reasons why NICaS cannot be used were excluded.

Subject recruitment and informed consent process

2.2.4 Ethical consideration

Ethical approval was obtained from the Oxford Tropical Research Ethics Committee (OxTREC) in the UK (Reference number 03-18), and the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam (number CS/BND/18/27)

2.2.5 Subject recruitment and informed consent process

Patients admitted to PICU at HTD with a clinical diagnosis of DSS during routine working hours on Monday to Thursday were eligible for recruitment to this study. Potential study participants were identified by the medical staff in PICU, who notified a member of the study team who were available at the study site. Since this was an emergency situation requiring fluid resuscitation as soon as possible, obtaining written consent for the full study was deferred until the child was cardiovascularly stable and the family were able to concentrate on the information being presented to them.

At the time of initial study enrolment, written consent was obtained from the parent/guardian by a study nurse or doctor for Phase 1 of the study – i.e. for the echo studies to be performed at baseline and one hour later, for the additional non-invasive monitoring, and for permission to

use residual plasma from standard lab tests to confirm dengue infection. Only a very brief description of these activities were given at this time, and no additional blood was taken, but the family was informed that they have the right to refuse consent without compromising the child's care. In addition, a written information sheet was provided describing the full study, should the family members wish to read it later.

Fluid resuscitation commenced as soon as practicable, following the standard management guidelines of the PICU and directed entirely by the attending physicians. Echocardiography to evaluate intravascular volume status was done as close as possible to the start of the fluid infusion, and then again one hour later. These echos were performed by the research staff who ensured that they did not interfere in any way with the clinical care of the patient. The research nurse recorded the time the fluid infusion started and the time the echo was performed. The other non-invasive study device NICaS was connected to the patient alongside routine monitoring systems.

Subsequently, when the child was stabilised within 3 hours from PICU admission, a full description of the study was provided to the parent/guardian, describing in detail the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. If the parent or guardian was happy for the child to continue to participate in the study, they were invited to consent to Phase 2 of the study, and the study processes will continue as described below. However, if the parent or guardian declined to consent to Phase 2 activities, the study processes stopped, and all study devices were taken off and no additional data or blood samples were collected. The families of the small number of children who had not improved by 3 hours were not approached for the Phase 2 consent, so the treating clinicians were able to discuss the serious problems the child was facing with their families, and it was felt they should not be burdened with other complex information about research at this time; there were no further study-related activities for these children beyond the initial activities covered by the Phase 1 consent.

The decision whether to approach the parent/guardian for Phase 2 consent was at the discretion of the treating clinician. In principle, the following criteria were considered, but the treating doctor may decide for any reason that it was inappropriate to burden the family with research information when their child was in a critical condition:

- Unstable hemodynamic status requiring more aggressive intervention such as urgent blood transfusion or switch to colloid therapy
- Endoscopic therapy to stop gastric bleeding
- Need for respiratory support
- Convulsions, coma, lethargy or un-explained agitation
- Any serious condition that the clinician felt indicated a poor prognosis.

2.2.6 Data collection instrument

When the child was clinically stable, a case report form (CRF) was completed using data from the onset of resuscitation, and then daily for all patients in the study. The CRF for this study included demographic data, hemodynamic and clinical parameters as well as a detailed record of fluid balance. The CRF was filled out daily by the study staff.

2.2.7 Clinical evaluation

Research hemodynamic tests were carried out at the bedside, including portable echocardiograms and continuous cardiac output monitoring through limb sensors using a commercial bio-impedance device- NICaS (further details in Chapter 6).

In this study, all management decisions were made by the ward clinicians, following HTD guidelines and using routinely collected monitoring information – i.e. without access to the specialised haemodynamic monitoring data. All therapeutic interventions were recorded, including the amount, type, and rate of infusion of all IV fluids administered, using a specially designed 24-hour observation chart.

A full blood count was performed daily for up to 5 days and a biochemistry sample was performed at enrolment and at any other time deemed necessary by the attending clinician. An additional research blood sample was collected daily for DSS pathogenesis studies.

The patient was asked to attend follow-up when they were discharged from the hospital, on day 14 to 21 from the start of their illness. If the patient was still in hospital at this time, they were followed up on the ward. At this visit, they had a final blood test for full blood count, convalescent serology and a final research blood sample. They also had the final hemodynamic and cardiac assessment.

2.2.8 Echocardiography

Echos were performed at the bedside by one investigator trained in this technique (HTT, HQC, TKH), using portable echo machines (SAMSUNG MEDISON Mysono Inc. and GE Vivid IQ) with cardiac settings. The echos were performed pre and post fluid bolus and during DSS management at 1, 3, 6, 12, 24 hrs. If the patient developed recurrent shock, echocardiograms were repeated similarly for the next 48 hrs. The exams included two-dimensional, M-mode and Doppler studies. All images were stored digitally and a selection was reviewed by SY and CB.

This study did not interfere with the clinical management and fluid therapy followed current HTD guidelines. Study doctors were not involved in clinical care or management decisions for the patients enrolled in the trial. One study doctor performed the whole series of echos assessing intravascular volume, fluid response, cardiac function and pleural effusion. A detailed protocol on ultrasound will be described in chapter 6.

2.2.9 Bio-impedance (NiCaS®)

NICaS (NI Medical) is a non-invasive FDA approved impedance cardiography device used for continuous cardiac output monitoring. It utilizes whole-body impedance technology to calculate stroke volume, heart rate and cardiac output. NICaS transmits a small electrical signal (1.4 mA) through the body through two sensors arranged in a wrist-to-ankle configuration. The majority of the signal flows through the blood in the arterial system. With each heartbeat, the

volume of blood in the arterial system changes and this results in a change in the body's electrical resistance. These changes are measured and transformed in real-time through internal algorithms to cardiac output, stroke volume and peripheral vascular resistance.

After peripheral intravenous (IV) lines were inserted for fluid resuscitation, a study nurse fixed a disposable sensor onto the patient's wrist which had no IV line and another disposable sensor on the opposite ankle. The sensors were connected to laptop at the bedside and the following data was entered: patient's study number, height, weight, sites of sensors and position. Data was recorded for the duration of the ICU stay or for a maximum of 4 days. The study data was masked from the general ward staff by obscuring the monitor's screen.

2.3 Dengue diagnosis and case definition (Both protocols)

Diagnostic serological assays (capture ELISA, Panbio Australia) were performed on the enrolment and follow-up samples which were batched and tested together at a later time point. Viremia levels were measured in enrolment plasma samples by qRT-PCR to measure viraemia levels.

Patients were confirmed dengue as having a positive RT-PCR or positive IgM at enrolment or IgM seroconversion between paired samples. Primary infection was defined as patients who had IgM positive or IgM seroconversion with negative IgG on paired samples. Secondary infection was defined as patients who had both IgM and IgG positive at enrollment or IgM or IgG seroconversion on paired samples.

2.4 Statistical analyses

2.4.1 Sample size calculation

In these studies, among patients with DSS we wished to identify hemodynamic parameters derived from different novel non-invasive techniques associated with specific clinical endpoints, including fluid responsiveness following the first bolus, recurrent shock and respiratory distress. Without previous data, a formal sample size could not be calculated for the prognostic potential of the various hemodynamic assessments to predict endpoints. These

studies were very labour intensive and for practical reasons we planned to enrol 50 cases/dengue season over two years, giving a total of 100 patients for each study.

2.4.2 Clinical endpoint definitions

Each clinical study had different primary and secondary endpoints, however, all clinical endpoints had the following definitions:

- Shock was defined as a narrowing of the pulse pressure (PP) < 20 mmHg or low blood pressure for age (systolic blood pressure - SBP < 80 mmHg for children at 5 years old or below, SBP < 90 mmHg for children more than 5 years old), combined with clinical signs of reduced peripheral perfusion (capillary refill time greater than 2 seconds, or cold extremities).
- The initial shock recovery time was defined as the time of onset for the first episode of shock to when the patient achieved cardiovascular stability, which was defined as the time point when the pulse pressure was > 25 mmHg for at least 6 hours. After recovering from the first episode of shock, patients either remained stable and recovered, or they experienced another episode of shock, termed reshock.
- Fluid response using echocardiography was defined as more than 10% increase in SVI following the first fluid bolus
- Clinical fluid response was defined as PP>25 mmHg, with a decrease in HR and decreased Hct, which resulted in a reduction of the fluid rate <15 ml/kg/h in the next hour.
- Reshock was shock re-occurring after the first 6 hours from initial cardiovascular stability.
- Respiratory distress was defined as any patient who developed respiratory compromise, including: using respiratory muscles/ increased respiratory rate/ required more oxygen or respiratory support (increase oxygen cannula support with FiO2 40% or NCPAP or MV).

- Acute kidney injury was defined as serum creatinine after fluid resuscitation > 1.5 times the upper normal limit for age. The upper normal limit for age: ≤ 1 year: 35 umol/L, 1-2 years: 44 umol/L, 3-10 years: 61 umol/L, 11-16 years: 79 umol/L
- Liver impairment was defined as AST or ALT ≥ 1000 U/L
- Severe bleeding was clinically defined when a patient had bleeding resulting in shock or death or involving a critical organ (brain, heart, spinal cord), or requiring an intervention (blood transfusion or procedure to stop bleeding, for example, nasal packing)

2.4.3 Statistical analyses (both protocols)

Numerical descriptive summaries were performed using median (interquartile range) for continuous variables and frequency (percentage) for categorical variables. Graphical descriptions of CRI/SVI and haemodynmic parameters were based on a time-plot of the individual's trajectory of the parameter of interest and loess scatter-plot smoothers. Partial correlations between continuous variables were based on Spearman correlation of residuals obtained from linear regression models with continuous variables of interest as outcomes and confounding variables (age, gender, day of illness of measurement) as covariates.

The relationship between continuous data (CRI, SVI) measured at different time points ahead of a low pulse pressure event (pulse pressure ≤ 20 mmHg) or shock was investigated using logistic regression models. Generalized estimating equations with independence covariance structure were used to take into account repeated measurements per patient. The results were summarized with odd ratios and the corresponding 95% confidence interval with and without adjustment for the time of CRI/SVI measurement, age, gender, and body weight. We also evaluated the overall predictive value of CRI/SVI using AUC as well as specificity and sensitivity at different cut-offs. We use logistic regression to find association between lactate clearance and outcomes.

CHAPTER 3

A SYSTEMATIC REVIEW OF FLUID RESUSCITATION FOR DENGUE SHOCK SYNDROME AND SEPTIC SHOCK IN CHILDREN

3.1 Introduction

There is controversy around the use of resuscitation fluids for children with dengue shock syndrome (DSS) in regards to both the fluid type and fluid rate. The WHO guideline for DSS management recommends using a rapid bolus fluid, followed by 1-2 hours of a high volume of colloid fluid for decompensated shock and that is evidenced-base by a few randomised control trials. For recurrent shock cases, colloids are recommended to use with a duration of between 1-2 hours based upon expert opinion (World Health Organization 2009). Colloids have greater volume expansion capacities and longer durations of volume expansion compared to crystalloids (mentioned in chapter 1, the section of fluid management for DSS). The use of colloids was expected to reduce the total volume of fluid resuscitation, improve shock resolution and reduce respiratory complications based on results of several experiments on animals (Di Filippo A. et al. 2006, Verheij J. et al. 2006, Margarido C. B. et al. 2007, Huang C. C. et al. 2009). In some settings, prolonged use of colloids for cases with severe plasma leakage cases, therefore, becomes a standard of care (Children's Hospital 1 2019). On the other hand, strong evidence of harmful effects of using colloids, especially hydroxyethyl starch (HES) in sepsis shock in adults (Perner A. et al. 2011, Perner A. et al. 2012, Haase N. et al. 2013, Haase N. et al. 2013), results in the scarcity of synthetic colloid production globally. Without an adequate supply of synthetic colloids for complex cases with severe plasma leakage, doctors in dengue-endemic countries are struggling with fluid choices. Adults and children are different, the results of studies in adults may not be rationally generalised to paediatrics; dengue shock and septic shock are also different though they share some similarities in the endothelial

dysfunction (McBride A. et al. 2020). With few RCTs in dengue shock management, a thorough review of research on fluid management for septic shock in children would provide some suggestions for future research in dengue. It is necessary to review all the studies on fluid resuscitation in children focusing on DSS and septic shock particularly to explore the remaining questions, strengths, and weaknesses in the methodologies of those studies. This would help to provide a direction for future research on fluid management for DSS in children.

3.1.1 Evidence for the current recommendation on fluid resuscitation for dengue shock syndrome and septic shock in children

As mentioned in chapter 1, DSS was managed differently depending on shock severity. Patients with compensated shock would receive crystalloid fluid at a rate of 10-20 ml/kg/h followed by a lower rate of crystalloid infusion. Patients with decompensated shock would receive a bolus of 20 ml/kg of crystalloid or colloid over 15-30 minutes followed by another bolus of 10-20 ml/kg/hour of colloid. The recommendations were based on expert opinions rather than strong evidence (World Health Organization 2009). There has been several clinical trials to support the use of synthetic colloids (Dextran, hydroxyethyl starch, and gelatin) for DSS (Dung N. M. et al. 1999, Ngo N. T. et al. 2001, Wills B. A. et al. 2005). The pilot study on 50 children with DSS showed a dramatic improvement in cardiac output in patients using synthetic colloids (Dung N. M. et al. 1999). Among them, 6% Dextran 70 was shown as the most effective volume expansion fluid. In a larger RCT, the use of synthetic colloids or crystalloids had similar outcomes on reshock occurrence. The benefits of using colloids versus crystalloids for patients with decompensated shock have not been studied due to ethical issues (Wills B. A. et al. 2005). The most recent WHO guideline for dengue management is from 2009 in which the dengue classification for dengue cases is different from the guideline published in 1997, however, the fluid management aspect has not been changed.

Regarding fluid resuscitation for children with septic shock, the Surviving Sepsis Campaign (SSC) 2016 and 2020 recommended fluid bolus of 40-60 ml/kg balanced solutions in the first hour for patients being managed in health care settings with the availability of intensive care

facilities (Weiss S. L. et al. 2020). The recommendation is based on the results of multiple observational studies that show the improved outcomes of patients (shorter duration of hospital stay, lower mortality) with bolus fluid therapy at initial resuscitation (Carcillo J. A. et al. 1991, Han Y. Y. et al. 2003, Carcillo J. A. et al. 2009, Paul R. et al. 2012, Evans I. V. R. et al. 2018). The Fluid Expansion as Supportive Therapy (FEAST) was the largest RCT to date providing evidence of increased mortality with bolus fluid therapy in the settings without advanced supportive care. According to the results of this trial, SSC recommends against bolus fluid for patients with hypotension who are managed in healthcare settings without intensive care availability (Weiss S. L. et al. 2020). This study also shows no benefit of using albumin compared to normal saline. The fluid type recommended from SSC is crystalloid for septic shock. Balanced crystalloid is preferred based on the results of large multicenter trials in adults in which the use of balanced crystalloid lowered mortality and renal dysfunction rate (Self W. H. et al. 2018, Semler M. W. et al. 2018). The SSC also recommends against the use of hydroxyethyl starch (HES) or gelatin for fluid resuscitation, based on RCTs in adults and a trial in children (Upadhyay M. et al. 2005, Myburgh J. A. et al. 2012, Perner A. et al. 2012). The conclusion of SSC acknowledged the relatively low quality of evidence resulting in the weak recommendations. There is a need for future research to establish the benefit of using those recommendations to improve outcomes.

In summary, fluid management for septic shock and dengue shock in children has not been studied well enough to provide strong recommendations on which fluid type or which fluid rate would induce better outcomes in resource-rich settings and resource-poor settings.

3.1.2 Vascular leakage in dengue and sepsis

Microvascular damage is seen both in bacterial and viral infections (Steinberg B. E. et al. 2012). The microvascular impairment results in a reduction in tissue oxygen delivery preceding multi-organ failure. However, dengue and sepsis have differences in the pathophysiology of

microvascular impairment, the level of plasma leak in dengue is far greater (Wills B. A. et al. 2004) which explains the distinct response with fluid resuscitation.

In sepsis, bacterial proteins, toxins, and overactivated inflammation phenomenon directly damaged endothelial cells which leads to a massive inflammatory reaction. The activation of lympho B and T cells releases cytokines and inflammatory mediators that induce glycocalyx damage and disruption of the tight and adherence junction between endothelial cells (McBride A. et al. 2020). These disruptions of glycocalyx lead to vascular dysfunction and plasma leakage. In addition, the coagulation cascade is also activated resulting in the formation of microthrombi in the vascular network that generates a systemic flow obstruction. In dengue, the mechanism of glycocalyx disruption has not been fully understood. The dengue non-structural 1 protein released from infected cells induces the expression of enzymes that degrade glycocalyx components including heparanases, and sialidases (Puerta-Guardo H. et al. 2016). This leads to impairment of the integrity of the endothelial layer. Additionally, pro-inflammatory cytokines, TNF alphas, and multiple vasoactive mediators are produced massively in secondary infection causing glycocalyx injury (Anderson R. et al. 1997). Side-stream dark-field videomicroscopy is one of the techniques that help to visualise images of how red blood cells flow in the microvascular system. A study using this method to observe sublingual microcirculation in dengue patients has shown a reduction in microvascular perfusion during the critical phase, together with the decrease of glycocalyx depth and elevation of glycocalyx components in plasma (Lam P. K. et al. 2020). Levels of syndecan-1 (one of products of glycocalyx degradation) are multiple times higher in dengue shock compared to septic shock reflecting the greater magnitude of plasma leak in dengue (Suwarto S. et al. 2017, Uchimido R. et al. 2019); meanwhile, L-arginine decreases and arginase increases suggesting impairment in the stabilisation of microvascular tone (Yacoub S. et al. 2017). In summary, plasma leakage in dengue and sepsis is due to disruption of adherents and tight junctions between endothelial cells as well as glycocalyx shedding but the plasma leak is more dominant in dengue.

3.1.3 Reasons to do a systematic review on fluid resuscitation strategies of dengue shock syndrome and septic shock in children

There are two main reasons to perform a systematic review on fluid resuscitation strategies for DSS and septic shock. Firstly, there has been no new update on the guidelines of fluid management for DSS for nearly a decade while the guideline for septic shock has been updated almost every year. After the FEAST trial, bolus fluid is not recommended for patients with shock and managed in settings without intensive care. According to that recommendation, children with DSS who often approach the primary care centres without advanced ventilatory support, should not receive a high volume of fluid bolus. Though septic shock and dengue shock are different in pathogenesis, the guideline should be reviewed. Secondly, in the current situation when the production of synthetic colloids is limited globally, there are fewer choices for fluid resuscitation for severe plasma leakage cases. We need to have a general view of evidence from previous RCTs on fluid resuscitation to explore the limitations and propose better proposals for future research in order to improve outcomes.

3.2 Objectives of the systematic review

I carry out this systematic review with the aim is firstly to have a synthesis of fluid strategies and their clinical outcomes on children with DSS and septic shock, and secondly to explore the knowledge gaps and limitations of previous RCTs and to propose potential future research.

3.3 Methods

The review was conducted in January 2020, based on relevant research articles identified from two major scientific databases (PubMed and Embase). The protocol of this review was prepared following the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) 2015 recommendation (Moher D. et al. 2015) but was not prospectively registered. The reporting format of this review follows the PRISMA 2020 statement (Page M. J. et al. 2021).

3.3.1 Information source

The search was performed on the two major medical literature databases, PubMed and Embase. PubMed is the most commonly used biomedical literature database and search engine, and comprises more than 34 million citations since 1966 [https://pubmed.ncbi.nlm.nih.gov/]. According to a recent analysis of more than 85,000 publications included in all Cochrane reviews from 2012 to 2016, PubMed has coverage of 70.9% (95% CI 68.4-73.3) of all the included publications, and this coverage varies across specialities and over time (Frandsen T. F. et al. 2019). Embase is a biomedical bibliographic database that included more than 41 million records since 1947 (Elsevier 2022) and is usually used as a supplement to PubMed. In a recent analysis, supplementing PubMed with Embase could increase coverage of included publications by 6-7% (Frandsen T. F. et al. 2021). Together with the Cochrane Central Register of Controlled Trials (CENTRAL), PubMed and Embase are considered to be the most important sources that are needed to be used to search relevant literature for biomedical intervention reviews (Higgins JPT et al. 2022).

3.3.2 Eligibility criteria

The inclusion criteria were original articles of randomised controlled trials in children (age ≤ 18 years) with dengue shock syndrome or septic shock receiving intravenous fluid resuscitation. I excluded studies in newborns as well as studies of fluid resuscitation in surgery, burn, trauma, and dehydrated diarrhoea.

3.3.3 Search strategy

Search terms and their combinations are summarised in Table 3-1. I developed these terms from keywords in objectives and their commonly used synonyms. Search results were restricted to clinical trials, randomised controlled trials in humans, and children (infants, preschool children 2-6 years, school children 6-12 years, adolescents 12-18 years), and English-language publications.

Table 3-1. Summary of search strategies

Boolean	P	I	C	O
OR	dengue shock syndrome	fluid therapy	(no limits)	(no limits)
OR	"Shock"[Mesh] AND "Dengue"[MESH]	fluid resuscitation		
OR	DHF	fluid strategies		
OR	DSS	fluid strategy		
OR	dengue shock	albumin		
OR	hypovolaemic shock	colloid		
OR	hypovolemic shock	crystalloid		
OR	septic shock	volume replacement		
OR		"volume"[All Fields] AND "replacement"[All Fields]		

On PubMed, the search was performed as below:

((((((((((dengue shock syndrome) OR (("Shock"[Mesh]) AND "Dengue"[Mesh])) OR dengue hemorrhagic fever) OR DHF) OR DSS) OR dengue shock) OR hypovolemic shock) OR hypovolaemic shock) OR septic shock)) AND (((((((((fluid therapy) OR fluid resuscitation) OR fluid strategies) OR fluid strategy) OR albumin) OR colloid) OR crystalloid) OR volume replacement) OR (volume[All Fields] AND "replacement"[All Fields])) Filters: Humans; Infant: birth-23 months, Preschool Child: 2-5 years, Child: 6-12 years, Adolescent: 12-18 years, Clinical Trial, Randomised Controlled Trial

On Embase, the search was performed as below:

1. Exp dengue/or exp dengue hemorrhagic fever/or exp dengue shock syndrome/or exp severe dengue/
2. exp shock/or exp capillary leak syndrome/or exp dengue shock syndrome/or exp hypovolemic shock/or exp septic shock/
3. Fluid therapy/or exp fluid resuscitation/
4. Fluid therapy.ti,ab.
5. Fluid strategy*.ti,ab.
6. ((fluid volume) adj3 replace*).ti,ab.
7. Exp albumin/or albumin.ti,ab.
8. Colloid.ti,ab. Or exp colloid/
9. Exp crystalloid.ti,ab or exp crystalloid/
10. (1 OR 2) AND (3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9)

Filters included: humans, infants: birth-23 months, preschool child: 2-5 years, child: 6-12 years, adolescents 12-18 years, clinical trials, and randomised control trials.

3.3.4 Selection process

The search results were exported and saved in Excel format (version V0). HTT and HQC were the two assessors who independently selected studies in the Excel file V0 by comparing

information obtained from title/abstract to the inclusion and exclusion criteria. Reasons to exclude the study were noted in one column of the Excel file and this file was saved as version V1. After finishing the selection, each assessor sent Excel files V1 to the third assessor (BW). BW reviewed the Excel files V1 of the two assessors and combined them to check and resolve disagreements. The selected studies were then checked for duplications and looked up for full texts in English.

3.3.5 Data collection process and data items

The two assessors HTT and HQC independently collected data from each article into the same data extraction form (in Excel format) created by HTT. Extracted information included data on:

- The publication: authors, published year.
- The study: country, study designs (randomization, allocation, masking), study period.
- The participants: disease category, number of patients, age range.
- The intervention: fluid type, fluid rate, duration of fluid intervention, comparators
- The outcome: primary endpoints, secondary endpoints, mortality, respiratory distress, respiratory support, inotrope requirement, hematocrit change for normal saline-Ringer's Lactate-Gelatin, -Dextran 70- Dextran 40- 10% HES 10% 200/0.5- 6% HES 200/0.5, reshock, organ failure.

If no information was reported from the full text, that cell was left blank. After finishing data extraction, there was a meeting of 3 investigators to review information from the files of HTT and HQC to check the accuracy and missing information. The final extraction information file was saved for further summary and report.

3.3.6 Risk of bias assessment

Each full text was independently assessed the risk of bias by HTT and HQC using the risk of bias tool RoB-2 version (Sterne J. A. C. et al. 2019). The following domains were evaluated: 1/ bias arising from randomisation process; 2/ bias due to deviation from intended interventions;

3/ bias due to missing outcome data; 4/ bias in the measurement of the outcome; 5/ bias in the selection of the reported results. In each domain, assessor used information written in the publication to decide whether the risk of bias for each signalling questions is yes, probably yes, probably no, no, and no information. I used the RoB-2 Excel toolkit (Altman Douglas 2019) to summarise results following the standard algorithm and report in figure the level risk of bias for each domain and overall risk (high, low, some concerns). The disagreements were discussed and finalised by all three assessors (HTT, HQC, and BW).

3.3.7 Synthesis of results

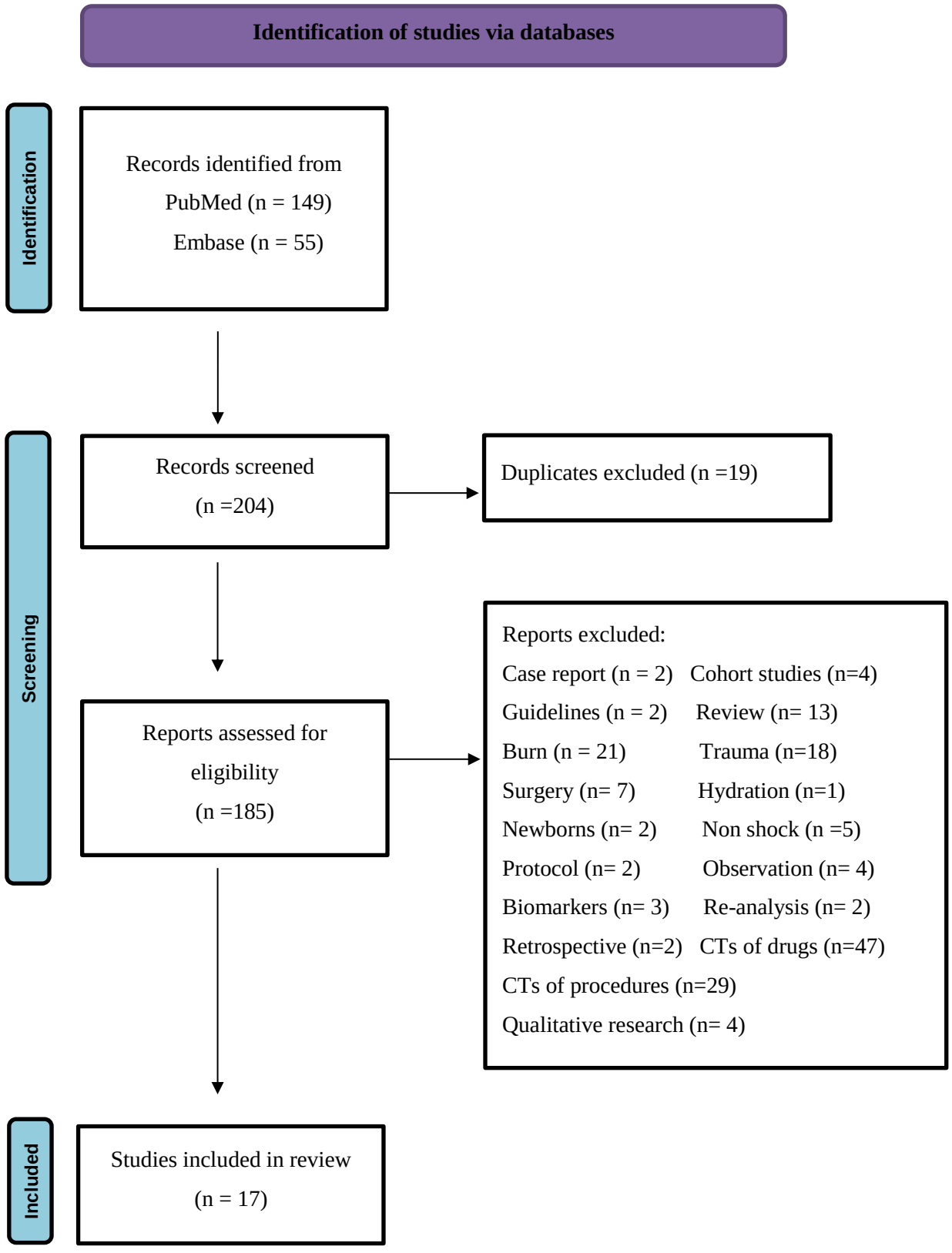
The extracted information was filtered based on disease category (dengue, sepsis) and then summarised and synthesized by intervention and outcome. Main characteristics of each intervention (fluid type, fluid rate, duration of fluid intervention) were reported in detail, while other characteristics were briefly listed. Results of endpoints were presented in descriptive tables. As this review focused only on qualitative synthesis of available fluid resuscitation strategies, I did not apply meta-analysis or any review software to synthesise the data. As participant characteristics (type of disease, age, baseline severity at enrolment, ethnicity, nutritional or immunological status), fluid interventions, and study procedures (outcome measurements, randomisation process, protocol deviation, missing data) varied across studies, both clinical and methodological heterogeneity of studies in this review is expected to be high. These heterogeneities are summarised, however, I did not use any statistical method to quantify and explore possible causes of them.

3.4 Results

3.4.1 Study selection

There was a total of 204 articles identified from the search on PubMed (149 items) and on Embase (55 items). The screening step excluded 19 duplicates and 168 articles that fullfiled the exclusion criteria. Finally, 17 randomised control trials with full text were included for data extraction. The study selection process is shown in Figure 3-1.

Figure 3-1. Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 flow diagram



3.4.1 Study characteristics

The summary of study characteristics is shown in Table 3-2. There were 5 RCTs on DSS management. Three of them were double-blind RCTs performed in Vietnam and another two single-blind RCTs were in Thailand and Indonesia nearly 10 years ago. All studies were done in a single centre. Different fluid types and fluid rates were investigated. The common outcomes were recurrent shock, decrease Hct after 1-2 hours of fluid bolus, and cardiovascular improvement. One trial in Indonesia chose a marker of endothelial inflammation as the primary endpoint. There were no consistencies for outcome definitions among studies about the severity of shock, the occurrence of a subsequent episode of shock, shock resolution time, fluid overload, respiratory distress, and indication of diuretic use. The summary of outcome definition is shown in Table 3-4.

There were 12 RCTs in children with septic shock and/or severe malaria.The studies on malaria include concomitant bacteremia, therefore, they were not excluded. Only 3 studies were performed in high-income countries (UK, America, and China). Other studies were in low-middle-income countries (Kenia, India and Thailand). Participants were in different age ranges and received intervention with different fluid rates and types. Mortality (overall mortality, 48-hour mortality, 28-day mortality) was the most common outcome; however, other outcomes were also reported, including shock resolution time, improvement of acidosis, improvement in haemodynamic status after 6 hours- 12 hours; 4 hour-8 hours, inotrope requirement, total volume of fluid, pulmonary oedema, need of intubation, length of ICU stay. All RCTs were open-label. The majority of studies did not score baseline severity at shock.

Table 3-2. Summary of study characteristics

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
DSS	Dung, 1999 (Dung N. M. et al. 1999)	Vietnam	Randomized trial, double blind, opaque envelopes containing only treatment pack number	50 children DSS from 5-15 years, not received fluid resuscitation	NS (n=12), RL (n=13), Gela (n=13), Dex70 (n=12) Fluid rate: 20ml/kg x 1h, then 10ml/kg x 1h	Duration of shock Number of shock episodes	No difference of recurrent shock. Colloid improve rapidly cardiac output
DSS	Nhan, 2001 (Ngo N. T. et al. 2001)	Vietnam	RT, double blinded, opaque envelopes containing only treatment pack number	230 children with DSS form 1-15 years	Dex70 n=55, 3%Gela n= 56, RL n=55, NS n=56 Fluid rate: Grade 3: 20ml/kg/h, Grade 4: 20ml/kg for 15 mins then 20ml/kg/h.	Initial pulse pressure recovery time; Occurrence and timing of subsequent episodes of shock, decreases in HCT and pulse after 1 hour, total volume Dex70 required after the first hour, total volume of fluid administered until full recovery, complications of fluid therapy	No difference in recurrent shock rate between 4 groups
DSS	Wills, 2005 (Wills B. A. et al. 2005)	Vietnam	RT, computer generated random numbers allocation, study fluid sealed inside specially prepared cardboard containers, sealed	512 children with DSS from 2-15 years	Group 1: RL n=128, HES200 n=129, Dex70 n=126, Group 2: Dex70 n=67, HES200 n=62 15ml/kg/h then 10 ml/kg/h	Rescue colloid; the time taken to achieve initial cardiovascular stability, the time taken to achieve sustained cardiovascular stability, the volumes of rescue colloid and total parenteral fluid required, change in the hematocrit at T2, and the number of days in the hospital.	No difference in recurrent shock

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
			envelope containing identity of study fluid attached to study file in case of emergency,				
DHF, DSS (80%)	Siripen, 2008 (Kalayanarooj S. 2008)	Thailand	Randomised trial	104 children with DHF (n=21) or DSS (n=83)	Dex40(n= 57), HES20010% (n = 47) Fluid rate: 10 ml/kg x 1h	Reduction of Hct after each bolus dose and the total amount usage, the severity of DHF illness, the impact on renal function (amount of urine, BUN, creatinine) and the coagulogram study (PT, PTT and TT) and fluid overload	10% HES 200 was safe. Concerns: Proportion of respiratory distress and severe bleeding were higher than before trial
DSS	Somasetia, 2014 (Somasetia D. H. et al. 2014)	Indonesia	Single blind, randomised control trial	50 children 2-14 years	RL (n= 25), Hypetonic sodium lactat solution (HSL) Fluid rate: HSL 15 ml/kg for 15 mins then 1 ml/kg/h x 12 hrs RL 20 ml/kg for 15 mins then 10-7-5-3 ml/kg/h (complicated study protocol for nonresponders and reshock)	Effectiveness of HSL to decrease endothelial cell inflammation assessed by sVCAM-1 measurements during the first 48 hours of DSS. Effectiveness of HSL to restore hemodynamic and tissue-oxygenation parameters, fluid balance, acid–base status, sodium and chloride balances, as well as in-hospital survival.	sVCAM-1 decreased significantly. Mortality is 20% much higher than literature (<1%), no clear explanations

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
Severe malaria	Maitland, 2003 (Maitland K. et al. 2003)	Kenya	prospective phase 1 trial (descriptive)	53 children with severe malaria (severe anemia, severe acidosis), unclear age range	NS n= 7-15-10 for 3 severe groups 4% Alb n= 6-7-7 for 3 groups 10-40ml/kg/1h, then maintenance fluid4ml/kg/h 5% Dextrose and 0.18% Saline	percentage reduction in base excess between admission and 8 hrs after admission Concerns: Aug 2001 -Dec 15, 2001 using NS; Dec 2001 to Mar 2002 using Albumin;	Bolus fluid improve BE. no comparison haemodynamic changes between fluids, only describle BE decrease level; no comparison on total volume fluid, routine care without bolus
Septic shock	Upadhyay,2004 (Upadhyay M. et al. 2005)	India	Prospective, randomized, open-label trial	60 children from 1m-12years	NS n=31 Gel n= 29 20ml/kg/10-20mins every 10-20 mins until normal BP	Restoration of plasma volume, improvement in hemodynamic status at the end of 6 hours and 12 hours of initial fluid resuscitation, the need for vasoactive drug therapy (number, maximal dose and duration) and survival	No difference in mortality; No different time of fluid resuscitation. Similar rate of respiratory distress and organ failure
Septic shock	Sathanam, 2008 (Santhanam I. et al. 2008)	India	randomised control, open label	147 children from 1m-12years	20ml/kg/20 mins x 3 times /1 hour (n= 73) 40 ml/kg/15min (n=74)	Hospital survival, shock resolution: peripheral warmth, CRT shorter than 2 seconds, normal BP for age and urine output >1 mL/kg/h, complications: hypoxia, hepatomegaly, and intubation at 20, 40, and 60 minutes for each regimen.	Similar outcomes, higher total volume use in study group (20ml/kg/20m x 3) Higher rate of respiratory distress in study group
Severe malaria	Akech, 2010 (Akech S. O. et al. 2010)	Kenya	open-label randomised controlled	79 children more than 6 months	Dex70 n= 39, HES130 n=40 20-40 ml/kg/h Duration: 1-2h	resolution of shock over 8 hours, resolution of acidosis, in-hospital mortality, adverse events (allergic reactions,	No difference in shock attenuated, total volume used, Acidosis improvement is

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
						pulmonary oedema, neurological sequelae)	better in HES group after 4h, 8h Mortality 2/40 (5%) for intervention group, Respiratory distress is higher in Dex70 group
Septic shock/severe malnourish (49% HIV)	Akech, 2010 (Akech S. O. et al. 2010)	Kenya	open-label randomised controlled	62 children >6 months	Half-strength Darrows/5% dextrose (HSD/5D) n = 26 Versus RL n=29, Alb n=6 Alb or RL: 20ml/kg/h; HSD 15 ml/kg/h	resolution of shock at 8 and 24 hours, mortality	Higher mortality 58% in HSD group vs 45% in RL group, higher oliguria rate in HSD at 8h, higher rate of respiratory distress, all fluid were safe
Septic shock, severe malaria	Maitland, 2011 (Maitland K. et al. 2011)	Kenya	multicenter, open randomised control trial	3141 children 1m-12years	Stratum A, 20ml/kg/h saline or human-albumin Stratum B: 40 ml/kg/h of albumin or saline or no bolus.	mortality at 48 hours after randomization, mortality at 4 weeks, neurologic sequelae at 4 and 24 weeks, episodes of hypotensive shock within 48 hours after randomization, and adverse event	mortality of bolus groups were higher than non- bolus in all subgroup
Septic shock	Chopra, 2011 (Chopra A. et al. 2011)	India	randomised open label trial	60 children from 2-12 years	NS: 20 ml/kg/ 15 min up to 60ml/kg/h (n=30) 3% saline: 15 ml/kg over 30 min then 0.9% saline was given if still at shock in volumes of 5–10 ml/kg	Changes in heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, capillary refill time, urine output, SpO2 and need for ionotropic drugs, duration of stay in ICU and mortality.	Total volume is higher in NS but no significant difference, no difference in inotrope requirement, similar mortality (33% in NS vs 27% in HS)

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
					guided by central venous pressure (n=30)		
Septic shock	Liu, 2015 (Liu S. et al. 2015)	China	Randomised open label single center	44 children from 2-104 months	Up to 60ml/kg/h NS vs 3% HS 6 ml/kg/10-15 minutes	hemodynamic changes, volume fluid, requirement of inotrope, organ impairment, mortality	Similar fluid response, OI improved at 3hours, volume fluid, inotrope requirement, organ impairment, 28-day mortality.
Septic shock	Sankar, 2017 (Sankar J. et al. 2017)	India	Randomized control, open label	96 children < 18years	NS 20mL/kg /15–20 minutes (n= 45) VS that over 5–10 minutes (n= 51)	Need for mechanical ventilation and/or impaired oxygenation—increase in oxygenation index in the initial 6 and 24 hours, proportion of clinical fluid overload (hepatomegaly and/or rales), proportion of ventilation for fluid overload alone, changes in OI, respiratory rates, Fio2 requirement and saturation from baseline to up to 24 hours, total fluids at 6 and 24 hours, and fluid overload at 24 hours.	Similar shock attenuation at 1h, 4h, 8h; mortality were 40% in group 20mins bolus vs 33% in group 10mins bolus, impaired oxygenation higher in group 10mins bolus. Stop trial in middle related to safety of 10m arm with higher rate of worse respiratory
Septic shock	Inwald, 2018 (Inwald D. P. et al. 2019)	UK	pragmatic, open, multicentre pilot RCT.	73 children <16years	Balance crystalloid solution (RL, Hartman, plasmalyte) 10 ml/kg/15mins vs 20mL/kg/15mins	Recruitment and retention, protocol adherence, separation, potential trial outcome measures, and parent and staff perspectives	Total volume lower 44% significantly in restricted fluid group; 2 cases in group using 20ml/kg bolus develop signs of OAP. It was not

Disease	Author, year	Country	Methods	Participants	Interventions	Outcomes	Main findings
					up to 4hours if still in shock.		feasible to continue the trial as mortality was zero in both 2 groups
Septic shock	Balamuth, 2019 (Balamuth F. et al. 2019)	America	Open-label pragmatic randomized controlled trial at a single academic children's hospital of Philadelphia	50 children >6months	RL or NS randomly assigned for suspected septic shock for 48hours RL n=24 NS (control n= 26) Volume of fluid depend on clinician	Proportion of eligible patients; acceptability to patients, families and clinician using EFIC for enrolment; adherence to study group assignment with demonstrable separation between the groups in the type of fluid used	Feasibility to run similar study in many centres
Septic shock	Ananthasit,2020	Thailand	double blinded randomised controlled trial, single center	61 children from 1m-18years	NS or BS bolus 20ml/kg/15-20m,up to 40-60 ml/kg/60-90m	Incidence of hyperchloremic metabolic acidosis; prevalence of AKI, need for RRT, length of pediatric ICU, in-hospital mortality.	No difference mortality 22.6% NS group vs 26.7% BS group, NS has hyperchloremia but no difference in clinical outcomes

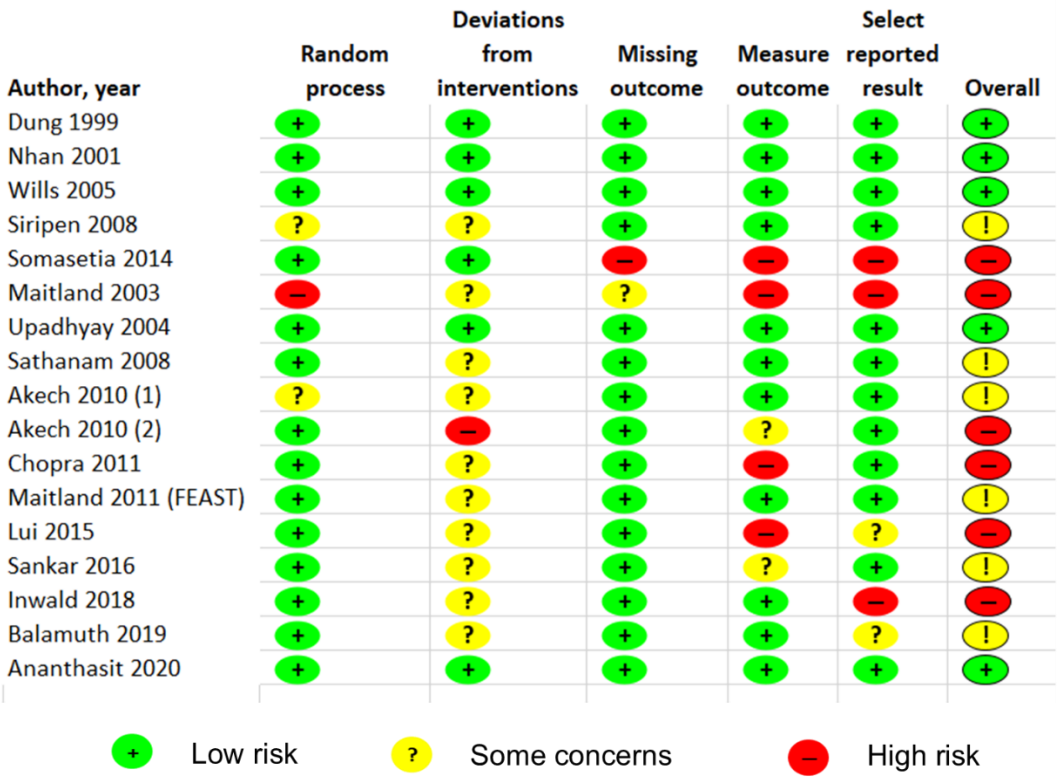
NA Normal saline, BS balanced solution, RL Ringer's Lactate, Dex40 Dextran 40, Dex70 Dextran 70, AKI Acute Kidney Injury, ICU Intensive Care Unit, HSL Hypertotic Sodium Lactate, HS Hypertonic saline, EFIC Exception from Informed Consent.

3.4.1 Risk of bias assessment

The risk of bias assessment using Risk of bias tool RoB 2 is presented in Figure 3-2. Of five RCTs in dengue, one had concerns of bias arising from randomisation and protocol deviation (Kalayanarooj S. 2008), and one study had and high risk of bias in missing outcome data, measurement of outcome and selection of reported results (Somasetia D. H. et al. 2014). There were no explanations for inappropriate numbers of recurrent shock and HES bolus, the cause of deaths, the volume of HES contributing to the total fluid balance and its effects on outcomes (the number of HES bolus was higher in the intervention group).

RCTs in septic shock have overall high risks of bias or some concerns of bias, especially arising from protocol deviation, selection in reporting results. The study on fluid response with albumin or saline bolus in severe malaria children (Maitland K. et al. 2003) did not randomly provide fluid to study patients; did not describe enough information on other routine management for severe anaemia and malaria; did not report full data on organ impairment (bilirubin level, coagulation profile). Chopra et al. reported outcomes of hypertonic saline versus normal saline on vital signs but not a full profile of electrolyte and acid-base changes during the management course (Chopra A. et al. 2011). There was also a lack of information on etiologies, organ impairment and routine care which may affect outcomes, especially for a single centre trial with a small sample size (expected sample size was about 4000 cases versus 60 study patients in this trial). The study on restricted fluid bolus volume could not describe clearly about respiratory support (when, which type of support), inotropic use (when, which agents, duration) due to missing information (Inwald D. P. et al. 2019).

Figure 3-2. Risk of bias assessment for all included studies using Risk of bias tool Rob-2



3.4.2 Study findings

Studies on fluid management for DSS and septic shock were different both in designs, fluid strategies and outcomes. The summary of fluid strategies is described in Table 3-3, definitions of outcomes are summarized in Table 3-4 and the summary of outcomes is shown in Table 3-5.

Fluid management for DSS

There were different fluid strategies for DSS management regarding fluid rate, fluid types and duration. Therefore the description of haemodynamic changes, Hct reduction and outcomes were not completely comparable. Regarding the fluid types and fluid rate, synthetic colloids reduced hematorit significantly compared to crystalloids. The fluid rate ranged from 10- 20 ml/kg/h improving the haemodynamic parameters and did not result in respiratory failure. Although colloids improved cardiac output rapidly (Dung N. M. et al. 1999), the use of colloid or crystalloid for compensated shock had similar outcomes.

The definitions of outcomes were different in clinical trials, for example, initial shock recovery time was slightly different regarding the pulse pressure cutoff of 30 mmHg (Ngo N. T. et al. 2001) or 25 mmHg (Wills B. A. et al. 2005). The definition of reshock or recurrent shock was based on the use of "rescue colloid" at any time point after the bolus of study fluid (Wills B. A. et al. 2005) or after a normal blood pressure with pulse pressure ≥ 30 mmHg (Ngo N. T. et al. 2001). There was no definition for respiratory distress or respiratory compromise but this outcome was mentioned in all RCTs of DSS without details. The term "fluid overload " was mentioned in one RCT (Kalayanarooj S. 2008) but there was no definition for it. The diuretic use was reported in these RCTs to reflect fluid overload status but no definitions for this indication in all studies.

About the association between fluid strategies and outcome, there were no differences in outcomes of reshock, respiratory distress, volume of fluid or organ impairment though colloids decreased significantly Hct and pulse. The mortality was low in 4 RCTs except for a small RCT in Indonesia with a mortality rate of 16% (Somasetia D. H. et al. 2014) in which hyperosmotic solution was used.

Fluid resuscitation for septic shock

RCTs on septic shock resuscitation compared different fluid types and different fluid rates for initial resuscitation. Overall, all types of fluid resuscitation provided similar outcomes including shock resolution, respiratory compromise, organ impairment and mortality.

Regarding fluid rate, small trials used high rate of fluid bolus ranging from 20-40ml/kg/15-20 mins. There was one trial using fluid bolus of 20 ml/kg/10 mins. Except trials that were conducted in Kenya without ventilatory support, other trials were performed in PICUs with well equipment for intensive care and haemodynamic monitoring. A bolus of 10-20 ml/kg/15-20 minutes is likely safe for children with septic shock in settings with advanced ventilatory support. Importantly, the fluid rate of 20 ml/kg/10 minutes was recommended not to be used as it resulted in respiratory failure and required invasive mechanical ventilation. A large RCT in Africa showed bolus fluid of 20-40 ml/kg/hours of 5% albumin or 0,9% saline solutions caused

33% higher mortality compared to no bolus. This trial was performed in settings without the availability of ventilator support, therefore, mortality was high. It raised concern about using fluid bolus for children with shock in poor-resource settings. After this trial, there was a multi-centres RCT comparing bolus rates of 20ml/kg/15 mins and 10 ml/kg/15 mins for children suspected septic shock and managed in ICUs in UK. The mortality was zero, thus, this pilot trial concluded that similar protocol would not be feasible to be studied in UK. Patients in the 20 ml/kg arm had a higher rate of mechanical ventilation support compared to the 10 ml/kg arm (25% vs 11%, respectively).

About fluid types, different colloids (albumin, gelatin, dextran) and crystalloids (normal saline, hypertonic saline, balanced solution) were studied for patients with septic shock with or without malaria. There were no differences in outcomes between different fluid types though the volume of colloid or hypertonic saline was reported significantly lower.

Regarding outcomes of RCTs on septic shock, mortality is the most common outcome. Other important outcomes associated with shock resolution, organ impairment and complications associated with fluid bolus were also studied. In different RCTs, authors used definitions of shock from guidelines of WHO or American College of Critical Care Medicine (ACCM) or Paediatric Advanced Life Support (PALS). The mortality ranged from 5% to 69%. A summary of mortality and respiratory distress in different RCTs were shown in Table 3-6.

.

Table 3-3. Summary of fluid strategies

Disease	Study	Fluid type	Fluid rate	Duration
Dengue shock	Dung 1999	NS, RL, Gel, Dex70	20 ml/kg x 1h then 10 ml/kg x 1h	2 h
Dengue shock	Nhan 2001	NS, RL, Gel, Dex70	20 ml/kg/h x 1 h	1 h
Dengue shock	Wills 2005	RL, 6% HES 200, Dex70	15 ml/kg x 1h, then 10 ml/kg x 1h	2 hrs
DHF and dengue shock	Siripen 2008	Dex40, 10% HES 200	10 ml/kg x 1 h	1 h
Severe dengue shock	Somasetia 2014	RL, HSL	HSL 5 ml/kg for 15 mins, then 1ml/kg/h x 12hs RL 20 ml/kg for 15 mins, then 10-7-5-3 ml/kg/h	15 mins, 12 hrs
Severe malaria (6% concomitant bacteremia)	Maitland 2003	NS, 4% Alb	10-40 ml/kg x 1 h	1 h
Septic shock (37% pneumonia, 12% dengue shock)	Upadhyay 2005	NS, Gel	20 ml/kg for 10-20 mins until achieve normal blood pressure	1 h
Septic shock	Santhanam 2008	RL	20ml/kg for 20 mins x 3 times 40 ml/kg for 15 mins	1 hour 15 mins
Severe malaria	Akech 2010	Dex70, 6% HES 130	20-40 ml/kg/h	1-2 hrs
Septic shock/severe malnourish (49% HIV)	Akech 2010	HSD/5D, RL, 4.5%Alb	4.5%Alb , RL : 20 ml/kg/h HSD 15 ml/kg/h	1h
Septic shock	Maitland 2011	NS, 5% Alb	NS, 5% Alb 20 ml/kg/h NS 5% 40 ml/kg/h	1h

			No bolus	
Septic shock (18/60 DSS)	Chopra 2011	NS, 3% saline (HS)	NS 20ml/kg/15 mins up to 60 ml/kg/h HS: 15ml/kg/30 mins	1h
Septic shock	Liu 2015	NS, 3% saline (HS)	NS 20-60 ml/kg/h HS: 6ml/kg/10-15 mins x 2 times	1h
Septic shock	Sankar 2017	NS	NS 20 ml/kg/15-20mins NS 20 ml/kg/5-10 mins	10-20 mins
Septic shock	Inwald 2018	RL, Hartman, Plasmalyte	10 ml/kg/15 mins 20 ml/kg/15 mins	15 mins
Septic shock	Balamuth 2019	RL, NS	Standard of care	-
Septic shock	Ananthasit 2020	NS, Ringer's acetate	20 ml/kg/15-20 min, up to 40-60 ml/kg/60-90 mins	1h

Alb Albumin, Dex70 Dextran70, Dex40 Dextran 40, HSL Hypertonic Sodium Lactate, HS Hypertonic saline, HES200 Hydroxyethyl starch 200/0.5, Gel Gelatin, NS Normal saline, RL Ringer's Lactate, Min Minute, h hour

Table 3-4. Definition of several important outcomes

Outcome	Study	Definition
Dengue shock		
Recovery from shock	Dung 1999	Clinical improvement with pulse pressure ≥ 20 mmHg
Initial pulse pressure recovery time	Nhan 2001	The time from the start of fluid therapy until the pulse pressure reached ≥30 mm Hg.
Initial cardiovascular stability time	Wills 2005	The time in hours from study entry until the pulse pressure reached and was maintained at ≥25 mm Hg with a systolic pressure of ≥80 mm Hg for a minimum of two hours
Subsequent episodes of shock or "reshock"	Nhan 2001	Clinical status which has association with tachycardia and coolness of extremities, the pulse pressure narrowed to ≤20 mm Hg, having previously reached a level of >30 mmHg.
Recurrent shock	Wills 2005	Clinical status in which patients required supplemental intervention with rescue colloid at any time after the infusion of the study fluid
Sustained cardiovascular stability	Wills 2005	The time in hours from study entry to reach and maintain these cardiovascular indexes indefinitely without further intervention
Clinical fluid overload	Siripen 2008	(describe in the results but not in the method) Clinical manifestations of fluid overload include swollen eyes, dyspnea, crepitation, rhonchi, wheezing, fluid overload, lasix given
Diuretic use		(Mentioned in the result of all trials in DSS but no studies described the indications for diuretic use).
Reduction in Hct after 1h, 2h 2h	Nhan 2001, Siripen 2008	Percentage of Hct change after 1 hour or 2 hours of fluid boluses

Outcome	Study	Definition
	Dung 1999, Wills 2005,	
Reduction in pulse rate after 1 hours	Dung 1999, Nhan 2001	Percentage of pulse decreased after 1 hour
Total volume of colloid after the first hour Further colloid infusion	Nhan 2001 Dung 1999	Volume of colloid use in ml or ml/kg after the first hours
Total volume of IV fluid	All studies	Total volume of fluid from shock
Mortality	All studies	Proportion of deaths
Septic shock		
Resolution of shock at 8 hrs, 24 hrs	Akech 2010	Adapting ACCM/PALS endpoints, defined as the absence of all the following features: severe tachycardia (>180 if aged<12months, >160 if aged 1-5 or >140 if aged ≥ 5); hypoxia (SpO2 <95%); hypotension (SBP <70 for <12 months, <80 for >1 year) or delayed CRT ≥ 3 seconds at 8h, 24h
Resolution of shock at 6 hrs, 12 hrs	Upadhyay 2005	Blood pressure returned to normal, perfusion improved or central venous pressure was >10 cm H2O at 6h, 12 h
Haemodynamic stability	Chopra 2011 Ananthasit 2020	Defined as blood pressure above 5th percentile, with pulse rate within normal range for age, urine output more than 1 ml/kg/h and CRT <3 seconds Include all of the following: normal heart rate for age, normal mental status, , SBP >5th percentile for age, urine output >1 mk/kg/h and no futher increase doses of inotrope or vasopressor required

Outcome	Study	Definition
Fluid refractory shock	Sankar 2017	Defined as per the surviving sepsis campaign guidelines definitions as - inability to achieve therapeutic endpoints after receiving fluids in the initial hour of resuscitation of up to 60 ml/kg or developing features of fluid overload such as rales or hepatomegaly during volume resuscitation without attaining therapeutic endpoints
Refractory shock	Sankar 2017	Defined as failure to attain therapeutic endpoints after receiving maximal recommended dosages of vasoactive medications for catecholamine resistant shock. In other words along the algorithm, persistent catecholamine-resistant shock was considered to be refractory shock
Fluid overload	Sankar 2017	Presence of any of the features following fluid resuscitation- enlarging liver size (increase in liver size from baseline), bilateral basal rales, anasarca, evidence of fluid overload on chest X-ray (new infiltrates or worsening of previous x-ray picture
Increase in Oxygenation index	Sankar 2017	OI= Mean airway pressure × Fio2 × 100/Pao2. Increase in oxygenation index of 5% from baseline during or up to 6 hours following fluid resuscitation will be considered clinically significant.
Mortality at 48 hours, 28 days (4 weeks)	Maitland 2011	Proportion of deaths at 48 hrs, 28 days.
Length of stay in PICU	Multiple studies	Days from ICU admission until discharge
Potential complications of volume resuscitation	Akech 2010	Include pulmonary oedema, raised intracranial pressure (SBP > 90th centile for age in association with a falling heart rate, or papilloedema or brain stem feature of transtentorial herniation)
Acute kidney injury Need for renal replacement therapy	Ananthasit 2020	Defined according to KDIGO (the kidney disease: improving outcomes): creatinine 2-2.9 times increases from baseline or urine output below 0.5 ml/kg/hour for 12 hours Needs for a new continuous renal replacement therapy, hemodialysis or peritoneal dialysis

Outcome	Study	Definition
Hyperchloremic metabolic acidosis	Ananthasit 2020	Defined as strong ion difference below 40 mEq/L and serum chloride more than 108 mmol/L
Hyperchloremia	Balamuth 2019	Clo level >110 mEq/L
Vasoactive Inotropic Score	Ananthasit 2020	Equal to dopamine dose (ug/kg/min) + dobutamine dose (ug/kg/min) + 100 epinephine dose (ug/kg/min) + 100 Noradrenaline dose (ug/kg/min) +10 Milrinone dose (ug/kg/min) + 10,000 vasopression dose (U/kg/min)

Table 3-5. Summary of primary outcomes

Disease	Study	Strategy	Outcome	Median (range) or n(%)	Effect size	p	Notes
DSS	Dung 1999	30 ml/kg/2hrs of NS, RL, Gel, 6% Dex70	Duration of shock Number episode of shock	NS 1.5(1-18) RL 5(1-18.5) Gel 7(1-29.5) Dex70 2.8(1-20) NS 1 (1-2) RL 1 (1-3) Gel 1 (1-3)) Dex70 1 (1-3)		0.36 0.46	Though Dextran 70 was associated with greater fall in Hct, there were no differences between 4 groups in shock re-occurrence.
DSS	Nhan 2001	20 ml/kg/h NS, RL, Gel, Dex70	Initial pulse pressure recovery time	Dex70 0.5(0.25-3) hrs Gel 0.5(0.25-2) hrs RL 0.75(0.25-7) hrs NS 0.75(0.25-3) hrs	-	0.03	Decrease Hct, pulse after 1 hours were significant different. However reshock rate was similar. The width of pulse pressure is the most significant factor associated with clinical fluid response.
DSS	Wills 2005	25 ml/kg/2hrs RL, 6% HES 200, Dex70	Reshock (rescue colloid)	Dex70 29 (23%) Starch 43(33%) RL 38 (33%)		0.18	Though the deduction in Hct significantly higher after using Dex70 or starch, the reshock rate was the same for all 3 fluid types.

Disease	Study	Strategy	Outcome	Median (range) or n(%)	Effect size	p	Notes
DSS	Siripen 2008	10 ml/kg/1h Dex40, 10% HES 200	Reduction in Hct Total volume of fluid	Dex 40 7.5% 10%HES 8.1% Dex40 119.4 m/kg HES 128.3 ml/kg		0.381 0.227	There were no significant differences in Hct change, total IV fluid, BUN, creatinine, urine volume, fluid overload, coagulation profile, electrolytes and liver function.
Severe DSS	Somasetia 2014	HSL 5 ml/kg/15 mins RL 20 ml/kg/15 mins	Decrease sVCAM-1 after 48hrs	HSL: ~ 3500 RL ~ 4000		0.0024	Significant decrease sVCAM-1 in HSL compared to RL at 48th hour. The clinical outcomes were similar.
Severe malaria	Maitland 2003	10-40 ml/kg/1 h NS or 4.5% Alb	Reduction in BE at 4hrs	Alb: -19% to --41% Saline: -19 to -35%	-	-	Small sample size study found a rapid improvements in haemodynamic status
Septic shock	Upadhyay 2005	20 ml/kg/10-20 mins Gel, NS	Restore plasma volume	NS 52.5 (51-55) ml/kg Gel 52.2(51-54)	-	>0.1 0.018	Total body water, extracellular fluid volume, haemodynamic stability at 6 and 12 hrs, organ failure, requirement of vasoactive drugs, survival were similar between 2 groups. Only fluid volume used were different (50 (20-128) ml/kg in

Disease	Study	Strategy	Outcome	Median (range) or n(%)	Effect size	p	Notes
							saline group vs 30 (20-70)ml/kg in Gel group
Septic shock	Santhanam 2008	20ml/kg/20 mins x 3 40 ml/kg/15 mins	Hospital survival	17.6% for both 2 strategies	-	1	Patients who did not achieve reverse shock and required ventilation had the highest mortality. Aggressive fluid protocol needs to be avoided in resource-poor settings.
Severe malaria	Akech 2010	20-40 ml/kg/h Dextran or HES	Resolution of shock	Dextran 20/39 (51) HES 20/40 (50)	-	0.91	No differences in vital signs, mortality, or kidney injury.
Septic shock/severe malnourish	Akech 2010	4.5%Alb , RL : 20 ml/kg/h HSD 15 ml/kg/h	Number with shock at 8h, 24h,	HSD/SD 15/22 (68); 14/18(78) RL 14/25(56), 14/25(56)	-	0.39 0.14	There were no differences in heart rate, creatinine, tachypnea, base deficit and mortality. The trial was terminated prematurely as the mortality above 50%
Septic shock	Maitland 2011	NS, 5% Alb 20 ml/kg/h NS 5% 40 ml/kg/h No bolus	Mortality at 48 hrs	Alb 111 (10.6) Saline 110(10.5) No bolus 76 (7.3)	1.45(1.13-1.86)		Mortality at 4ws: 1.39(1.11-1.74) for the bolus Neurologic sequelae or death: 1.33(1.09-1.64) significantly higher than no bolus

Disease	Study	Strategy	Outcome	Median (range) or n(%)	Effect size	p	Notes
Septic shock	Chopra 2011	NS 20ml/kg/15 mins up to 60 ml/kg/h 15ml/kg/30 mins	Haemodynamic stability (HR, SBP, DBP, CRT, urine, Spo2 , needs of inotropes	Haemodynamic parameters were similar Need of inotrope was similar		>0.05 for all compariso ns	No differences in haemodynamic parameters. Only the change in serum sodium level after HS bolus was higher significantly compared to NS bolus (10 meq/Lversus 5 meq/L)
Septic shock	Liu 2015	NS 20-60 ml/kg/h 3% saline: 6ml/kg/10-15 mins x 2 times	Haemodynamic improvement after 1-3-6-24h	improvement of heart rate, MAP, OI after 3, 6, 24 hours of fluid resuscitation	-	-	There was an improvement in haemodynamics after 3 hours of fluid resuscitation but no significant differences were found between 2 fluid strategies for all outcomes of pulse, MAP, OI, lactic acid clearance, multi-organ dysfunction score and mortality.
Septic shock	Sankar 2017	i/NS 20 ml/kg/15-20mins ii/NS 20 ml/kg/5-10 mins	Newly ventilated at first hour Time to initiate MV	i/ 5(11) vs ii/ 18 (35) i/215(52-340) mins vs ii/25 (15-180) mins	0.31(0.13-0.78)	0.006 <0.0001	Fast administering fluid resuscitation over 5-10 mins increases the risk of intubation. There were no significant differences in mortality.
Septic shock	Inwald 2018	i/BS 10 ml/kg/15 mins	Total number of bolus	i/58 vs ii/ 48	-	-	Research without prior consent worked smoothly

Disease	Study	Strategy	Outcome	Median (range) or n(%)	Effect size	p	Notes
		ii/BS 20 ml/kg/15 mins	Total volume of fluid	i/14.5(SD 11.1) vs ii/ 25.7(SD 12) ml/kg			Despite PALS, ACCM continue to recommend 20ml/kg boluses but many clinicians favoured 10 ml/kg bolus Small sample size. Mortality was zero.
Septic shock	Balamuth 2019	Standard of care NS vs RL	Feasibility Total fluid	85% LR 107(60-155) ml/kg NS 98(63-128) ml/kg	-	-	A pilot study with the primary endpoint on feasibility. The acceptance of EFIC was high (exception from informed consent regulation)
Septic shock	Ananthasit 2020	BS or NS 20 ml/kg/15-20 min, up to 40-60 ml/kg/60-90 mins	Incidence of hyperchoremic acidosis	NS: 17 (55) BS 10 (33)	-	0.091	There was similar proportion of AKI, RRT, length of stay in PICU and mortality

Table 3-6. Results of mortality and respiratory distress in patients with septic shock

Author, year	Fluid types	Fluid rate	Mortality rate (%)	Respiratory distress
Maitland, 2003 (Maitland K. et al. 2003)	Normal saline for 3 severity groups (n= 7-15-10)	10-40ml/kg/1h, then 4ml/kg/h 5% Dextrose in 0.18% Saline	1/13 (8%) - 1/22(5%) – 2/18 (12%) for three severity groups, respectively. No description of fatal outcome belonging to which group.	Only describe respiratory rate which decreased after fluid bolus
	4% Alb for 3 severity groups (n= 6-7-7)			
Upadhyay, 2005 (Upadhyay M. et al. 2005)	Normal saline (n=31)	20ml/kg/10-20mins every 10-20 mins until normal BP	9/31 (29%)	0/31
	Gelatin (n=29)		9/29 (31%)	1/29
Sathanam, 2008 (Santhanam I. et al. 2008)	Ringer's lactate	20ml/kg/20 mins x 3 times /1 hour (n= 73)	13/73(17.8%)	Intubation rate 28/73 (38%)
		40 ml/kg/15min (n=74)	13/74 (17.6%	37/74 (50%)
Akech, 2010 (Akech S. O. et al. 2010)	Dex70 n= 39	20-40 ml/kg/h Duration: 1-2h	2/39 (5%)	No pulmonary aedema
	HES 130 n=40		2/40 (5%)	
Akech, 2010 (Akech S. O. et al. 2010)	Half-strength Darrows/5% dextrose (HSD/5D) (n = 26)	15 ml/kg/h	15/26 (58%)	No clinical pulmonary aedema
	Ringer's Lactate (n=29)	20 ml/kg/h	13/29 (45%)	
	4.5% Albumin (n=6)	20 ml/kg/h	3/6 (50%)	
Maitland, 2011 (Maitland K. et al. 2011)	Normal saline	20 ml/kg/h (n=1041)	110/1041(10.5%)	Pulmonary aedema 6/1047 (0.6%) for NS group 14/1050 (1.3%) for Albumin group
	5% human albumin	20 ml/kg/h (n=1050)	111/1050 (10.6%)	
	Normal saline	40 ml/kg/h (n=16)	9/16 (56%)	

Author, year	Fluid types	Fluid rate	Mortality rate (%)	Respiratory distress
	5% albumin	40 ml/kg/h (n=13)	9/13 (69%)	
		No bolus (n=1044)	76/1044 (7.3%)	6/1044 (0.6%)
Chopra, 2011 (Chopra A. et al. 2011)	Normal saline	20 ml/kg/ 15 min up to 60ml/kg/h (n=30)	10/30 (33%)	No information
	3% saline	15 ml/kg/30 min. then 0.9% saline (n=30)	8/30 (27%)	
Liu, 2015 (Liu S. et al. 2015)	3% saline (n=20)	6 ml/kg within 10 to 15 minutes, 1-2 times	1/20 (5%)	ARDS 13/20 (65%)
	0.9% saline (n=24)	Up to 60ml/kg/h NS vs	2/24 (8.3%)	12/24 (50%)
Sankar, 2017 (Sankar J. et al. 2017)	Normal saline	NS 20mL/kg /15–20 minutes (n= 45)	18/45 (40%)	Require intubation within 215 mins
		20ml/kg/5–10 minutes (n= 51)	17/51 (33%)	Required intubation within 25 mins
Inwald, 2018 (Inwald D. P. et al. 2019)	Balance crys solution (RL, Hartman, plasmalyte)	10 ml/kg/15mins (n=39)	0	Mechanical ventilation 4/36(11%)
		20mL/kg/15mins (n=34)	0	8/32 (25%)
Balamuth, 2019 (Balamuth F. et al. 2019)	Ringer's Lactate (n=24)	Volume of fluid depend on clinician	-	-
	Normal saline (n= 26)		-	-
Ananthasit,2020	Normal saline	20ml/kg/15-20m, up to 40-60 ml/kg/60-90 mins	22.6%	
	Balanced solution		26.7%	

3.5 Discussion

Different fluid types and fluid rates were used in RCTs for dengue shock syndrome and septic shock management. The high level of clinical and methodological heterogeneity of studies makes it difficult to synthesize and derive good conclusions on which fluid type or fluid rate would provide better outcomes. Each RCT refers to a specific population which varies across studies. For RCTs on DSS, all patients were confirmed dengue and no coinfection was reported. However, for RCTs on septic shock, the population included shock due to dengue, malaria, pneumonia, meningitis or coinfection malaria and bacteremia (Upadhyay M. et al. 2005, Maitland K. et al. 2011). As each aetiology of infection has specific pathophysiology which requires different fluid management strategies, "one size cannot fit all" and personalised management should be the future direction to improve outcomes.

3.5.1 Fluid type and fluid volume for initial resuscitation

RCTs in DSS showed the safety and efficacy of using synthetic colloids (Dextran, HES, Gelatin) for children with DSS. In the three RCTs in Vietnamese children, there was only one death (Wills B. A. et al. 2005) and few allergic reactions without cardiopulmonary impairment. The allergic reactions include fever, and rigour occurring in patients receiving Dextran and urticarial rash in patients receiving HES. The results of these trials supported WHO guidelines for dengue shock management. Of note, the total volume of colloid fluid in these trials was below 50 ml/kg for the whole management course, therefore, it is unknown the safety of these synthetic colloids if the total volume use is more than that volume. At present, dextran and starch used in those RCTs have been limited for production globally due to high caution on using them in septic shock. Meanwhile nearly a decade, there have been no studies on fluid management for DSS to provide evidence of using albumin or human plasma or new balanced crystalloid solutions. Another issue is that the management for decompensated shock has not changed or updated after the results of the FEAST trial.

On the other hand, outcome definitions were not consistent across the studies which make difficulties in comparing results. The respiratory distress and fluid overload were not defined in any RCTs on DSS management, therefore the results could not be comparable as these outcomes relied on clinicians subjectively and could vary very much in each centre. The term 'fluid overload" should be defined rationally to fit with the international perspective. As patients with DSS have severe levels of plasma leakage, presentation of oedema does not mean high intravascular volume status. The term fluid overload should not be confused with oedema. Patients with DSS often have oedema during fluid management but are accompanied by low intravascular volume. In future studies, these outcomes need to be defined clearly and rationally.

The bolus of colloids or crystalloids for children with septic shock need to be carefully considered as it may increase the mortality of children admitted to resource-poor settings. Large sample size could reveal the occurrence of an uncommon event that a small one could not do. Although the results of the FEAST trial (Fluid Expansion as Supportive Therapy) were questioned for high mortality which is against the results of previous RCTs with small sample size, we cannot ignore the real, true and important warnings on harmful effects of high rate fluid bolus to severely ill patients. There is a need for future research comparing restricted fluid strategies to current guidelines to provide better guidance for septic shock resuscitation. A lower rate of the fluid bolus of 10 ml/kg/20 mins can be considered. After the FEAST trial, the feasibility of RCTs using high volume versus low volume fluid resuscitation needs also to be assessed in the early phase. In good intensive care settings, the mortality can be low, therefore, the endpoint should target other important outcomes, including the need for respiratory and organ support, duration of support and health economics.

3.5.2 Limitations of RCTs included in this review

Besides the population heterogeneity, the methodology is another limitation of RCTs which results in weak conclusions. Except for the FEAST trial, other trials both in dengue and septic

shock were designed in one centre and with a small sample size. Limitations of the single centre trial include lack of external validity which is necessary for the generalisation ability for the application in different settings (Bellomo R. et al. 2009). A meta-epidemiologic study including 421 RCTs (223 single-centre and 198 multi-centre trials) concluded that single-centre RCTs showed larger effects of the intervention arm than did multi-centre RCTs with the ratio of odds ratio of 0.73 (95% CI 0.64 – 0.83). The ratio of odds ratio is often used to illustrate the difference in the estimated intervention effect (its value below 1 indicates a larger estimation) (Dechartres A. et al. 2011).

Another limitation is that there was no haemodynamic monitoring either for macrocirculation or microcirculation used in RCTs as comparators with clinical signs. Therefore the thoughtful explanations for unexpected outcomes were difficult. Clinical manifestations of shock can be challenging in pediatric patients and would not be perfectly correct, especially during the compensated period. Thus for future research, there is a need of applying advanced techniques to monitor patients. That is not only for research purposes itself but also to assure the safety of patients who are already in serious status. The continuous haemodynamic monitoring would also allow understanding of the physiological response of different patients to different fluid types (colloid versus crystalloid) as well as different volume fluid strategies (liberal or restricted strategies). This area has limited data on pediatric patients and is my particular interest which I will present and discuss in other chapters of this thesis.

This review also found the limitation in describing the outcome of patients with shock. The conventional outcome is mortality but in our new era, other important outcomes should be targeted, toward patient-centred care including length of ICU stay, number of organ support, rehospitalisation rate, health economics and other assessments for mental health and recovery status.

3.5.3 Challenges for intervention studies in children

Children are vulnerable to research as they are not people who respond agree or disagree with the informed consent. In LMICs where the healthcare settings lack advanced supportive care, intervention studies on critically ill patients would carry the risk of complications and high mortality. The survival of a severely ill patient in ICU does not depend only on fluid resuscitation but relies on multiple factors: organ support capacity, medicine, nursing care, standard protocols of ICU procedure, nutrition, physical therapy, rehabilitation and so on. The ethical issue will be the biggest challenge for intervention research. The time-sensitive nature of intervention for shock resuscitation is often impossible for formal written informed consent (which would take a haft of an hour to fully explain the protocol to the parents). Deferred consent or verbal consent or multiple phases of a consent process should be investigated for the feasibility of future research. The successful use of "research without prior consent" and " the US federal exception form informed consent regulation" in two pilot studies in UK and US may not be accepted in other countries. Children should be protected in the trial rather than in future applications, therefore, there should have multiple activities prior to clinical trials on the consent process.

Another challenge is that children are naturally a heterogenous population with a wide range from neonates to adolescents. They have different physiological responses to diseases and therapies. Large sample size is needed to answer a research question in this population. Multicenter and pragmatic designs would be the ideal option for trial in children. However, it would require strong collaboration and the adaptivity of the research platform at different research sites to increase the feasibility of the study.

3.5.4 Strengths and limitations of this review

This systematic review provided an overview of fluid resuscitation strategies for DSS and septic shock in children. It revealed the limitations of previous studies, raise concerns about interpretation results, and suggested important outcomes that need to be focused on in future research in order to amend the guidelines. This review has several limitations. Firstly, the search

strategy was performed in only two databases which may miss many important studies in this field. Secondly, we select only RCTs to limit the number of final articles but this also misses important observational studies which could provide more evidence of using different fluid types and fluid rates for resuscitation. Only English publications were included for this review, which might miss studies in dengue-endemic countries where local languages were used and might contain important results. Last but not least, I could not pull data for a meta-analysis or use a good statistical method to assess heterogeneity qualitatively.

3.6 Conclusion

Synthetic colloids are safe and effective for DSS management in children. The fluid bolus of 10-20 ml/kg/h was safe and had no requirement for respiratory support. However there were few studies in DSS management and all were single-centre trials, hence the results might overestimate the effects. For septic shock, there was weak evidence for fluid choice. A high rate of fluid bolus of more than 20 ml/kg/15 minutes was harmful and required mechanical ventilation support within the first hour for children with septic shock. The bolus fluid of 20-40 ml/kg/hour needs to be carefully considered as it increased mortality in the setting without ventilatory support. The restricted fluid strategy will be the protocol of future research. Regarding endpoints of fluid management, it should not only focus on cardiovascular stability but also on the need for organ support, the total volume use and the management cost (World Health Organization 2009) to widen the study application into resource-poor settings. This systematic review suggests a direction for future research on fluid resuscitation which should apply a new methodology to increase the sample size (adaptive trial, multiple centres, multiple arms, online platform and simplified protocol). This kind of research would require strong collaborations across the endemic regions, the introduction of a new approach to the consent process; various tools of hemodynamic monitoring. The feasibility of conducting RCTs in children needs to be addressed, especially for patients with DSS, as the mortality reported from excellent centres was low but that reflects only the iceberg tip of the global issue.

CHAPTER 4

A RETROSPECTIVE STUDY OF FLUID MANAGEMENT FOR CHILDREN WITH DENGUE SHOCK SYNDROME IN ASIA

4.1 Introduction

As described in Chapter 1, most individuals experience dengue as an influenza-like febrile episode, typically lasting 3 to 7 days, but in a small proportion of cases potentially life-threatening complications develop. The most notable problem, particularly prevalent in children and young adults, is a systemic vascular leak syndrome that can progress to hypovolaemic shock (dengue shock syndrome, DSS) if severe (World Health Organization 2009, Paz-Bailey G. et al. 2022). Thrombocytopenia and coagulopathy are almost invariably present, often associated with minor clinical bleeding, but severe haemorrhage does sometimes occur, usually in association with profound shock (Wills B. A. et al. 2002). Managing a clinical case of severe dengue with shock is not easy although there are guidelines for dengue management established in many countries, which are usually based on WHO 2009 guidelines.

4.1.1 Challenges related to fluid management for dengue shock syndrome

Successful management of DSS relies on good supportive care, primarily judicious use of intravenous fluids to maintain adequate intravascular volume for the critical 24-48 hours when capillary permeability is markedly increased. Recurrent hypovolaemic episodes (re-shock) due to ongoing leakage can occur during this time (World Health Organization 2012). Around 20-30% of DSS patients develop re-shock, often 6 to 12 hours after initial cardiovascular stabilisation, and among these individuals, about 30% will have further episodes; repeated episodes substantially increase the risk of death (Ngo N. T. et al. 2001, Lam P. K. et al. 2013, Yacoub S. et al. 2017). Meticulous attention to detail is necessary to limit iatrogenic complications associated with fluid resuscitation, in particular the development of respiratory compromise due to fluid overload. The World Health Organization (WHO) dengue

management guidelines recommend prompt infusion of a 10-20 ml/kg crystalloid fluid bolus for children with established DSS, followed by a tapering crystalloid fluid regimen that can be supplemented by boluses of synthetic colloid solutions for recurrent shock (World Health Organization 2009). If managed in a dedicated HDU/ICU environment by experienced staff mortality rates below 1% are achievable (World Health Organization 2022). However, the economic and resource burdens for local health services are considerable. For example, managing a severe dengue case with fatal complications has been estimated to cost 84,730 USD per case (Shepard D. S. et al. 2016).

In endemic settings where DSS case numbers are high and HDU/ICU facilities with the capacity to provide respiratory support are scarce, the mortality of severe dengue with respiratory failure requiring mechanical ventilation can be 100% (Mohamed A.N et al. 2013, Gupta S. et al. 2020). Additionally, in managing severe dengue cases with coagulopathy the risk of significant bleeding is much higher compared to septic shock or other types of shock; as a result, invasive procedures for haemodynamic monitoring are less likely to be applied during the early stage of management but are instead delayed and only used in profound or refractory shock cases with established multi-organ dysfunction. Therefore the guidelines for advanced haemodynamic monitoring that are generally recommended for septic shock management are not applied to DSS.

Another issue is that the evidence base for the current WHO guidelines is limited, with the recommendations based primarily on expert opinion and three blinded RCTs of initial fluid management for children with DSS that were performed in Vietnam (Dung N. M. et al. 1999, Ngo N. T. et al. 2001, Wills B. A. et al. 2004, World Health Organization 2009). The first was a pilot study in 50 children with DSS, who were assigned to receive one of four fluids (6% Dextran70, 3% gelatin, Ringer's lactate or 0.9% saline) for 2 hours at initial resuscitation. The fluid rate was 20 ml/kg/h in the first hour then 10 ml/kg/h in the second hour. This study provided preliminary safety information about the use of colloids in DSS management. The

second, a double-blind RCT in 230 children with DSS, compared 6% Dextran 70, 3% gelatin, Ringer's Lactate and normal saline. Although haematocrit values dropped more rapidly in the dextran and gelatin recipients (11.5% and 7.1%, respectively) after a bolus of 20 ml/kg over 1 hour compared to recipients of normal saline and Ringer's lactate (6.5 and 5.7%, respectively), the recurrent shock rate was similar across all four groups (31% for dextran, 27% for gelatin, 36% for Ringer's Lactate and 30% for normal saline) (Ngo N. T. et al. 2001). The study suggested that patients with pulse pressure (PP) <=10 mmHg should be managed initially with colloid because this group had a higher re-shock rate (41%) compared to that of the group with PP > 10 mmHg (25%). This study was followed by a larger double-blind RCT performed in 512 Vietnamese children with DSS, in which patients received Ringer's Lactate or 6% dextran 70 or 6% Hydroxyethyl starch (HES) 200/0.5 for resuscitation over 2 hours, 15ml/kg/h in the first hour then 10 ml/kg/h in the second hour. However, enrolment was stratified by initial severity, with patients presenting with profound shock (PP <=10 mmHg) not eligible to receive the crystalloid solution. This study showed that a balanced crystalloid solution was as effective as either the dextran or the starch-based colloid for initial resuscitation of children with moderately severe DSS (Wills B. A. et al. 2005). Among those with profound shock, the two colloid solutions performed similarly, although more adverse effects were observed in the dextran group. Since then, no research has been carried out to formally evaluate the effectiveness of the overall WHO fluid management guidelines, or to investigate optimal regimens for decompensated or recurrent shock; currently such cases are typically managed with plasma and/or synthetic colloids with the choice based mainly on local expert opinion.

On the other hand, during this time a series of major publications from high-income settings has highlighted a number of adverse events associated with the administration of synthetic colloid solutions for volume expansion (Myburgh J. A. et al. 2012, Perner A. et al. 2012). An RCT involving 798 adult ICU patients with severe sepsis, comparing the use of 6% HES 130/0.42 and Ringer's acetate for resuscitation, showed that HES increased 90-day mortality and the use of renal replacement therapy (RRT) (Perner A. et al. 2012). A much larger RCT of

7000 adult ICU patients compared resuscitation with 6% HES 130/0.4 and normal saline. Results of this trial showed that RRT was required more frequently among patients who received HES although the mortality rates were similar between the two groups (Myburgh J. A. et al. 2012). Since then many systematic reviews and meta-analyses have concluded that the use of HES increases mortality, acute kidney injury (AKI), need for RRT, and/or need for blood transfusion in adult ICU patients requiring volume resuscitation (Gattas D. J. et al. 2013, Haase N. et al. 2013, Zarychanski R. et al. 2013, Serpa Neto A. et al. 2014). The resulting controversy led to an announcement by the European Medicines Agency in 2014 that HES solutions must not be used for critically ill patients with burns or septic shock (European Medicines Agency 2014).

In scenarios when isotonic crystalloids alone are felt to be inadequate for shock resuscitation, albumin solutions are now the preferred choice, often combined with inotropic agents (Xu J. Y. et al. 2014). However, in low/middle-income settings where albumin solutions are prohibitively expensive synthetic colloids continue to be widely deployed as rescue therapy for DSS, in the belief that their usage reduces the overall volume of fluid required and thus minimises the need for respiratory support. Many countries have developed recommendations that are loosely based on the WHO guidelines but vary considerably in the specific details (type of fluid, bolus volume, duration) of the regimens used (Table 4-1). Prolonged colloid therapy for re-shock episodes (4-6 hours versus 1-2 hours) is becoming established in some settings (Pan American Health Organization 2016, Children's Hospital 1 2019, Vietnam Ministry of Health of 2019), potentially increasing the risk for adverse effects on renal function and coagulation disturbances.

Table 4-1. Examples of the different fluid strategies used for DSS management in selected Asian countries

	Malaysia	Myanmar	The Philippines	Thailand, Sri Lanka, India	Vietnam
Preferred crystalloid	- 0.9% saline - Ringer's Lactate - Hartmanns	- 0.9% saline	- 0.9% saline - Ringer's Lactate - Sterofundin - Plasmalyte	- 0.9% saline with/without dextrose 5%	- Ringer Lactate - 0.9% saline
Suggested Colloids *	- 3% Gelatin - HES (no details) - 5% Albumin	- 3% Gelatin - Platelet-rich plasma	3%, 6%, 10% Hetastarch 6%, 10% Pentastarch 6% Tetrastarch 6%, 10% Dextran 70 3% Dextran 60 2.5-4% Gelatins (various)	- 10% Dextran 40 - Gelatins (no details) - 6% HES (various) - 5% Albumin	- Dextran 40 (no details) - Dextran 70 (no details) - HES (no details)
Initial fluid rate for compensated shock	10 ml/kg	10 ml/kg	10 ml/kg	10 ml/kg	15-20 ml/kg
Duration of colloid use for shock/reshock	2 hours	2 hours	2 hours	1-2 hours	3-6 hours

* The colloid choice depends on the availability of fluids in each country. Preferred solutions in Malaysia, Thailand are listed in order. In other countries there are no specific recommendations on concentration and/or molecular weight for the different colloids.

Another challenge for clinicians is the management of DSS in high-risk groups, including infants, the elderly, pregnant women, obese individuals and patients with comorbidities (Paixao E. S. et al. 2018, Werneck G. L. et al. 2018, Zulkipli M. S. et al. 2018, Maneerattanasak S. and Suwanbamrung C. 2020, Macias A. E. et al. 2021). Several local guidelines mention that high-risk groups should be closely monitored as they may develop severe complications, but typically no details for specific management are provided, meaning that clinicians must manage such patients on a case-by-case basis.

4.1.2 The need for evidenced-based strategies for DSS fluid management

In summary, management recommendations for DSS vary considerably in different dengue-endemic countries. The protocols used for paediatric DSS in Asian countries, which carry the highest burden globally, differ in terms of recommended initial fluid type and rate of administration, as well as the type of colloid and duration of use suggested for profound or recurrent shock. These different protocols for management are based primarily on local expert opinion, aiming to reduce the occurrence of re-shock episodes as well as the development of respiratory distress and mortality, but with limited formal research evidence to support the recommendations. Many clinicians tend to increase the volume of fluid given or prolong the use of colloids, in order to decrease the reshock rate. However, this may exacerbate plasma leakage, which in turn results in respiratory distress, increasing the need for high-level ventilatory support and potentially resulting in a high fatality rate. Conversely, the use of low fluid volumes and infusion rates may not resolve shock and the ongoing cardiovascular compromise may lead to multi-organ failure. There is a clear need to explore how different fluid management strategies affect outcomes of paediatric DSS, ideally via a large randomised controlled trial platform involving multiple centres in dengue-endemic settings, to provide robust and generalisable evidence that is relevant to the population most at risk. However, such trials are complex and expensive to perform. As a preliminary step to engage with clinicians across relevant Asian countries and facilitate the development of a formal trial protocol, the

aim of this study was to carry out a large scale review of current fluid management for paediatric DSS across multiple centres of excellence in Asia.

4.2 Objectives

In this study, I aimed to describe the fluid management strategies actually used for paediatric DSS at different centres of excellence across Asia and to investigate the potential effects of these strategies, in particular the use of colloid versus crystalloid solutions, on important outcomes.

4.3 Methods

4.3.1 Study design

This was a large-scale retrospective study in which I and a small team of interested clinicians reviewed the in-patient files of sequential patients discharged with a diagnosis of DSS from selected centres of excellence across Malaysia, Myanmar and Vietnam, and subsequently used the de-identified data to explore relationships between fluid use and outcome. Ethical approval for the retrospective file review was obtained from the appropriate Ethics Committee at each participating hospital.

Potential cases were identified from the central electronic hospital register, if available, or from direct examination of relevant ward admission/discharge record books. In hospitals with a very high case burden, we limited the case selection to one or two specialist dengue wards, plus the high dependency or intensive care units at each facility. Subsequently, the in-patient records of all potential cases were obtained and reviewed by one of the study clinicians to confirm the discharge diagnosis and apply the exclusion criteria.

4.3.2 Sample size

There was no formal sample size calculation as this was the first study to collect detailed information on intravenous fluid use for DSS across multiple countries; we anticipated that each hospital would manage patients differently and that the clinical definitions for the various outcomes of interest might also be different. To gain a representative overview of paediatric

cases managed within the 3 countries, we attempted to gather information on all sequential cases managed at the designated hospitals over 1 or 2 dengue seasons. Dengue seasonality varies geographically so we selected overlapping years in the different countries: Malaysia (4 hospitals in Kuala Lumpur, 2015-2016); Myanmar (1 hospital in Yangon, 2016); and Vietnam (2 hospitals in Ho Chi Minh City, 2016-2017).

4.3.3 Inclusion criteria

We selected the records of all patients sent home from the selected wards in the designated hospitals in the three countries with a diagnosis of dengue shock syndrome at discharge (ICD code A91.c1, A91.c2).

4.3.4 Data collection instrument

I designed a unique case report form (CRF) to extract the following information from all cases identified: clinical history and baseline severity indicators at presentation with DSS; all therapeutic interventions throughout the hospital course, in particular, detailed information on all intravenous fluids given (timing, type, rate of administration); all cardiovascular observations from the onset of DSS for up to 72 hours or until discharge or death (in most centres pulse and blood pressure were measured every 1-2 hours until stable, depending on staff capacity, then with gradually reducing frequency over 24 hours); all adverse events including occurrence and timing of episodes of reshock, development and severity of bleeding, respiratory distress, organ impairment; and final outcome. A unique code number was assigned to each record to maintain anonymity. In each country, a small team of dengue-experienced doctors and nurses were trained to extract the information, closely supervised by myself and a core Vietnamese study team. CRFs were subsequently transferred to HCMC where they were reviewed for completeness and query validation with site staff, before data entry into a secure electronic database designed to maintain patient and site anonymity.

4.3.5 Exclusion criteria

In Vietnam adolescents aged 16 or more are admitted to adult wards. We therefore excluded all records for patients aged >16 years across the 3 countries, as well as referred cases who had received fluid resuscitation before admission to the designated hospital and also files with incomplete or conflicting information on fluid management.

4.3.6 Classification of fluid types and fluid management strategies

Each hospital had its own general guidelines for immediate (1-2 hours) management of DSS, typically informed by a rapid severity assessment performed by junior medical staff. Subsequent management was determined by the response to the initial fluid resuscitation and usually involved consultation with senior staff. Different treatment patterns were apparent at the different centres, particularly in the preference for early versus late intervention with colloids, and the length of time that colloids were given after achieving cardiovascular stability.

To gain insight into the possible effects of these different colloid/crystalloid treatment regimens on outcomes we classified the strategies applied for each individual in two ways: a) Immediate Response Strategy: either crystalloid alone or mixed crystalloid and colloid fluids, given during the first 2 hours after DSS onset; and b) General Management Strategy: for apparently stable patients (those who improved within 2 hours and did not develop re-shock within a 6-hour window from DSS onset) we categorised the fluid therapy given within this 6 hour period as follows – Group 1, crystalloid only; Group 2, crystalloid plus use of any colloid for up to 4 hours; and Group 3, continuous use of a colloid solution for more than 4 hours. We used these 3 groupings as a proxy for the physicians' attitudes towards continuing use of colloid in cardiovascularly stable patients.

4.3.7 Outcome definitions

The main outcomes of interest were the occurrence of a) re-shock and b) respiratory compromise, more than 6 hours after recovery from the initial episode of shock. We also assessed the time to initial shock recovery, the number and timing of re-shock episodes, and the time to final shock recovery – i.e. the time to achieve stable hemodynamic status (no further

re-shock episodes) while receiving zero or low dose IV fluids and without inotropic support. Definitions for all outcomes, all of which were derived by applying algorithms to the electronic dataset, are presented below:

- Re-shock was defined as an episode of cardiovascular decompensation recorded by the attending clinician in the hospital file, that occurred at least 6 hours after stabilization following the previous episode and resulted in a change in management.
- Initial shock recovery time was defined as the time from DSS onset to first achieving cardiovascular stability. Criteria of stable vital signs (PP >= 25mmHg and SBP >= 80 (age <= 5) or SBP >= 90 (age > 5)) for at least 6 hours, without use of inotropes. A single low measurement (PP < 25 or low SBP) was accepted within the 6 hour period provided there was no change in the intravenous fluid regimen used.
- Final shock recovery time was defined as the time from DSS onset to achieving sustained cardiovascular stability – i.e stable vital signs (PP, SBP limits as above) without subsequent cardiovascular deterioration, with an intravenous infusion fluid speed <=4 mL/kg/h at this time and subsequently, and with no inotropes or whole blood or packed red cells given after this time.
- Respiratory distress was defined as one or more of the following signs recorded in the file – shallow breathing, retractions, grunting, nasal flaring, accessory muscle use – usually with an increased respiratory rate for age
- Respiratory compromise was defined as any of the following: a) one or more signs of respiratory distress recorded in the file together with an increase in nasal oxygen flow; or b) need for respiratory support with NCPAP or c) need for mechanical ventilation.
- Acute kidney injury was defined if any creatinine level was more than 1.5 times the upper limit of normal (ULN) for age. ULN for age: <=1 year: 35 umol/L; 1-2 years: 44 umol/L; 3-10 years: 61 umol/L; 11-16 years: 79 umol/L. Premorbid creatinine values were not available.

- Severe liver impairment was defined as any AST or ALT level ≥1000 IU/L. Premorbid liver enzyme values were not available.
- Severe bleeding was defined as any bleeding during the illness course which resulted in shock, and/or necessitated a blood transfusion or an intervention to stop bleeding (eg nasal packing), and/or involved a critical organ (brain, spinal cord), and/or resulted in death.

4.3.8 Economic evaluation

We also assessed the relative cost of different fluid management strategies. For this, we focused only on Vietnam as the costs of the various interventions were the same at the different study sites. For each patient we included all expenditure for bed costs, routine laboratory tests, intravenous fluids, blood products, respiratory support and antibiotic therapy, using the Vietnamese MoH pricelist 02/2017/TT-BYT issued in March 2017. We did not include the costs for nursing services, physician services, extra laboratory tests, food services and indirect costs. Prices were summarised in Vietnamese Dong and then converted to USD (Table 4-2).

Table 4-2. Cost of fluids, procedures and therapeutic agents used in the management of paediatric dengue shock syndrome cases in Vietnam, in USD*

	Dosing Form	Price in USD
Resuscitation Fluid		
Lactated Ringer's	500ml/bottle	0.4
Dextrose Saline	500ml/bottle	0.4
Sodium Chloride 0.9%	500ml/bottle	0.3
Sodium Chloride 3%	100ml/bottle	0.3
Albumin 20%/100ml	100ml/bottle	73.8
Glucose 5%	500ml/bottle	0.3
Glucose 10%	250ml/bottle	0.4
Glucose 30%	250ml/bottle	0.5
Refortan 6% 200/0.5	500ml bottle	6.2
Gelofusin	500ml bottle	4.7
Voluven 6% 130/0.5	500ml bottle	8.3
Blood products		
PRBC from 350ml whole blood	175ml/bag	43.6
Whole blood 250ml	250ml/bag	38.8
Concentrated Platelet - 6 units	320ml/ 6 units	176.5
Cryoprecipitate 50 ml	50ml/bag	16.0

	Dosing Form	Price in USD
Fresh Frozen Plasma 150ml	150ml/bag	7.9
Sedation drugs		
Midazolam 5mg/1ml	5mg/1ml	0.7
Fentanyl 100ug/2ml	100ug/2ml	0.4
Antibiotics		
Oral Antibiotics		
Amoxcillin/clavulanic acid	1200mg	1.9
Amoxycillin (Flamox)	1500mg	3.0
IV Antibiotics		
Cefotaxime 1g	1000 mg	2.8
Cefepime 1g	1000mg	0.9
Ceftriaxone	1000 mg	8.1
Ceftazidime 1g	1000 mg	3.4
Colistin 1MU	1MU	16.0
Ciprofloxacin	500mg	11.0
Clindamycin 0.6g	600mg/4ml	4.7
Oxacillin	1000 mg	0.7
Cefoperazone/sulbactam	1000mg	2.1
Gentamicin	80 mg	0.1
Acyclovir IV 250mg	250 mg	15.6
Cloxacillin 1g	1000 mg	0.7
Meropenem 500 mg	500 mg	20.8
Metronidazole 500mg/100ml	500 mg	0.4
Ampicillin/sulbactam 1g/500mg	1500 mg	3.0
Imipenem	500 mg	15.0
Levofloxacin 500mg/100ml	500 mg	5.5
Tazocin 4g/0.5g	4.5g	10.0
Vancomycin 1g	1000 mg	4.7
Amikacin 500mg/2ml	500 mg	3.4
Inotropes		
Adrenaline 1mg/1ml	1mg	0.3
Dobutamine 250mg/20 ml	250mg	2.6
Dopamine 200 mg/5ml	200mg	0.9
Noradrenaline 4mg/4ml	4mg	3.1
Support for complications		
Nasal prong	1 hour	0.1
Mask	1 hour	0.1
NCPAP	1 day	23.8
Mechanical ventilation	1 day	23.8
Intubation	1 time	24.8
Arterial line procedure	1 time	6.0
Hemofiltration	1 time	282.4
Duosol/Hemosol	1 bag	29.1
ICU bed	1 day	28.3

	Dosing Form	Price in USD
Emergency bed	1day	15.0
Laboratory tests		
CBC		2.0
Hct - bedside		0.8
Dextrostix		1.0
AST		0.9
ALT		0.9
BUN		0.9
Creatinine		0.9
Na,K,Cl		1.3
Albumin		0.9
Blood gas		9.5
Arterial Lactate		4.3
Coagulation screen (aPTT, PT, Fib, TT)		10.7
Dengue NS1		5.6
Ultrasound		2.2
Chest Xray		3.1

** 1 USD = 22370 VND (exchange rate in 2017)*

ICU bed cost is allocated for DSS patients requiring CPAP or mechanical ventilation during their hospitalization. Emergency bed cost is allocated for DSS patients who did not require support with CPAP or mechanical ventilation during their hospitalization.

4.3.9 Statistical analysis

Descriptive statistics are presented as median and interquartile range (IQR) for numeric variables, and frequency and percentage for categorical variables.

We used Cox proportional hazard models to compare the time-to-event outcomes across the various fluid intervention groups – i.e. time to initial shock recovery, time to first re-shock episode, and time to final shock recovery. For binary outcomes such as the development of respiratory compromise, severe bleeding, and severe liver impairment, we used logistic regression models, while negative binomial regression models were used for numeric outcomes such as the number of re-shock episodes and length of hospital stay. Hazard ratios, odds ratios, and risk ratios, together with their 95% confidence intervals, are reported for the time-to-event, binary, and numeric outcomes, respectively.

In recognition of the fact that the patient's initial severity at presentation with DSS is highly likely to influence management decisions, we developed an inverse probability weighting (IPW) score to adjust for the potential lack of equivalence between patients. This type of weighting can reduce confounding and some forms of selection bias as described by Robins in 1998 (Robins J. M. et al. 2000). For the immediate response strategy, we used a logistic regression model to calculate the IPW, incorporating variables that are recognized in the international literature as indicators of DSS severity (World Health Organization 2009). The nine input variables we included were age, body weight and day of illness, plus heart rate, systolic blood pressure, pulse pressure, hematocrit level, platelet count and respiratory compromise, all assessed at onset of DSS. For the general management strategy, we used all the previous variables plus four additional variables (maximum heart rate, minimum systolic blood pressure, minimum pulse pressure, and maximum hematocrit, all within the first 6 hours) to calculate the IPW. Confidence intervals were calculated using a robust sandwich-type variance estimator. All cases with missing data for any of the variables selected for the IPW calculations were excluded from the adjusted analyses.

All the analyses were performed with R version 3.4.1. The IPW calculations were done with the 'ipw' package.

4.4 Results

4.4.1 Process of file screening

Data were extracted from a total of 915 files among the 927 potential DSS cases identified from the initial screen of hospital records (Figure 4-1). For this analysis I focused on children with DSS managed throughout at the same facility, excluding adults, referred cases, and patients for whom data were incomplete or conflicting. Thus the patient population used for the analysis of immediate response strategy comprised 691 cases. Among these patients, 79 did not achieve cardiovascular stability within 2 hours and 65 decompensated again within 6 hours, leaving 547 children in the analysis population for evaluation of general management strategy.

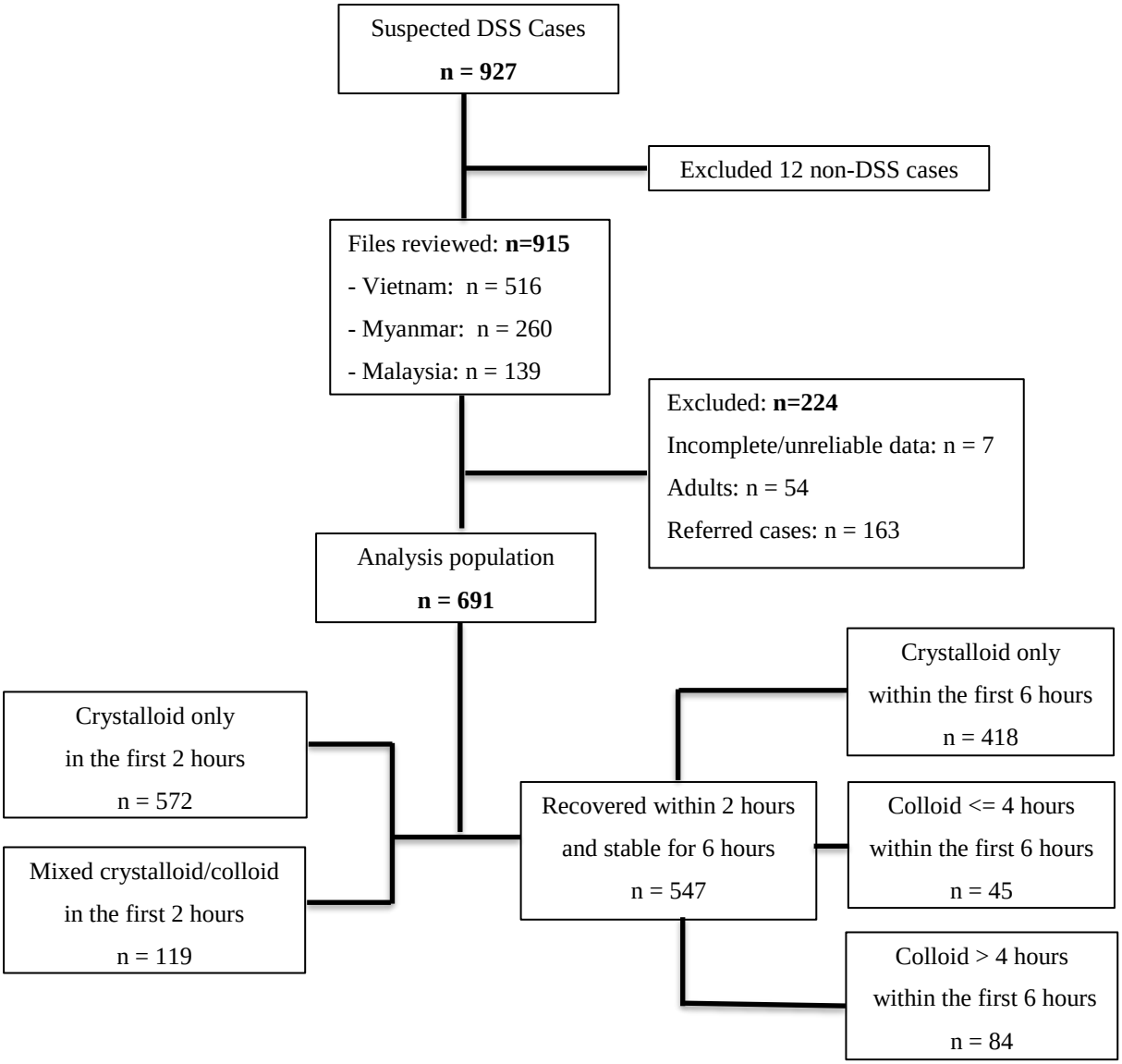


Figure 4-1. Study flowchart

4.4.2 Quality of the data

Baseline characteristics

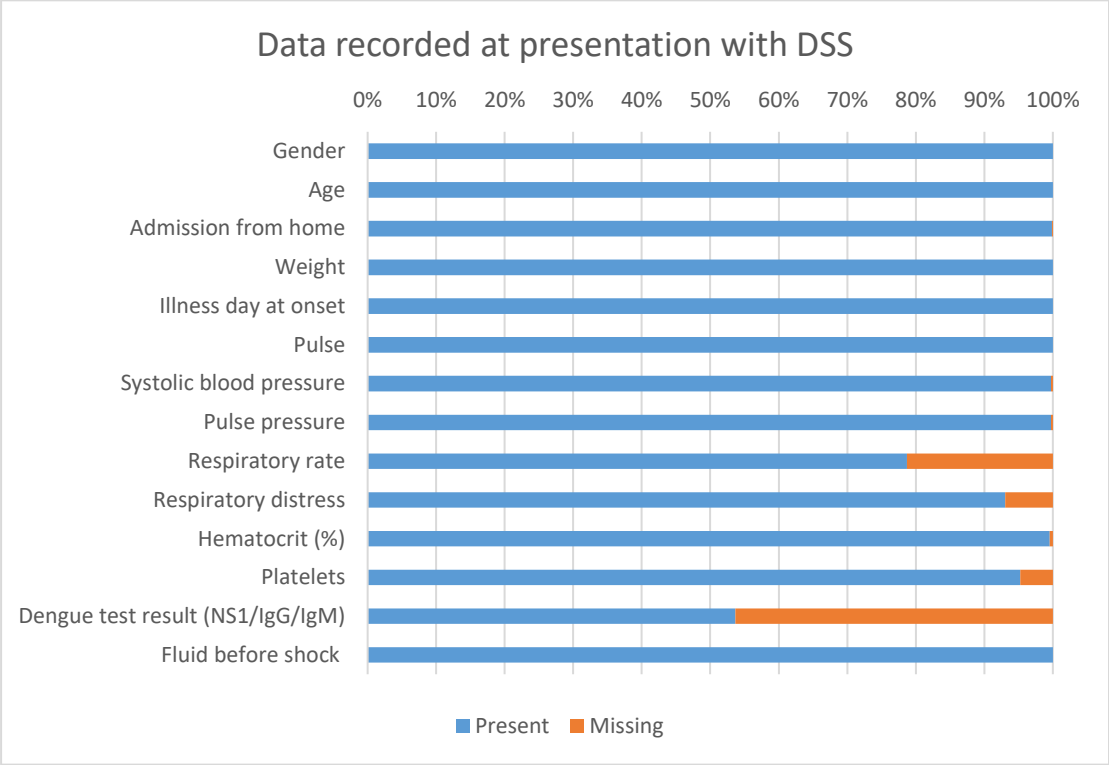
Data for most baseline characteristics were available apart from information on respiratory rate, respiratory distress and the results of confirmatory dengue diagnostics (Figure 4-2).

In dengue-endemic countries, the diagnosis of DSS is usually made clinically, especially when patients arrive at hospital with a typical history, symptoms and signs. Diagnostic tests are frequently not available (especially during epidemics), may be expensive (with the cost generally borne by the family), and tend to be used primarily for atypical DSS cases when clinicians are considering alternative diagnoses. A 10-year prospective study at one hospital (HTD) in Vietnam has demonstrated that among unselected patients undergoing laboratory diagnostic testing, a clinical diagnosis of DSS was correct in 95% cases (Lam P. K. et al. 2013).

Patients with dengue shock are typically alert and, unlike septic shock, rarely have overt signs of respiratory distress; thus the focus of clinical staff in busy centres (where 5-10 DSS cases may present each day during the season) is directed toward recording cardiovascular parameters rather than respiratory rate. However, data were missing on blood pressure in a small number of cases at presentation, due to the patients being profoundly hypotensive on arrival with no measurable blood pressure. Information on presence/absence of respiratory distress was missing in 48 cases, but none of these patients received any respiratory support during their hospital stay, and all recovered and were discharged home.

Haematocrit (Hct) measurements are an essential part of the assessment of DSS cases, as they reflect how severe haemoconcentration is and are routinely used to assess the effects of fluid therapy. Meanwhile, platelet measurements are often thought of in terms of the risk for severe bleeding, which is uncommon in children (Rosenberger K. D. et al. 2016, Paz-Bailey G. et al. 2022). Thus Hct values were almost invariably recorded (only 3 missing values) while platelet values were missing from the files in 33 cases.

Figure 4-2. Data availability for baseline characteristics recorded at presentation with shock for the 691 DSS patients included in the analysis.



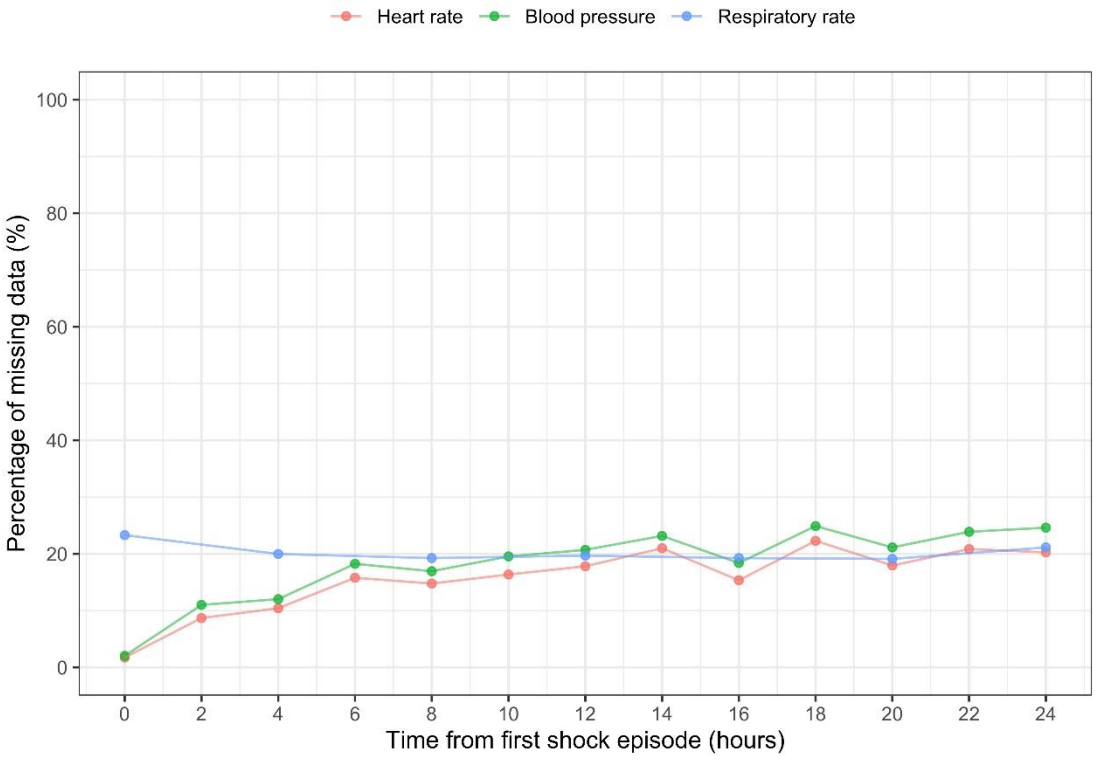
Vital signs data recorded during the initial period of shock management

The proportion of missing data during the first 24 hours of observation after presentation with shock is shown in Figure 4-3, summarized in 2 hourly intervals (-1/+1) for pulse and blood pressure and 4 hourly intervals (-2/+2) for respiratory rate. These intervals were selected as being broadly representative of clinical practice across the 3 countries. Although in many centres the recommendation is for hourly observations for all DSS cases until cardiovascular stability is achieved, in practice the capacity for frequent monitoring is determined by workload and staff availability. Therefore, depending on the monitoring capacity at each study centre, the time interval for pulse and blood pressure measurement was from 1 hour to 4 hours, while the time interval for respiratory rate monitoring was typically every 3-4 hours.

As shown in the figure, heart rate and blood pressure values were recorded at least every 2 hours in around 90% of cases initially, falling to around 80% of cases from 6 hours after shock

onset, remaining around that level for the rest of the 24 hour period. Respiratory rates were recorded less frequently, and were missing in around 20% of cases during each 4-hour interval.

Figure 4-3. Missing data for heart rate, blood pressure and respiratory rate during the first 24 hours from shock onset.



Outcome data

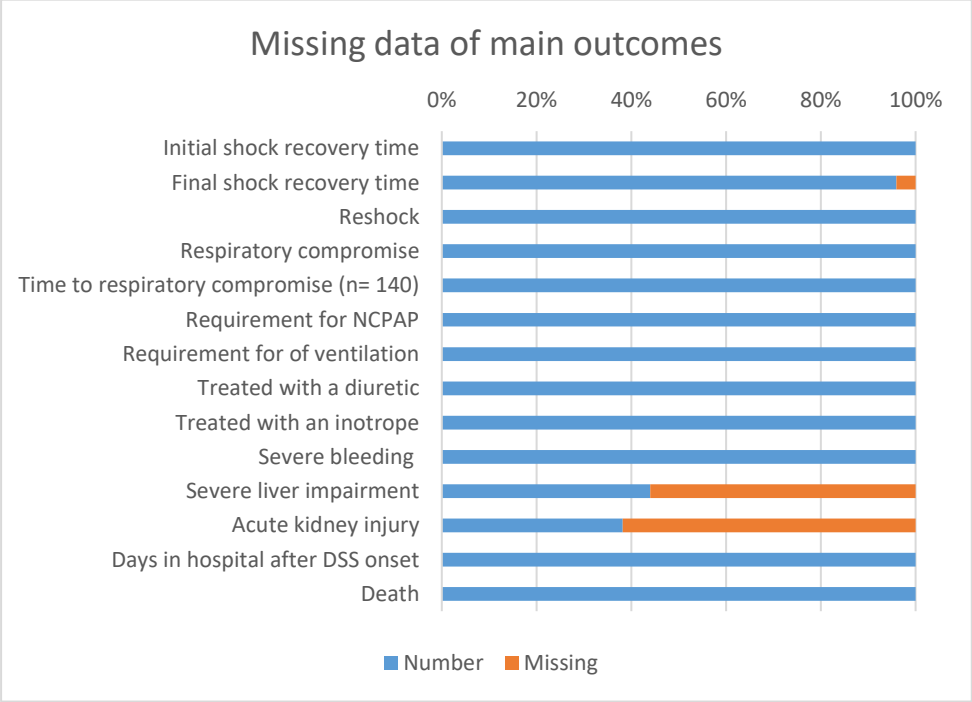
Overall, the amount of missing data for the outcomes of interest was small, apart from for severe liver impairment and acute kidney injury, suggesting that the associations identified with the different fluid strategies used were reasonably robust despite the retrospective nature of the study.

In addition to death, major outcomes such as development of reshock, respiratory distress or severe bleeding are easily recognized by experienced clinicians and considered important enough to be clearly recorded in the hospital file. However, to derive the outcome "final shock recovery time", a number of criteria must be satisfied: survival, normal SBP for age, pulse pressure ≥ 25 mmHg, no reshock, no blood transfusion, no inotrope use, and fluid speed ≤ 4

ml/kg/h after that time point. For 28 patients (4%) it was not possible to satisfy all these criteria and thus this outcome is missing; aside from the 11 deaths, in many cases this was because very low dose inotropes or IV fluid at 4 ml/kg/h were maintained to the end of 72-hour- data collection.

More than 50 % of data on acute kidney injury (AKI) and severe liver impairment are missing (Figure 4-4). In children, the most common complication of dengue infection is severe plasma leakage progressing to hypovolaemic shock but organ impairment is unusual, except in severe cases with profound or prolonged shock. Therefore, in many situations, clinicians do not routinely check renal or liver function. Liver transaminase and creatinine are tested in complex cases with decompensated shock, prolonged shock or repeated reshock, which explains the high proportion of missing data on AKI and severe liver impairment.

Figure 4-4. Missing data for the main outcomes of interest among the 691 patients included in the final data analysis



4.4.3 Fluid management for individual children during the first 72 hours

Individual fluid management for all 691 children over the first 72 hours from shock onset is presented in Figure 4-5, demonstrating the wide range of fluid strategies given in terms of fluid type, rate and duration.

About half of the patients received crystalloids for the whole period but 380/691 (55%) received a colloid infusion at some point during this time. The latter group also received blood more frequently. Some patients (25/691) received a continuous colloid infusion for more than 24 hours.

4.4.4 Baseline characteristics of paediatric patients with dengue shock syndrome

Baseline characteristics at onset of DSS were similar across the groups (Table 4-3a and Table 4-3b). There was no gender imbalance, and the majority of children developed shock on day 5 of illness.

The width of pulse pressure is associated with severity of DSS in children. A study of 230 Vietnamese children with DSS showed that pulse pressure ≤ 10 mmHg was associated with profound shock and worse outcomes than DSS patients with pulse pressure > 10 mmHg (Ngo N.T.et al 2001). Therefore we classified patients with PP ≤ 10 mmHg as profound shock/severe DSS. At the first shock episode 587/691 (85%) patients were diagnosed with compensated shock with PP>10 mmHg, while the remaining 102 children were diagnosed with profound shock (PP<10mm Hg). Nine cases (1%) presented with respiratory distress at the first shock. The median hematocrit level was 47% (IQR 44-50) in the group that received crystalloid only and 49% (IQR 45-52) in the group that received mixed fluid for initial resuscitation. The median platelet counts were 34,000 and 28,000 per microliter respectively, for the two immediate response groups. Among the 371 patients for whom dengue diagnostic tests were performed, 347 cases (94%) were positive. Among all 691 patients, 91 (13%) had received some parenteral fluid before the onset of shock, with a median volume of 13 ml/kg (IQR 4-40) in the crystalloid only group versus 20 ml/kg (IQR 5-66) ml/kg in the mixed fluid group (Tables 4-3a and 4-3b); in 5 children colloids were included in the pre-shock fluid treatment.

Severe liver impairment and acute kidney injury were recorded in only a small number of cases: in all 28 patients with severe liver involvement and in 5/7 cases with acute kidney injury the abnormalities were already noted at presentation with shock.

Figure 4-5. Overview of fluid use among all 691 children with dengue shock syndrome.

The vertical axis indicates the number of patients, with each line representing all the fluid used for one patient. On the horizontal axis, time=0 is the time of onset of DSS. Blood includes whole blood and blood products (packed red cells, plasma, cryoprecipitate, platelets, fresh frozen plasma, and platelet-rich plasma when used for coagulation/bleeding problems). Colloid solutions include HES, gelatin, albumin and platelet-rich plasma when used for volume expansion. Crystalloids include Ringer's Lactate, 0.9% saline, 5% dextrose in 0.9% saline, Sterofundin, and Hartmann solution. Other fluids include glucose 10% -30% with or without added electrolytes, nutritional support etc.

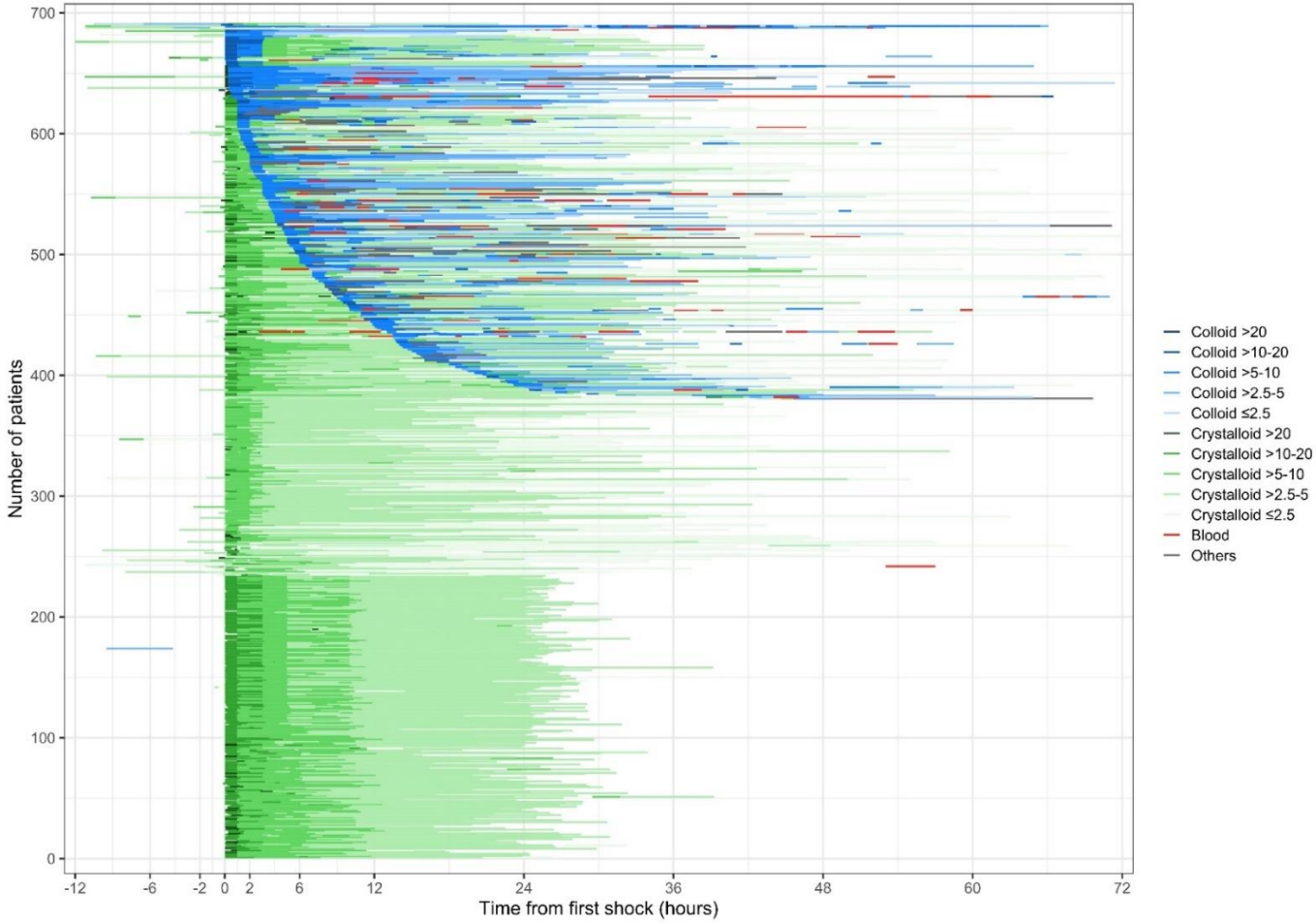


Table 4-3a. Baseline characteristics of children with DSS classified by the choice of fluid during the first two hours (immediate response strategy).

Characteristics	Immediate Response Strategy			
	n	Crystalloid only N=572	n	Mixed fluid N=119
Gender (Male)	572	286 (50)	119	62 (52)
Age (years)	572	9 (5, 11)	119	8 (5, 10)
Admission from home	572	521 (91)	118	98 (83)
Weight (kg)	572	27 (18, 40)	119	25 (16, 36)
Illness day at onset	572	5 (5, 6)	119	5 (5, 6)
Pulse	572	120 (107, 130)	119	122 (116, 140)
Systolic blood pressure	571	90 (85, 100)	118	90 (80, 100)
Pulse pressure	571	20 (20, 20)	118	20 (5, 20)
- PP<=10		54 (9)		48 (41)
- PP>10		517 (91)		70 (59)
Respiratory rate	441	25 (22, 28)	103	28 (24, 30)
Respiratory distress	532	5 (1)	111	4 (4)
Hematocrit (%)	570	47 (44, 50)	118	49 (45, 52)
Platelets (K/uL)	540	34 (19, 53)	118	26 (18, 44)
Dengue test positive (NS1/IgG/IgM)	286	268 (94)	85	79/85 (93)
Received fluid before shock	572	63 (11)	119	28 (24)
- Crystalloid		57 (98)		24 (86)
- Colloid, Crystalloid		1 (2)		4 (14)
- Volume of pre-shock fluid (ml/kg)	58	13 (4, 40)	28	20 (5, 66)

Summary statistic is absolute count (%) for categorical variables and median (inter-quartile range) for continuous data

Table 4-3b. Baseline characteristics of children with DSS classified by the choice of fluid in the first 6 hours (general management strategy)

	General Management Strategy					
Characteristics	**n**	**Crystalloid only (n=418)**	**n**	**Colloid <=4h (n=45)**	**n**	**Colloid >4h (n=84)**
Gender (Male)	418	210(50)	45	21 (47)	84	52 (62)
Age (years)	418	9 (6, 12)	45	9 (7, 11)	84	8 (4, 10)
Admission from home	418	389 (93)	45	38 (84)	84	64 (76)
Weight (kg)	418	29 (19, 41)	45	28 (20, 38)	84	26 (16, 37)
Illness day at onset	418	5 (5, 6)	45	5 (5, 6)	84	5 (5, 6)
Pulse	418	120 (105, 130)	45	120 (110, 136)	84	121 (114, 144)
Systolic blood pressure	417	92 (90, 100)	45	90 (80, 100)	83	90 (80, 100)
Pulse pressure	417	20 (20, 20)	45	15 (5, 20)	83	20 (15, 25)
- PP<=10		29 (7)		25 (56)		16 (19)
- PP>10		388 (93)		20 (44)		67(81)
Respiratory rate	331	24 (22, 28)	39	24 (20, 28)	79	26 (23, 30)
Respiratory distress	400	1 (0)	42	1 (2)	82	4 (5)
Hematocrit (%)	417	47 (44, 50)	45	49 (46, 52)	84	46 (44, 51)
Platelets (K/uL)	390	34 (19, 55)	45	25 (19, 44)	84	28 (18, 48)
Dengue test positive (NS1/IgG/IgM)	191	176/191 (92)	24	23/24 (96)	52	49/52 (94)
Received fluid before shock	418	42 (10)	45	7 (16)	84	19 (23)
- Crystalloid		34 (97)		7 (100)		13 (87)
- Colloid, Crystalloid		1 (3)		0 (0)		2 (13)
- Volume of pre-shock fluid (ml/kg)	35	19 (5, 39)	8	13 (4, 46)	15	10 (4, 61)

Summary statistic is absolute count (%) for categorical variables and median (inter-quartile range) for continuous data

4.4.5 Effect of different fluid strategies on the outcomes of interest

Immediate response strategy (Table 4-4a and Table 4-5a, Figure 4-6)

There were 572 cases who received crystalloids only and 119 cases who received both crystalloid and colloid fluid within the first 2 hours. The total volume of fluid used in the first 24 hours was similar between the two groups (118 ml/kg (IQR 85-131) in the crystalloid only group, versus 123 ml/kg (IQR 96-143) in the mixed fluid group). However, the total fluid volume used within the first 72 hours was higher for the mixed fluid group (140 ml/kg (IQR 112-175) versus 127 ml/kg (IQR 105-154) in the crystalloid only group). Of notes, some patients in the crystalloid only group received colloids due to prolonged shock or reshock later on, therefore, volume of colloid used within 24 hours or 72 hours was accounted in the crystalloid only group. Although the mortality rate was similarly low in the two groups (9/572 [2%] in the crystalloid only group, versus 2/119 [2%] in the mixed fluid group), other outcomes were generally more favourable in the crystalloid only group in the unadjusted analyses (Table 4-5a). There was a significant difference in initial shock recovery times between the groups (HR 0.80, p = 0.028); final shock recovery time was shorter in the crystalloid only group (11 hours (IQR 9-21) versus 19 hours (IQR 10-28), HR 0.63, p <0.001). Similarly, the reshock rate was higher in the group receiving mixed fluids (21% versus 15%), and the time to the first reshock episode was longer in the crystalloid only group (14 hours (IQR 9-18) versus 10 hours (IQR 8-15), HR 1.54, p = 0.074).

With respect to respiratory outcomes, the mixed fluid group developed respiratory compromise more frequently (38% versus 14%) and earlier (12 hours versus 24 hours, HR 3.44, p <0.001) than the crystalloid only group, and had a greater need for NCPAP and mechanical ventilation (24% versus 4%, and 8% versus 2%, HR for either intervention 5.19, p <0.001). Severe bleeding was also observed more frequently in the mixed fluid group than the crystalloid only group but the difference was not significant. Severe bleeding was apparent at presentation in 2 cases (epistaxis and GI bleeding respectively), one in each group and occurred in the remaining 28 cases after a median time interval of 16 hours (IQR 5-25). In the majority of these cases, severe

bleeding was from the nose or upper GI tract, but a small number of children developed bleeding from multiple sites. In all cases with severe liver impairment, this was noted at presentation with shock. Two of the patients with acute kidney injury (crystalloid only group) were identified on tests obtained the day after initiation of fluid resuscitation, while all others already had impairment at presentation.

I acknowledge that the severity at shock presentation would influence management decisions and outcomes, hence, I used an inverse probability weighting (IPW) score to adjust for the potential lack of equivalence between patients. To calculate IPW score, I applied the logistic regression model for nine variables which are indicators of DSS severity (age, body weight, day of illness, heart rate, SBP, DBP, pulse pressure, Hct, platelet count and respiratory compromise at onset of DSS). After adjusting for differences in baseline characteristics using the IPW score, the following outcomes remained significantly worse among mixed fluid recipients than among crystalloid only recipients: respiratory compromise (HR 2.08, $p = 0.022$); requirement for NCPAP or mechanical ventilation (OR 2.34, $p = 0.045$); and days in hospital after DSS onset (RR 1.33, $p = 0.032$). In Figure 4-6, Kaplan-Meier curves summarise these findings for several of the important outcomes, highlighting the fact that respiratory compromise occurred more frequently and at an earlier timepoint among the mixed fluid recipients.

General management strategy (Tables 4-4b and Table 4-5b, Figure 4-7)

Baseline characteristics and a summary of fluid use and outcomes for the 547 cases included in the general management strategy analysis are presented in Tables 4-4b and 4-5b and Figure 4-7. The total volume of fluid used within 24 hours and 72 hours was the highest for Group 3 patients, i.e. those who received a colloid infusion for more than 4 hours during the 6-hour period used to classify the general management strategy (136 ml/kg (IQR 119-146) and 157 ml/kg (IQR 135-189). Groups 1 and 2 received similar overall fluid volumes within the first 24-hour period, but within the 72 hours window Group 2 patients received greater volume of

fluid compared to Group 1 patients (145 ml/kg (IQR 121-174) versus 125 ml/kg (IQR 105-143))

Individuals in Group 1, crystalloid only, were shown to have the shortest time to achieve final recovery (10 hours (IQR (9.17)); however all three deaths occurred in this group. Meanwhile final recovery times in Group 2 and Group 3 were 23 hours (IQR (11, 29)) and 15 hours (IQR (10, 26)), respectively. Notably, the reshock rate was lowest in Group 3, with a rate of only 1% in this group versus 13% and 36% in Groups 1 and 2 respectively. When compared with Group 1 (crystalloid only), the risk of developing reshock was higher among Group 2 patients (OR 3.8, $p<0.001$) but lower among Group 3 patients (OR 0.08, $p = 0.014$). Similarly, no individual in Group 3 experienced a second episode of re-shock, compared to 2% in Group 1 and 11% in Group 2. The risk ratios for development of multiple episodes of reshock in Group 2 and Group 3 patients versus those in Group 1 were 3.05 ($p<0.001$) and 0.08 ($p = 0.012$), respectively.

Conversely however, individuals in Group 3 showed significantly worse outcomes for all respiratory parameters assessed: respiratory compromise – 34/84 (40%) in Group 3, versus 26/418 (6%) in Group I and 8/45 (18%) in Group 2; need for respiratory support – 33/84(40%) in Group 3 versus 14/418 (3%) in Group 1 and 2/45 (5%) for Group 2; treatment with a diuretic – 40/84 (48%) in Group 3, versus 20/418 (5%) in Group 1 and 4/45 (9%) in Group 2. Few children required inotropic support, 7/418 (2%) in Group 1, none in Group 2 and 5/84 (6%) in Group 3.

Using the IPW score to adjust for differences in baseline severity, the following outcomes remained significantly different when comparing Group 3 to Group 1: final shock recovery time (HR 0.73, $p = 0.016$); number of reshock episodes (RR 0.13, $p = 0.043$); development of respiratory compromise (OR 8.84, $p <0.001$); time to respiratory compromise (HR 6.94, $p <0.001$); requirement for NCPAP or ventilation (OR 8.16, $p <0.001$) and days in hospital after DSS onset (RR 1.15, $p = 0.015$). When comparing Group 2 to Group 1 and adjusting for baseline severity, only the final shock recovery time was significantly different (HR 0.72, p =

0.005). The Kaplan-Meier curves presented in Figure 4-7 illustrate differences in the major outcomes visually

Table 4-4a. Summary of fluid use and outcomes for patient groups classified by the choice of fluid in the first 2 hours (immediate response strategy)

Outcome		Immediate Response Strategy		
	n	Crystalloid only (N=572)	n	Mixed fluid (N=119)
Fluid volume within first 24hrs (ml/kg)[&]	572	118 (85,131)	119	123 (96, 143)
Crystalloids	572	97 (72, 118)	116	50 (25, 70)
Colloids	182	43 (924, 75)	119	69 (35, 107)
Fluid volume within first 72hrs (ml/kg)[&]	572	127 (105, 154)	119	140 (112, 175)
Crystalloids	572	116 (84, 130)	116	61 (27, 88)
Colloids	192	55(28, 99)	119	70 (35, 122)
Time to initial shock recovery (hrs)	570	1 (1, 2)	117	1 (1, 2)
Time to final shock recovery (hrs)*	556	11 (9, 21)	107	19 (10, 28)
Reshock	572	83 (15)	119	25 (21)
Time to first reshock episode (hrs)	83	14 (9, 18)	25	10 (8, 15)
Number of reshock episodes	83		25	
- 1		66 (80)		18 (72)
- 2		15 (18)		7 (28)
- >2		2 (2)		0 (0)
Respiratory compromise	572	78 (14)	119	45 (38)
Time to respiratory compromise (hrs)	78	24 (15, 31)	45	12 (6, 22)
Requirement for NCPAP	547	24 (4)	110	26 (24)
Requirement for of ventilation	548	11 (2)	111	9 (8)
Treated with a diuretic	572	53 (9)	119	35 (29)
Treated with an inotrope	572	13 (2)	119	10 (8)
Start time for inotrope use (hrs)	13	20 (8, 42)	10	17 (11, 28)
Duration of inotrope use (hrs)	13	8 (0, 40)	10	106 (20, 200)
Severe bleeding**	572	22 (4)	119	8 (7)
Severe liver impairment***	224	19 (8)	80	9 (11)
Acute kidney injury***	192	6 (3)	72	1 (1)
Days in hospital after DSS onset	572	4 (3, 5)	119	5 (4, 6)
Death	572	9 (2)	119	2 (2)

Summary statistic is absolute count (%) for categorical variables and median (IQR) for continuous data. NCPAP – Nasal continuous positive airway pressure.
[&]Patients with prolonged shock or reshock received colloids, therefore colloids were accounted in the group using crystalloid only in the first 2 hours.* See text for details on missing data (section 4.4.2)** One patient in the crystalloid only group had severe GI bleeding at shock. One patient in the mixed fluid group had severe epistaxis at shock and required nasal packing. In the remaining cases, severe bleeding occured after shock resuscitation had commenced
***Patients with at least one relevant laboratory test (liver transaminase or creatinine) performed at first shock or during DSS treatment are included here; in the majority with abnormalities, these were already present on tests obtained at first shock (see text).

Table 4-4b. Summary of fluid use and outcomes for patient groups classified by the choice of fluid in the first 6 hours (general management strategy)

Outcome		Group 1		Group 2		Group 3
	n	Crystalloid only (n=418)	n	Colloid <=4h (n=45)		Colloid >4h (n=84)
Fluid volume within first 24hrs (ml/kg)	418	118 (85, 127)	45	119 (98, 137)	84	136 (119, 146)
Crystalloids	418	114 (80, 120)	45	70 (61, 82)	82	38 (20, 50)
Colloids	83	32 (14, 51)	45	35 (30, 65)	84	95 (76, 110)
Fluid volume within first 72hrs (ml/kg)	418	125 (105, 143)	45	145 (121, 174)	84	157 (135, 189)
Crystalloids	418	120 (98, 131)	45	90 (80, 106)	82	40 (20, 55)
Colloids	89	48 (21, 71)	45	36 (30, 70)	84	112 (83, 140)
Time to initial shock recovery (hrs)	418	1 (0, 1)	45	1 (1, 2)	84	1 (0, 1)
Time to final shock recovery (hrs)*	411	10 (9, 17)	44	23 (11, 29)	78	15 (10, 26)
Reshock	418	55 (13)	45	16 (36)	84	1 (1)
Time to first reshock episode (hours)	53	12 (9, 16)	16	12 (10, 17)	1	16 (16, 16)
Number of reshock episodes	53		16		1	
- 1		43 (81)		11 (69)		1 (100)
- 2		9 (17)		5 (31)		0 (0)
- >2		1 (2)		0 (0)		0 (0)
Respiratory compromise	418	26 (6)	45	8 (18)	84	34 (40)
Time to respiratory compromise (hours)	26	23 (20, 28)	8	21 (12, 31)	34	19 (11, 25)
Requirement for NCPAP	410	10 (2)	44	2 (5)	82	29 (35)
Requirement for Ventilation	410	4 (1)	44	0 (0)	82	4 (5)
Treated with a diuretic	418	20 (5)	45	4 (9)	84	40 (48)
Treated with an inotrope	418	7 (2)	45	0 (0)	84	5 (6)
Start time for inotrope use (hrs)	7	26 (14, 52)	0	NA	5	17 (14, 29)
Duration of inotrope use (hrs)	7	4 (1, 29)	0	NA	5	185 (27, 186)
Severe bleeding**	418	9 (2)	45	2 (4)	84	4 (5)
Severe liver impairment***	150	7 (5)	29	4 (14)	76	6 (8)
Acute kidney injury***	128	2 (2)	20	0 (0)	76	1 (1)
Days in hospital after DSS onset	418	4 (3, 5)	45	4 (4, 5)	84	5 (4, 6)
Death	418	3 (1)	45	0 (0)	84	0 (0)

Summary statistic is absolute count (%) for categorical variables and median (IQR) for continuous data. NCPAP – Nasal continuous positive airway pressure.

* See text for details on missing data

** One patient in the Group 1 had severe GI bleeding at shock. One patient in Group 2 had severe epistaxis at shock and required nasal packing

***Patients with at least one relevant laboratory test (liver transaminase or creatinine) performed at first shock or during DSS treatment are included here; in all individuals experiencing these two outcomes, the abnormalities were already present on tests obtained at first shock.

Table 4-5a. Associations between immediate response strategy (the choice of fluid in the first 2 hours) and the outcomes of interest

	Unadjusted analysis			Adjusted analysis*		
Outcomes	ES	95% CI	p-value	ES	95% CI	p-value
Time to initial shock recovery[a]	0.80	0.66 - 0.98	0.028	0.85	0.61 - 1.20	0.360
Time to final shock recovery[a]	0.63	0.51 - 0.78	<0.001	0.51	0.24 – 1.07	0.075
Time to first reshock[a]	1.54	0.98 - 2.4	0.060	0.94	0.57 - 1.56	0.819
Number of reshock episodes[b]	1.51	0.94 - 2.37	0.079	0.99	0.59 - 1.62	0.982
Time to respiratory compromise[a]	3.44	2.39 - 4.97	<0.001	**2.08**	**1.11 - 3.88**	**0.022**
Requirement for NCPAP or ventilation[c]	5.19	2.97 - 9.04	<0.001	**2.34**	**1.02 – 5.37**	**0.045**
Severe bleeding[c]	1.80	0.74 – 4.00	0.167	0.57	0.18 - 1.80	0.338
Days in hospital after DSS onset[b]	1.28	1.18 - 1.40	<0.001	**1.33**	**1.03 - 1.73**	**0.032**

The crystalloid only group is the reference group.

[a] The effect size is hazard ratio; [b] The effect size is risk ratio; [c] The effect size is odds ratio.

* All adjusted estimates are derived from models using the inverse probability weighting score. Among the adjusted analyses, statistically significant results are indicated in bold typeface.

Abbreviations: CI – Confidence interval; ES – Effect size; NCPAP – Nasal continuous positive airway pressure.

Table 4-5b. Associations between general management strategy (the choice of fluid in the first 6 hours) and the outcomes of interest

	Unadjusted analysis			Adjusted analysis*		
Outcome	ES	95% CI	P-value	ES	95% CI	P-value
Time to final recovery[a]						
- Colloid <=4h	0.54	0.40 – 0.74	<0.001	**0.72**	**0.57 – 0.91**	**0.005**
- Colloid >4h	0.58	0.46 – 0.74	<0.001	**0.73**	**0.56 – 0.94**	**0.016**
Reshock[c]						
- Colloid <=4h	3.80	1.9 – 7.40	<0.001	0.71	0.19 – 2.64	0.609
- Colloid >4h	0.08	0.25 - 1.22	0.014	0.14	0.02 – 1.07	0.058
Time to reshock[a]						
- Colloid <=4h	3.22	1.84 – 5.63	<0.001	0.73	0.21 – 2.53	0.618
- Colloid >4h	0.09	0.01 – 0.64	0.016	0.15	0.02 – 1.10	0.062
Number of reshock episodes[b]						
- Colloid <=4h	3.05	1.68 – 5.39	<0.001	0.95	0.27 – 3.32	0.939
- Colloid >4h	0.08	0.00 – 0.36	0.012	**0.13**	**0.02 – 0.93**	**0.043**
Respiratory compromise[c]						
- Colloid <=4h	3.26	1.3 - 7.45	0.007	0.62	0.14 – 2.75	0.531
- Colloid >4h	10.25	5.72 - 18.65	0.000	**8.84**	**4.27 – 18.27**	**<0.001**
Time to respiratory compromise						
- Colloid <=4h	3.04	1.38 – 6.72	0.006	0.62	0.15 – 2.60	0.516
- Colloid >4h	8.28	4.97 – 13.81	<0.001	**6.94**	**3.79 – 12.70**	**<0.001**
Requirement for NCPAP or ventilation[c]						
- Colloid <=4h	1.57	0.24 – 6.02	0.561	0.11	0.01 – 1.15	0.065
- Colloid >4h	17.84	8.80 – 38.27	<0.001	**8.16**	**3.00 – 22.17**	**<0.001**
Severe bleeding[c]						
- Colloid <=4h	2.11	0.32 – 8.53	0.348	0.15	0.01 – 1.63	0.120
- Colloid >4h	2.27	0.60 – 7.16	0.181	2.94	1.53 – 16.28	0.216
Days in hospital after DSS onset[b]						
- Colloid <=4h	1.20	1.04 – 1.37	0.010	1.01	0.91 – 1.12	0.847
- Colloid >4h	1.31	1.18 - 1.45	<0.001	**1.15**	**1.03 - 1.29**	**0.015**

The crystalloid only group is the reference group. CI Confidence interval; ES Effect size [a] The effect size is hazard ratio; [b] The effect size is risk ratio; [c] The effect size is odds ratio.*Using inverse probability weighting for the adjusted analyses, 95% confidence intervals were calculated using robust sandwich-type variance estimator. Among the adjusted analyses, statistically significant results are indicated in bold typeface

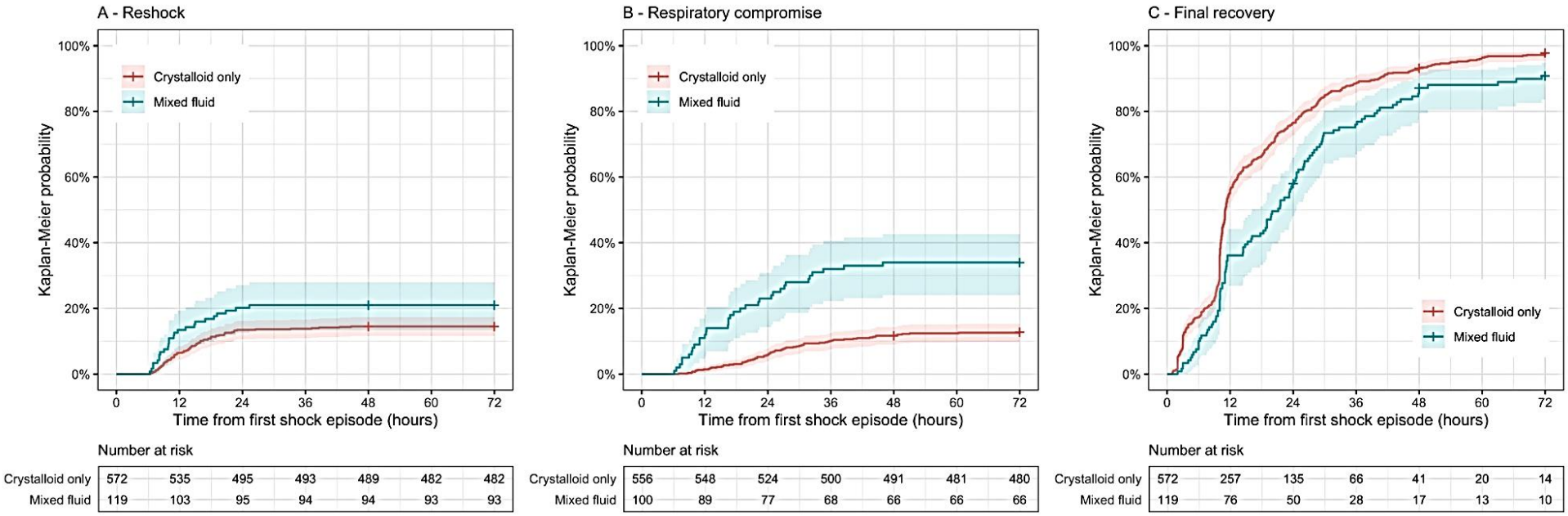


Figure 4-6. Kaplan-Meier curves for three major outcomes with respect to the immediate response strategy (the choice of fluid in the first 2 hours)

Panel A: Time to first reshock episode; Panel B. Time to onset of respiratory compromise; Panel C. Time to final recovery

Note that for respiratory compromise, the number at risk excludes cases with missing data and cases with respiratory compromise within the first 2 hours from shock onset (i.e. within the time-frame of the management strategy).

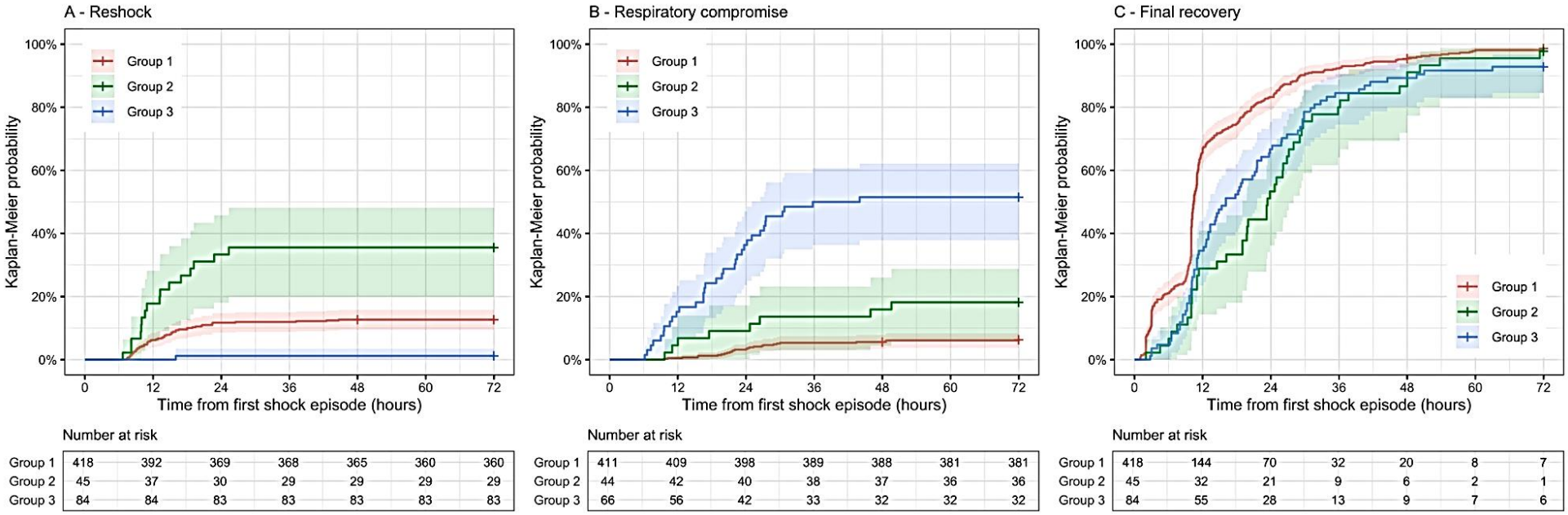


Figure 4-7. Kaplan-Meier curves for major outcomes with respect to the general fluid management strategy (the choice of fluid in the first 6 hours)

Panel A: Time to first reshock episode; Panel B: Time to onset of respiratory compromise; Panel C: Time to final recovery

Group 1: Crystalloid only; Group 2: Colloid ≤ 4 hours; Group 3: Colloid >4 hours.

Note that for respiratory compromise, the number at risk excludes cases with missing data and cases with respiratory compromise within the first 6 hours from shock onset (i.e. within the time-frame of the management strategy of interest).

4.4.6 Cost of different fluid strategies

Categorising by immediate management strategy, the median total cost for the mixed fluid recipients was approximately 1.7 times higher than the median total cost for the crystalloid only recipients (182.8 USD versus 106.2 USD, $p < 0.0001$), a major difference being in the costs of the fluids used (3.5 USD versus 63.8 USD, $p < 0.0001$). When classified by general management strategy, the median total cost for Group 3 patients was 2.3 times higher than Group 1 (223.5 USD versus 98.7 USD), and 1.4 times higher than Group 2 (137.3 USD versus 98.7 USD). The majority of the costs for Groups 2 and 3 were for fluid (45.6 USD and 87.2 USD, respectively) and ICU bed use (60.1 USD and 90.1 USD, respectively) (Table 4-6).

Table 4-7 presents the results after adjusting for baseline severity using the IPW score. For the immediate response fluid strategy, the estimated cost for those in the mixed fluid group remained significantly greater compared to the crystalloid only group (R square 0.398, $p < 0.001$). For the general management strategy analysis, Group 2 and Group 3 also had higher expenditure compare to Group 1 (R square 0.271, $p < 0.001$).

Table 4-6: Comparison of the costs of different fluid strategies used to treat Vietnamese children with DSS in USD (2017 prices)

	Immediate Response Strategy (The choice of fluid in the first 2 hours)			General Management Strategy (The choice of fluid in the first 6 hours)			
Variables	**Crystalloid only n=289**	**Mixed fluid n=62**	**p-value**	**Crystalloid only (n = 258)**	**Colloid <=4h (n = 26)**	**Colloid > 4h (n = 66)**	**p-value**
Lab costs	11.2 (7.9-15.8)	19.5 (12.6-32.4)	<0.0001	10.1 (7.8 15.5)	13.8 (10.0-17.0)	23.6 (14.3-34.3)	< 0.0001
Fluid costs	3.5 (2.4-26.4)	63.8 (43.9-122.7)	<0.0001	3.5 (2.4-4.6)	45.6 (28.1.0-63.8)	87.2 (55.9-122.6)	<0.0001
Respiratory support*	8.0 (6.4-9.7)	8.0 (6.4-41.7)	0.0094	8.0 (6.4-9.7)	6.4 (6.4-9.7)	9.7 (6.4-47.7)	<0.0001
Medication costs[&]	25.3 (8.1-42.2)	33.1 (9.6–98.9)	0.6417	25.3(8.1-42.0)	NA	33.1(12.6-43.8)	NA
Bed costs	75.1 (60.1-90.1)	75.1 (60.1-116.6)	0.0114	75.1 (60.1-90.1)	60.1 (60.1-90.1)	90.1 (60.1-119.2)	0.004
Total costs	106.2 (77.9-137.6)	182.8 (136.0-277.2)	<0.0001	98.7 (77.3-130.5)	137.3 (120.5-171.1)	223.5 (156.9-319.4)	<0.0001

Summary statistic is median (IQR)

NA: Not Applicable. All tests were non-parametric tests.

Fluid costs includes the costs for all colloids, crystalloids, blood and blood products used.

*: Includes costs for oxygen, NCPAP, ventilation. All patients are included here since nasal oxygen is mandatory for children with DSS according to national guidelines

[&]: Includes costs of inotropes, sedation, antibiotics and hemofiltration for patients who received any of these interventions. (n=23)

Table 4-7. Association between the costs of different fluid strategies in Vietnamese children with DSS – the figures are in USD (2017 prices)

Variables	Estimated value	P-value	Adjusted R-squares	P-value
Immediate response strategy (Choice of fluid in the first 2 hours)			0.398	<0.001
Intercept	111.1			
Crystalloid only (Reference)				
Mixed fluid	59.0	< 0.001		
General management strategy (Choice of fluid in the first 6 hours)			0.271	<0.001
Intercept	99.9			
Crystalloid only (Reference)				
Colloid <=4hours	59.7	0.09		
Colloid >4hours	141.1	< 0.001		

Multiple linear regression was used, with the analyses adjusted for severity at baseline using the inverse probability weighting score.

4.5 Discussion

Although WHO guidelines for dengue management have existed for many years and are widely disseminated, here I have described how the fluid strategies actually employed for DSS management vary considerably across dengue-endemic settings in Southeast Asia. The results of the study suggest that use of mixed colloid/crystalloid regimens within the first two hours for initial fluid resuscitation might not provide better outcomes compared to use of crystalloids alone, after adjusting for severity at baseline (age, body weight, day of illness, pulse, blood pressure, Hct, platelet levels and respiratory compromise at shock onset). Secondly, although the use of colloids for more than four hours continuously within the first 6 hours, among children who were cardiovascularly stable at 2 hours, reduced the number of reshock episodes, there was a concomitant increase in the risk of respiratory compromise and the need for ventilatory support. Prolonged use of colloids did not increase the risk of bleeding but did increase the length of hospital stay and the cost of management. These results suggest that clinicians should be more cautious in their use of colloid solutions, especially in settings without access to respiratory support.

4.5.1 Variation in management strategies for DSS - colloids versus crystalloids

The WHO guidelines recommend use of crystalloids for compensated shock and colloids for hypotensive shock, with the suggested duration for colloid use limited to one to two hours for the initial shock episode and for reshock. However, as indicated by this work, many clinicians rely more on their personal experience then the guidelines per se, potentially reflecting the limited availability of formal research to support the guidelines.

During the 1950s-1970s when DSS was first recognized in Bangkok, there was no established guideline for dengue case management; typically whole fresh blood or plasma were used for patients with shock and the case mortality rate was reported to be 14% in Thailand (Siripen K. 1999). The recognition that plasma leakage rather than haemorrhage was the pathognomonic feature of severe dengue resulting in hypovolemic shock, together with development of a standardised protocol for DSS management by the Thai doctors, led to a dramatic reduction in mortality. In many centres of excellence, mortality rates are now below 1% (Lam P. K. et al. 2013, World Health Organization 2022).

Historically, the general principles governing fluid resuscitation developed alongside evolving knowledge of human physiology and vascular biology. Administration of synthetic colloids became an accepted strategy for management of hypovolaemic shock during the latter half of the 20th century, theoretically aiming to boost colloid oncotic pressure and increase intravascular volume more than crystalloid solutions. Dextrans were already available at the time when Thai physicians were developing their guidelines and thus became incorporated into the original treatment regimens. By contrast HES solutions were not introduced until the 1960s, but then became the most commonly used synthetic colloid in western ICUs by the end of the 20th century, at a time when adverse effects with dextrans and gelatins were starting to be identified. Thus, in parallel with the progressive expansion in the geographical footprint of dengue around this time, many new HES solutions were becoming available allowing clinicians to make different choices depending on local availability and their personal experience with

particular preparations. Subsequently, even as very large trials in western ICUs began to demonstrate significant safety concerns around use of HES solutions, many expert clinicians managing DSS cases were establishing local guidelines that relied on use of these solutions.

Despite a virtual embargo on use of HES solutions in western ICUs following the big resuscitation studies described earlier (Perner A. et al. 2011, Gattas D. J. et al. 2013, Haase N. et al. 2013, European Medicines Agency 2014), there has been little change in practical DSS management in dengue-endemic countries in the last 10 years. The WHO guidelines continue to advocate use of synthetic colloids for profound or recurrent shock, and clinicians have become comfortable with use of HES solutions without observing apparent associations with mortality or adverse effects in their regular clinical practice. Dextrans and gelatins are still used in countries where such solutions remain available (typically countries with a historical precedent for their use for DSS management), but these solutions are now deployed so infrequently in the high-income countries where major fluid resuscitation studies take place, that up-to-date assessments of adverse effects/safety issues are lacking.

4.5.2 Potential benefits and risks of crystalloids versus colloids for DSS resuscitation

From a theoretical perspective, isotonic solutions of synthetic colloids with an average molecular weight similar to albumin (eg dextran 70) might be the preferred choice to replace the plasma proteins that leak in DSS, thus boosting intravascular colloid oncotic pressure and reducing the immediate rate of leakage, while larger molecules (eg HES 130/200) might remain for longer in the intravascular space potentially reducing the risk for subsequent reshock (Vink H. and Duling B. R. 2000, Nguyen-Pouplin J. et al. 2011). Indeed, a rapid effect of colloids in restoring cardiac index in DSS management was first demonstrated in the pilot randomized fluid trial in Vietnamese children (Dung N. M. et al. 1999). However, the randomized trials that followed showed no clear advantage in reducing the reshock rate or overall shock recovery time, for colloids over crystalloids when used for initial DSS resuscitation (Ngo N. T. et al. 2001, Wills B. A. et al. 2005). Notably however, in the 2005 report no differences were

observed between the crystalloid and colloid recipients in bleeding manifestations, coagulation derangements or the severity of fluid overload, all highly relevant outcomes in DSS. However, it is important to recognize that the adult studies in which HES related adverse effects were identified were much larger, involving thousands of patients rather than several hundred. On the other hand, the population at risk for DSS is rather different since most DSS patients are children and young adults without co-morbidities. Therefore, the use of colloids may bring different effects in this particular illness.

In this retrospective review, considering the immediate response strategy there was no apparent benefit to use of colloids in either the initial unadjusted analysis or after adjusting for baseline severity, actually with slightly worse respiratory outcomes in the mixed fluid recipients. The attending clinicians are likely to have based their initial severity assessment on WHO guidelines; although we devised the IPW score to adjust for differences in baseline severity such adjustments are invariably imperfect and it is probable that the crystalloid only group were still less severe than the mixed fluid group. However, the general findings on occurrence of reshock are in line with those observed in the 2005 randomized blinded trial (Wills B. A. et al. 2005). In that trial use of normal saline versus dextran or starch for initial resuscitation over 2 hours did not affect the need for rescue fluid subsequently (ie. fluid for reshock) among trial participants presenting with either moderate or severe shock. In the trial there was no difference in adverse events except for an increase in allergic reactions to dextran, between the different fluid groups. By contrast, in our retrospective study adverse respiratory outcomes still occurred more frequently in the mixed fluid group than the crystalloid group in the adjusted analysis.

The results of our study are more complex if we consider the overall management strategy, since prolonged colloid use (Group 3) largely prevented the occurrence of reshock and significantly reduce final shock recovery time but markedly increased the risk of developing respiratory compromise and the need for respiratory support. However, all 3 deaths occurred in Group 1 (crystalloid only), and Group 2 (<4 hrs) colloid recipients recovered significantly more

quickly than the Group 1 recipients but without an apparent increase in respiratory complications. Thus, while colloid use may confer some benefit, the findings suggest that prolonged use of colloids should be avoided in DSS patients, especially in settings with limited access to ventilatory support.

Development of renal, liver and bleeding complications were infrequent overall. Significant organ specific compromise is uncommon in DSS except in the elderly or those with profound shock, and renal/liver function were rarely monitored except in individuals with profound shock so it is possible that effects of the different fluid strategies on organ function might have been missed. On the other hand, most DSS patients are previously healthy children and young adults without co-morbidities, while the adult patients enrolled in the big fluid trials in which the main HES adverse effects were noted were a very heterogeneous group, many with associated co-morbidities as well as multi-organ impairment.

4.5.3 Other potential confounders

As discussed earlier one of the main potential confounders for this analysis, given the retrospective data collection method, is that the baseline severity of individual patients was not recorded systematically and might have influenced the fluid management strategy adopted in each case.

Another important factor to consider is the overall fluid volume (ml/kg) given over time as well as the high fluid rate. The continued high infusion rate in many DSS cases to achieve a target haematocrit probably caused fluid overload and respiratory compromise rather than the choice of fluid (colloid versus crystalloid). Normalizing haematocrit has been mentioned in local guidelines for DSS management (Children's Hospital 1 2019, Vietnam Ministry of Health 2019) which results in the prolonged use of a high fluid rate to maintain the haematocrit below 40%. This study shows the total fluid volume given over the first 24 and 72 hours after onset of shock increased in line with colloid use. Previous research has shown that, among individuals

with dengue, the risk for respiratory distress increases significantly as the infused volume over the preceding 24 hrs increases (Rosenberger K. D. et al. 2016). There is increasing interest in exploring whether restriction of intravenous fluid therapy, or use of specific balanced crystalloids, might be beneficial in critical care settings. In a recent randomized controlled trial among adult patients with septic shock in ICU, intravenous fluid restriction did not result in fewer deaths at 90 days than a standard fluid regimen (Meyhoff T. S. et al. 2022) but the difference in actual fluid volumes received between the two groups was relatively small. In another recent study the use of a balanced multi-electrolyte solution versus 0.9% saline in critically ill patients did not reduce the risk of acute kidney injury or death (Finfer S. et al. 2022).

Controversy also surrounds the use of fluid boluses, especially for paediatric shock resuscitation, following publication of the FEAST trial results in 2011 (Maitland K. et al. 2011). In that trial a significant increase in mortality was observed in African children with severe febrile illness (primarily malaria) and physical signs of impaired perfusion who received 20-40ml/kg boluses of either saline or albumin, compared to the no-bolus control group. However, many of the children were also malnourished and/or anaemic and whether most of them were truly hypovolemic remains uncertain. A recent review article outlines current knowledge on fluid management for severe malaria (Kalkman L. C. et al. 2022) and highlights the complexity of the problem and the need for additional research to optimize fluid therapy. Children with DSS automatically receive a fluid bolus in line with current WHO guidelines, but as our study shows, the type and volume of fluid given is highly variable. Although the pathogenesis of dengue is poorly understood it is apparent that, unlike malaria, plasma leakage is the crucial issue, with hypovolemia clearly evident in DSS cases. Rapid correction of profound hypovolemia has traditionally been accepted as a standard feature of shock resuscitation. However, in recent years considerable research efforts have been directed at improving our understanding of vascular physiology in health and disease, and there is now evidence to

indicate that aggressive fluid replacement in profoundly hypotensive states may actually be harmful to the microvasculature (Trung D. T. et al. 2020).

Although any or all of these factors may have influenced the outcomes in our study, the available data from this retrospective review do not allow further investigation. Like all therapeutic interventions, parenteral fluids have both beneficial and adverse effects, and these vary depending on the clinical context. The evolution of the crystalloid/colloid debate over the years, and more recently the focus on exploring the type/volume/rate of administration of crystalloid fluids in critical care scenarios, highlights the importance of continually re-evaluating the principles and practice of DSS management in the light of new knowledge.

4.5.4 Health economics

The economic burden of dengue illness in Vietnam has been estimated at between 115 USD and 278 USD per patient (2016 prices), with medical costs consistently making the largest contribution to overall costs (Hung T. M. et al. 2018). Here we present the first report that specifically focuses on DSS management in Vietnam. The major factors contributing to these costs reflect fluid costs and bed costs, since DSS patients in Vietnam are always managed in intensive care settings. Although provision of respiratory support is clearly expensive, the actual number of children requiring such support was small and thus the effect on overall costs was marginal. Comparing the costs between the different fluid strategies, the total cost among those receiving colloids for more than four hours was far more expensive than the two other groups. Notwithstanding potential benefits for individual children, adopting a general management strategy that encourages prolonged use of colloids for more than four hours did not improve overall outcomes but did increase the economic burden per patient significantly. In future research, this area needs to be carefully explored in order to better discriminate the effects of different fluid strategies to provide improved outcomes but also to ensure feasibility of application in LMIC settings.

4.5.5 Strengths and limitations

A particular strength of this study is that we describe for the first time how patients with DSS were actually managed in different centres of excellence across 3 countries where dengue is endemic. The work summarises the overall picture of the fluid therapy given to sequential patients, demonstrating that while the WHO guidelines and their respective local adaptations are often quoted in the published literature, in practice fluid management varies considerably in different settings. This variability is likely dependent in large part on the availability of different fluid types locally, as well as staff experience in using them, and highlights that the evidence base for the recommended regimes is limited. This study also explores the relationship between respiratory complications and prolonged use of colloids; especially in Vietnam prolonged colloid infusions is an adaptation that was fairly recently advocated for DSS management, with the intention of reducing recurrent shock and improving overall outcomes, but is clearly not without hazards.

Another important strength of the study is that the process of performing the review has brought together expert clinicians from several dengue endemic countries, who have worked alongside each other to address practical problems and thus created a network of dengue experts in many hospitals who all see the need for formal large-scale prospective evaluation of fluid management for DSS in the future. This could be a very valuable resource to tap into for future research efforts.

The major limitation of the study is its retrospective nature. Collecting data from hospitals with different fluid management and monitoring protocols, as well as variable systems for recording clinical information, inevitably raises concerns about the completeness of the dataset and the validity of analysing potentially inconsistent data together. However, the assessment of data quality showed that for most important variables the amount of missing information was actually quite small; the fact that these hospitals are experienced in managing DSS and that the WHO recommendations for monitoring DSS cases are well known in endemic areas is likely

to have helped. I defined all the outcomes of interest before beginning the data analysis, and used electronic algorithms to derive them, aiming to reduce personal bias. By using the IPW score I also tried to adjust for any lack of equivalence between patients in the treatment groups I investigated. However in a retrospective study like this uncertainty must remain about the link between patient severity and the fluid chosen, and about how this linkage could affect the outcomes of interest. Clearly a systematic prospective approach including randomization would be preferable to evaluate any intervention, but as a first step towards exploring potential effects of fluid management strategies on DSS outcomes the study has provided some interesting data.

Even if the patient groups were equivalent at baseline, differences in decision-making between clinicians at the different sites – regarding fluid type, volume, rate etc., when to intervene with respiratory support or give a blood infusion – make the analysis and interpretation complicated. On the other hand, it is partly because such differences exist that the study was possible. Despite the potential effects of the many possible confounders in a retrospective study, the data are interesting and suggest a need for a formal regional randomized trial to investigate the influence of fluid types and fluid rate on outcomes and create more of an evidence base for the WHO guidelines, which have not changed over the past 10 years.

4.6 Conclusions

This is the first study to investigate the influences of different fluid management strategies used throughout the illness course on outcomes from DSS, involving data from a large number of children cared for across the three Asian countries. The results suggest that colloid use in the first two hours of resuscitation did not provide better outcomes than the use of crystalloids alone. Prolonged use of colloids for more than 4 hours among children who appeared stable after the initial resuscitation did influence the reshock rate but also significantly increased the development of respiratory compromise and the overall costs of management. In a setting with limited respiratory support, clinicians need to be very careful when considering prolonged use of colloids for DSS patients.

The optimal fluid solution for initial DSS resuscitation as well as the best strategy for subsequent fluid management needs to be investigated by a large-scale trial in the future. With the recent development of innovative haemodynamic monitoring techniques, integration of clinical data and continuous haemodynamic data would provide a deeper understanding of how each individual responds with a different fluid resuscitation strategy. Additionally, a range of biomarkers of endothelial dysfunction have been reported (Yacoub S. et al. 2016, Vuong N. L. et al. 2021), which could be measured during the evolution of the DSS episode to assess the effects of different interventions on endothelial damage. Long-term follow up in combination with a full health economics assessment will also be important to explore the optimal fluid strategies for managing severe dengue in LMICs.

CHAPTER 5

THE COMPENSATORY RESERVE INDEX PREDICTS SHOCK IN PATIENTS WITH SEVERE DENGUE

This chapter has been published on BMC medicine with the entitled name “The compensatory reserve index predict recurrent shock in patients with severe dengue” (Trieu H. T. et al. 2022).

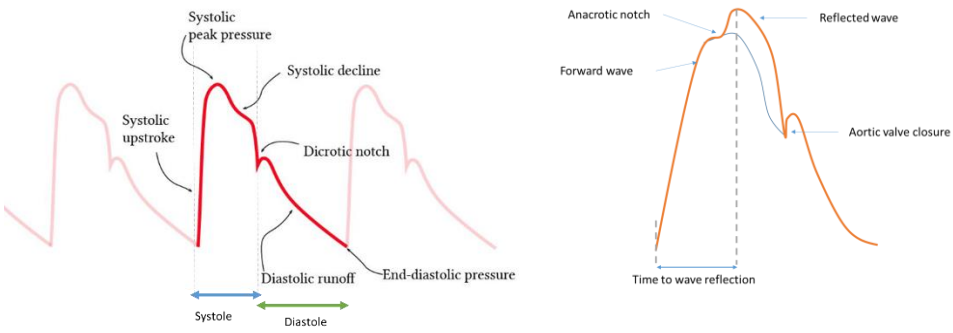
5.1 Introduction

5.1.1 Arterial pulse waveform

Arterial waveform represents the impulse of left ventricular contraction, conducted through the aortic valve and vessels. It includes several components: systolic upstroke, peak systolic, systolic decline, diastolic runoff and end-diastolic pressure (Figure 5-1).

Figure 5-1. Components of an arterial waveform

(reproduced from https://derangedphysiology.com/)



Panel A Panel B

Panel A illustrates the components of an arterial waveform. The upstroke limb includes features of systolic upstroke and systolic peak pressure. The down-stroke limb includes systolic decline, diastolic runoff and end-diastolic pressure. Panel B illustrates two waveforms generated during systolic ejection. The peak systolic pressure is shaped by the reflected wave, which will be low if the compliance of the vessel is good. Reflected waves become more prominent when moving further down the vascular tree.

Systolic upstroke represents ventricular ejection. Peak systolic pressure represents the maximum pressure in the central arteries, which is influenced by left ventricular contraction, arterial compliance and reflected pressure waves coming back from the vascular tree. The reflected wave shapes the peak of the arterial waveform, which changes with age (younger age-better compliance-lower reflected wave), length (distance from the artery to the aortic valve, longer distance- higher reflected wave) and vascular resistance (higher resistance-higher reflected wave). Other components of the arterial waveform in the down-stroke limb reflect the decrease of arterial pressure at the end of systole and after closing of the aortic valve. Diseases that affect the cardiovascular system will influence the shape of arterial waveforms.

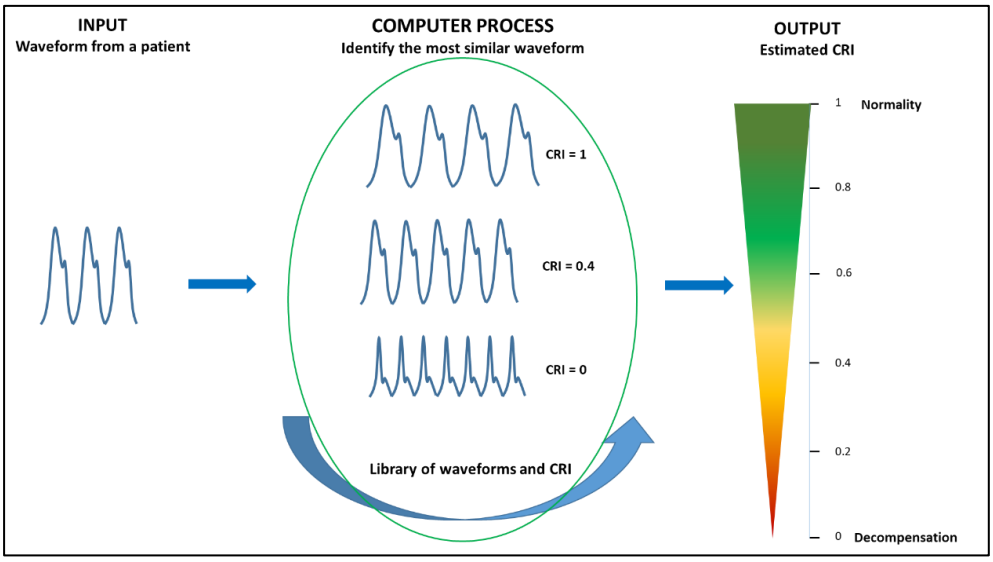
5.1.2 Compensatory reserve index (CRI)

The compensatory reserve index (CRI) is a novel physiological parameter that tracks real-time changes in central volume. CRI is derived from feature analysis of the arterial pulse waveform from PPG signals utilizing machine learning algorithms.). The pulse arterial waveform data is analysed by an algorithm to give the CRI value ranging between 0 and 1, where 0 represents the point of decompensated shock (SBP< 80 mmHg) and 1 indicates a normal volume status (Moulton S. L. et al. 2013).

As traditional vital signs (heart rate, blood pressure, arterial oxygen saturation) change relatively late in the course of hypovolemic/haemorrhagic shock there is a need for new more sensitive ways to assess intravascular hypovolemia and identify patients at risk of decompensated shock. To develop CRI, simulated model of hypovolaemia from studies in healthy subjects using a lower body negative pressure (LBNP) chamber was used. Continuous monitoring of pulse arterial waveforms was recorded for further analyses (Cooke W. H. et al. 2004, Moulton S. L. et al. 2013). The negative pressure results in a redistribution of blood which mimics actual haemorrhage in studies from humans and nonhuman primates (Hinojosa-Laborde C. et al. 2014, Hinojosa-Laborde C. et al. 2016). These studies were able to provide detailed analysis and demonstrate progressive changes in the arterial waveform during clinical

deterioration of the participants. Different subjects had different tolerance to varying levels of central blood volume depletion and were divided into 2 groups: low tolerant (failed to complete at least -60mmHg of LBNP) and high tolerant (completed at least -60 mmHg of LBNP). De-identified waveforms from all participants were analysed and models were developed along with data from volume and physiological changes. The accuracy of the models was checked using data from each subject as a true test subject. Data from other participants were used to build up the model. The data of the test subject was named test data and the data from other participants was named learning data. This provides a statistical estimation for the accuracy of the model for all participants. Data from all participants using this protocol were used to create a library of physiological signals from participants with different baselines. Over 12 years more than 260 healthy men and women participated in the LBNP studies, providing hundreds of thousands of waveform recordings (Convertino V. A. et al. 2013). The process to generate CRI values is described in Figure 5-2. Using machine learning algoithms the waveform from a person is compared with those from the library of waveforms with CRI values collected during model of blood loss experiments. Computer processing can rapidly identify the most similar waveform and correlative CRI then provide the real-time result.

Figure 5-2. Diagram illustrating the process of generating CRI value from waveforms obtained from a patient, reproduced from Victor Convertino (Convertino V. A. et al. 2016)



The features of arterial waveform patterns change during the central volume loss experiment, which can be analysed and provide real-time information on the individual's volume status. The arterial waveform includes 2 components, the ejection wave and the reflected wave. The ejection wave occurs when the heart contracts during the systolic period and the reflected wave is created by pressure reflecting back from the peripheral arteries. The feature of waveforms changes in hypovolemic states, with a smaller magnitude of height, width and longer distance between the 2 waves (Figure 5-3). Factors that influence cardiac output are contained in the features of ejected waves whereas mechanisms that influence vascular resistance are contained in the reflected waves. The sum of all compensatory factors of an individual with central volume depletion would therefore be captured in the morphology of both ejected and reflected waveforms.

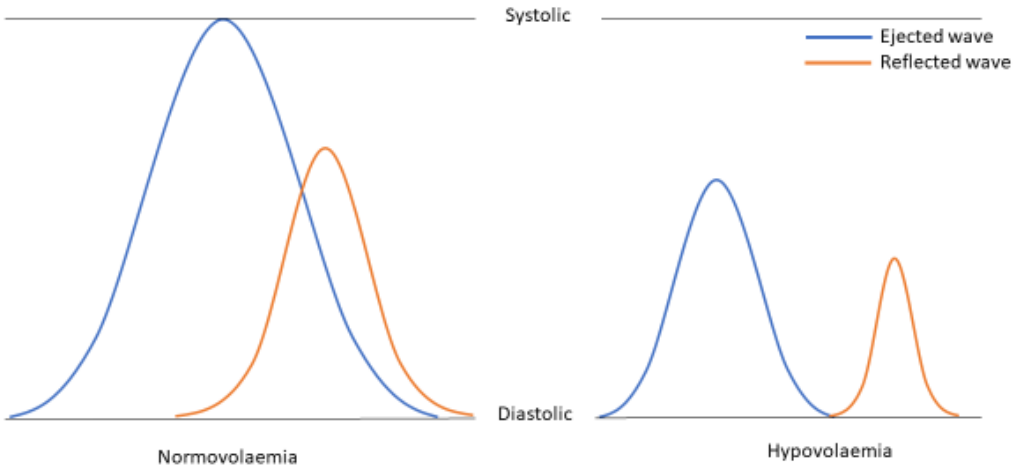


Figure 5-3. Different features of arterial waveforms from patients with normovolaemic and hypovolaemic status. Adapted from Victor Convertino (Convertino V. A. et al. 2016)

Research in this area is not new and the morphology of PPG waveforms has been investigated in combination with heart rate and blood pressure to estimate cardiac output and stroke volume (Moulton S. L. et al. 2013). A limitation in those studies is that the data was based on population averages and could not account for individual compensatory differences. Therefore those algorithms don't have a high sensitivity for detecting individual deterioration during central volume depletion and different levels of compensation.

The CRI algorithm however represents the individual compensation of a person with progressive blood loss and is defined as below:

$$\mathrm{CRI} = 1 - \frac{\mathrm{BLV\ (t)}}{\mathrm{BLV}(HDD)} \approx 1 - \frac{\lambda\,.\,\mathrm{LBNP}\ (t)}{\lambda\,.\,\mathrm{LBNP}(HDD)} = 1\text{-}\frac{\mathrm{LBNP}\ (t)}{\mathrm{LBNP}(HDD)}$$

BLV (t) is blood loss volume at time t and BLV(*HDD*) is blood loss volume at which the subject manifests haemodynamic decompensation (SBP <80 mmHg); LBNP (t) is lower body negative pressure at time t and LBNP(HDD) is the lower body negative pressure at which the subject manifest haemodynamic decompensation. This algorithm allows estimation of an individual's

compensatory mechanisms under a model of haemorrhage at certain time-points (Moulton S. L. et al. 2013).

To validate the CRI, it was hypothesized that CRI would be able to detect early physiological changes that occur during the compensatory phase of central volume loss, preceding changes in conventional haemodynamic parameters. CRI was measured on 230 soldiers who donated blood and 34 controls who did not donate blood. Following the blood donation, there were no changes in heart rate, diastolic blood pressure, oxygen saturation, whereas the systolic BP decreased by 3% and the CRI decreased by 16%. The AUCs for identifying mild blood loss was 0.84 using CRI; 0.73 for heart rate; 0.60 for systolic blood pressure (Nadler R. et al. 2014). In another prospective clinical trial on 50 trauma patients with different levels of bleeding, CRI also differentiated early those patients with active bleeding versus those with no bleeding (0.17 vs 0.56). A cut-off of 0.21 had a sensitivity of 0.97 and specificity of 0.83 for identifying active bleeding (Stewart C. L. et al. 2014).

CRI has also been shown to be specific for each individual; the algorithm has been developed to identify how each individual compensates differently with fluid loss (Convertino V. A. et al. 2015, Convertino V. A. and Koons N. J. 2020).

A study on 25 healthy non-smoking men and women volunteers in which blood withdrawal was performed, with up to 20% of total blood volume loss (1,300 ml for men and 1,000 ml for women) for a duration of 60-80 minutes, then reinfused back into the subject at the end of the protocol. CRI was recorded with other vital signs (heart rate, SpO2, cardiac output, stroke volume). CRI decreased by 33% and in a linear trend with the progression of blood loss whereas HR only increased by 15.6% and cardiac output and peripheral vascular resistance were unchanged. ROC AUC for CRI was 0.901 with a sensitivity of 0.80 and specificity of 0.78, which were higher than those of other traditional vital signs. This study suggested that CRI monitoring may help detect early deterioration due to bleeding. CRI was also validated in a similar study including 42 participants who underwent blood removal gradually with the same

protocol but with 4 PPG CRI monitors attached on 4 specific fingers. Seven participants decompensated and the mean CRI at the point of collapse was 0.15 ± 0.007. Asymptomatic participants who achieved 20% blood loss had a mean CRI of 0.611± 0.028. This study also demonstrated that the CRI correlates with intravascular volume as well as accounting for individual variability.

5.1.3 Factors that may influence the CRI

Derived from PPG waveform, CRI values may be influenced by external factors such as heat stress or exercise-induced dehydration. During the studies of simulated haemorrhage with LBNP, a two-step protocol was performed on 20 volunteers who underwent LBNP until developing decompensation status (systolic blood pressure < 80 mmHg). In protocol 1, core temperature was increased to 1.2^{o}C before the LBNP experiment or kept normal. In protocol 2, exercise-induced dehydration (90-minute-treadmill exercise with speed and inclination adjusted in a climate-controlled chamber at 41^{o}C and humidity of 25%) was performed with or without oral fluid replacement for dehydration (calculated by weighting body mass every 15 minutes). CRI prior to LNBP in the first protocol was 0.87 ± 0.09 then decreased to 0.42 ± 0.19 by controlled heating. CRI at the start of protocol 2 was 0.67 ± 0.19 and decreased to 0.47 ± 0.25 by exercise-induced dehydration. This study showed that higher temperatures and dehydration affect the CRI values (Gagnon D. et al. 2016).

Regarding the effect of pain and analgesia on CRI measurement, a study assessed CRI in healthy pregnant women before and during the active phase of labour. Results showed that CRI did not vary during the study period, CRI ranged from 0.82 to 0.89. These results suggest that pain and analgesia had minimal impact on CRI and therefore may have potential use for patients during peri-operation (Hinojosa-Laborde C. et al. 2017).

5.1.4 Application of CRI monitoring in different aetiologies of shock

CRI has been used in trauma patients, where it was able to distinguish the severity of bleeding. The mean CRI for actively bleeding patients was 0.17 versus 0.56 for non-bleeding patients.

CRI continued to decrease following fluid infusion in patients with ongoing bleeding and increased in non-bleeding patients (Stewart C. L. et al. 2016).

CRI has also been used for patients with congenital heart disease requiring transcatheter pulmonary valve replacement. The hemodynamic status changes rapidly pre and post procedure due to low cardiac output when the balloon is inflated at right ventricular outflow tract causing temporary occlusion. CRI was measured in 26 patients from 13 to 29 years old before and after balloon inflation, and the mean values were 0.66 ± 0.15 and 0.53 ± 0.16, respectively. These results are the preliminary evidence that CRI may be also helpful to monitor patients during cardiac procedures (Ehrmann D. E. et al. 2020). Another prospective pilot study used CRI monitoring in 23 congenital heart disease patients from preoperative baseline to the first 24-hour post-operation cardiac surgery (Ehrmann D. E. et al. 2021). The average CRI values of patients with longer ICU length of stay (LOS) ($\geq$ 3 days) were lower compared to those with shorter ICU LOS (< 3 days) throughout the monitoring period. This study additionally found that CRI monitoring was feasible and safe for both children and adult patients.

In another study, the CRI was also able to differentiate non-perforated vs perforated appendicitis (Choi Y. M. et al. 2018, Shahi N. et al. 2021). The study that was conducted between 2014-2015 at the Children's Hospital Colorado, recruited 94 patients from 2 to 17 years old who were diagnosed with acute appendicitis. The results showed that CRI values were significantly higher in children with simple acute appendicitis compared to those with perforated appendicitis at presentation to the emergency department (0.6 vs 0.3, $p < 0.01$). Multivariable regression models found that lower CRI was associated with the diagnosis of perforated appendicitis. A decrease of 0.1 of CRI increased the odds of perforated appendicitis by 40%.

Regarding the use of CRI for younger children, CRI was measured in 26 newborns with congenital diaphragmatic hernia. Eight of them required extracorporeal membrane oxygenation

(ECMO) and had lower CRI values prior to ECMO compared to those in the non-ECMO group (0.068 versus 0.112, respectively, p = 0.035). When compared to several prenatal prognostic factors including heart rate, lung to head ratio, percent predicted lung volume, total lung volume, a lower CRI had the highest predictive capability for ECMO with an AUC of 0.85 (95% CI 0.69- 1) for these neonates with congenital diaphragmatic hernia. A threshold of 0.054 of CRI correlated with the highest accuracy levels for indicating ECMO with a sensitivity of 0.88, specificity of 0.72 (Leopold D. K. et al. 2020).

These studies have demonstrated the utility of CRI in monitoring both adults and children from different age groups and clinical syndromes.

5.1.5 Preliminary evidence of the compensatory reserve index in dengue

Dengue shock syndrome is predominantly caused by intravascular hypovolaemia in the majority of cases, especially in children. A case series assessing CRI in three children with dengue shock syndrome, demonstrated its potential utility at tracking real time changes in intravascular volume (Moulton S. L. et al. 2016). In this study, deidentified PPG waveform data was recorded for 15 minutes per day for each patient, and then later, processed by the CRI algorithm. The median of CRI at admission was less than 0.2 in the first 2 days of shock increasing to above 0.4 from day 3 after the onset of shock, achieving normovolaemia (CRI > 0.75) by the end of the observational period. This study provides preliminary results of the potential use of CRI in monitoring patients with dengue shock.

5.2 Objectives and hypothesis

In this study, I aimed to illustrate dynamic changes in CRI in intensive care unit (ICU) patients with dengue shock syndrome and determine if CRI could predict the occurrence of reshock episode.

I hypothesise that the CRI would decrease prior to changes in traditional vital signs and identify patients at risk for developing reshock.

5.3 Methods

The full methods for this study is presented in chapter 2. Briefly, 103 patients were enrolled in a prospective observational study, which include paediatric and adult patients with suspected severe dengue and were admitted to intensive care units at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam (Yacoub S. et al. 2017). Individuals aged 15 or greater were admitted to an adult ICU and those less than 15 years old were admitted to paediatric ICU. CRI monitor was connected to the patients from the enrollment until ICU discharge or a maximum of 5 days. Pulse waveforms were sent from the finger probe to the laptop via Bluetooth. Clinical data including fluid therapy and hourly vital signs were entered into the CRI laptop. Waveform data was sent to the United States after finishing the data collection period for converting into CRI values. CRI data was integrated with clinical data for final data analyses.

5.3.1 Clinical endpoint definitions

Shock was defined as a narrowing of the pulse pressure (PP) $\leq$ 20 mmHg or low blood pressure (BP) for age, combined with clinical signs of reduced peripheral perfusion (capillary refill time greater than 2 seconds, or cold extremities).

The initial shock recovery time was the time from first episode of shock to when the patient achieved cardiovascular stability, which was defined as the time point when the pulse pressure was > 25 mmHg for at least 6 hours. After recovering from the first episode of shock, patients either remained stable and recovered, or they experienced another episode of shock, termed reshock. Reshock was defined as an episode of shock (defined above), followed by a period of cardiovascular stability.

5.3.2 Statistical analyses

To investigate the ability of CRI to predict shock, only patients who were admitted with DSS were included in the analysis. All reshock episodes occurred within 48 hours from the onset of shock, therefore, we only use data within this period of time for the data analysis.

The CRI monitor provides continuous values for every heartbeat. We used 5-minute averages of CRI to reduce erratic variations. Changes of CRI for each patient in the first 48 hours were plotted, together with data of systolic and diastolic blood pressure and fluid volume given to the patient over time.

Descriptive summaries were performed using median (interquartile range) for continuous variables, and frequency (percentage) for categorical variables. Graphical descriptions of CRI and haemodynamic parameters are based on a time-plot of the individual trajectory of the parameter of interest. Correlations between CRI and other haemodynamic parameters (heart rate, systolic blood pressure and diastolic blood pressure) were assessed using partial Pearson's correlation coefficient. These correlations were controlled for potential confounding variables including age, gender, and body weight. To perform these calculations, CRI and hemodynamic data (including data within the initial shock recovery period) were grouped either every 30 minutes (for CRI and heart rate) or 1 hour (for CRI and blood pressure) from the onset of the first shock. Overall correlation and correlation at each time-point were then estimated. The significance of partial correlations was based on their Fisher transformation and the bootstrap standard errors, using 1,000 resamples to account for repeated data.

All CRI data obtained after recovery from the first episode of shock and within the first 48 hours from enrolment were used to investigate CRI's ability to predict reshock. After excluding data during the initial shock recovery period, only patients with available CRI data were included in this analysis. For each CRI measurement, we derived the occurrence of the first reshock within 12 hours from its measurement as a primary outcome and the occurrence of pulse pressure <=20 mmHg within 12 hours from its measurement as a secondary outcome. The "12 hour" threshold allowed us to incorporate all information in our dataset, as the maximum duration from any CRI measurement to the first reshock was 11.3 hours. For prediction within a shorter period, we explored relationships between CRI measurements and outcomes of interest within 6 hours from CRI measurement. These analyses were based on

logistic regression models, and the results were summarized with odds ratios (ORs) and the corresponding 95% confidence intervals, with and without adjustments for the time of CRI measurement, age, gender and body weight. CRI's ability to predict outcomes was quantified using area under the ROC curve analyses, as well as sensitivity and specificity at different CRI cutoffs (0.2, 0.4, 0.6). To account for repeated measurements per individual, all confidence intervals for OR, AUC, sensitivity and specificity were calculated using the cluster bootstrap standard errors based on 1000 resamples of the original dataset. In addition, CRI and HR measurements were grouped and displayed at different 1-hour windows before the first reshock occurrence (for patients with reshock) and after enrolment (for patients without reshock). All analyses were performed with R version 4.0.3.

5.4 Results

There were 103 patients recruited into the study. After excluding non-DSS cases (patients with warning signs and stayed in ICU for monitoring), patients developed shock after enrolment and DSS cases without CRI data, there were 63 cases for the final analysis (Figure 5-4) which includes 50 children and 13 adults.

5.4.1 Characteristics of all study patients at enrollment

Clinical characteristics are shown in Table 5-1. Males and females shared similar proportions. The median age was 11 years (IQR 8-14). Five (8%) patients presented decompensated shock at the time of ICU admission (four had pulse pressures ≤ 10 mmHg and one had no measurable blood pressure). The median Hct was 48% (IQR 46-52). All patients had no fever and had normal respiratory rate at enrolment. The median platelet was 27,000/mm3 (IQR 17,000-35,000).

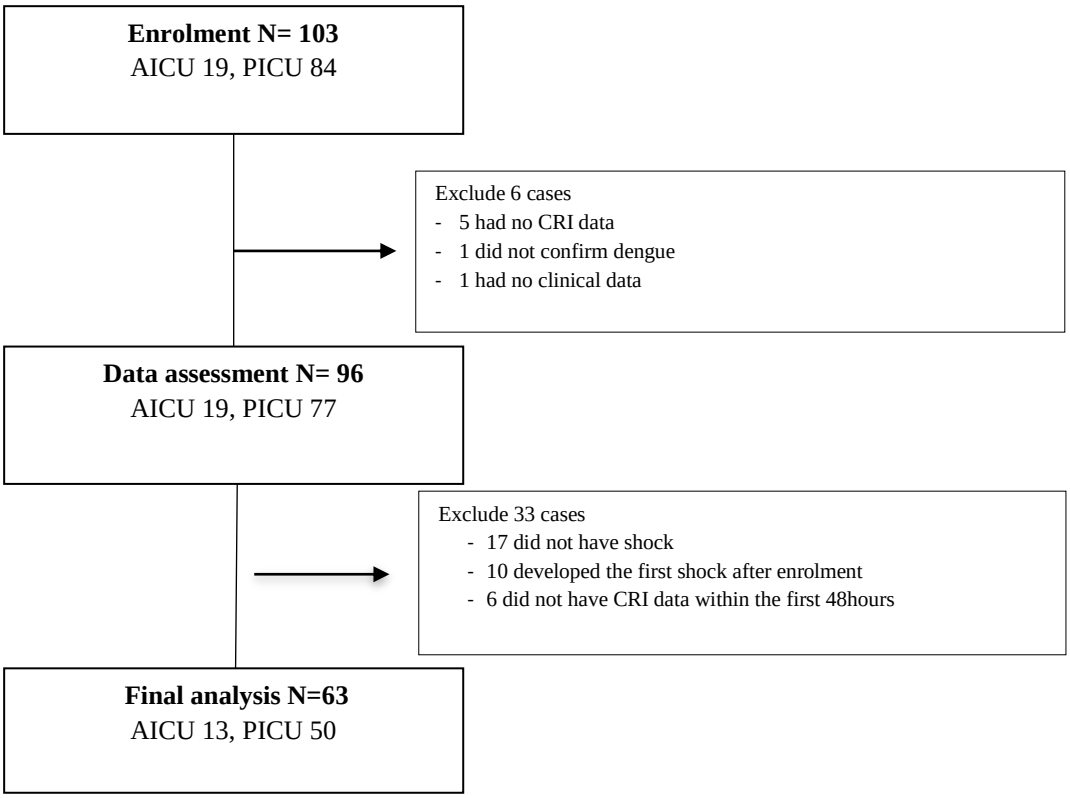


Figure 5-4. Flowchart of included patients

AICU Adult Intensive Care Unit, PICU Paediatric Intensive Care Unit

Table 5-1. Characteristics of study patients at enrollment

Characteristics	Included group (N= 63)	Excluded group (n=40)	Total (n=103)
Age (years)	11 (8, 14)	12 (8, 13)	11 (8, 14)
Female	27 (43)	22 (55)	49 (48)
Weight (kg)	40 (30, 49)	39 (30, 50)	39 (30, 50)
Day of illness at enrolment	6 (5, 6)	5 (4, 5)	5 (4, 6)
Heart rate (beats/min)	104 (92, 119)	100 (92, 120)	100 (92, 120)
SBP (mmHg)	100 (90, 100)	100 (90, 105)	100 (90, 100)
DBP (mmHg)	80 (70, 80)	80 (64,80)	70 (65, 80)
Temperature (ºC)	37 (37, 37.5)	37 (37, 37)	37 (37, 37)
Respiratory rate (breaths/min)	20 (20, 24)	20 (20, 24)	20 (20, 24)
Pulse pressure <=10 mmHg	5 (8)*	1 (0)	6 (0.1)
Haematocrit (%)	48 (46,52)	50 (46, 52)	49 (46, 52)
Platelet at enrolment (×1,000 cells/μL)	27 (17, 35)	32 (19, 47)	29 (18, 41)
WBC (k/μL)	4.40 (3.14, 6.28)	4.61 (3.07, 5.75)	4.59 (3.11, 6.05)
AST (IU/L)	196 (114, 464)	142 (90, 251)	165 (104, 378)
ALT (IU/L)	100 (56, 189)	68 (41,116)	91 (44, 172)
Venous lactate (mmol/L)	3.0 (2.2, 4.4)	2.7 (2.2, 3.7)	2.9 (2.2, 4.1)

Statistics presented: median (IQR), n (%). SBP systolic blood pressure, DBP diastolic blood pressure, WBC white blood cell, AST aspartate aminotransaminase, ALT alanine transaminase. *There were 4 cases with pulse pressure <=10 mmHg and 1 case with unmeasurable blood pressure

5.4.2 Summary of fluid management and outcomes of patients with dengue shock syndrome

Reshock occurred in 15/63 (24%) patients. Among them, four patients had two episodes of reshock and one patient had 3 episodes of reshock. The median time from the first shock to the first reshock was 12 hours (IQR 10-14). Respiratory distress occurred in eight patients (13%), however, no patient required ventilatory support. Three patients required inotropes, and three patients required blood transfusion. There were no deaths (Table 5-2).

Table 5-2. Parenteral fluid management and outcomes of study patients included in the final analysis

Management and outcomes	Median (IQR) or n (%) N= 63
Duration of fluid management (hours)	26 (22, 31)
Total volume of fluid resuscitation (ml/kg)	112 (78,143)
Hours from the shock onset to initial haemodynamic stability	7.0 (6.5, 10.7)
Reshock	15 (24)
Number of reshock episodes	
1 time	*10 (48)*
2 times	*4 (27)*
3 times	*1 (7)*
Hours from enrolment to first reshock	12 (10, 14)
Respiratory distress*	8 (13)
Severe bleeding**	4 (6)
Deaths	0 (0)

Statistics presented in median (IQR), n (%)

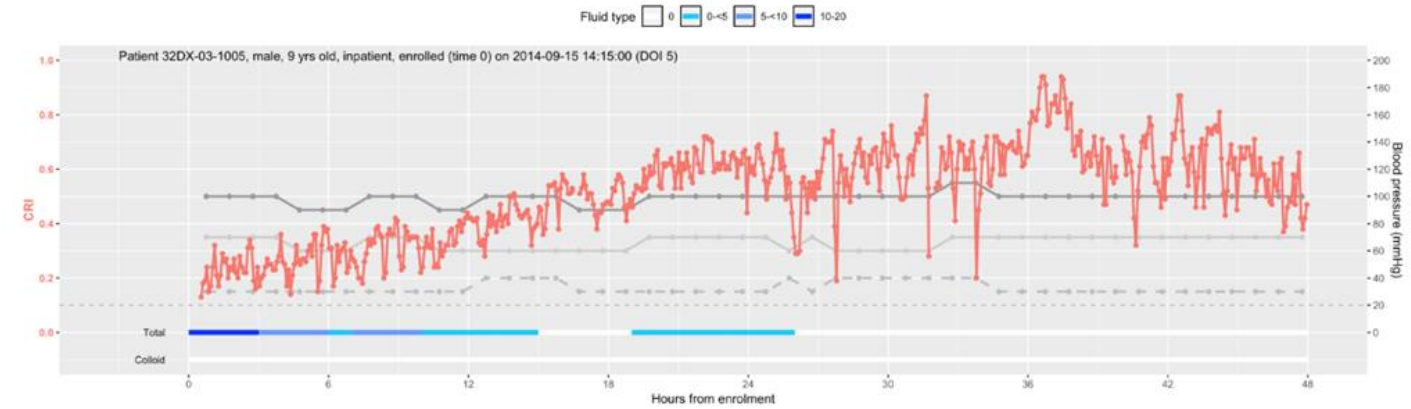
*Respiratory distress was defined clinically if having signs of using accessory muscles and/or requiring respiratory support. ** Severe bleeding was defined as bleeding that required blood transfusion or procedures to stop bleeding.

5.4.3 Dynamic changes of CRI during 48-hour-fluid resuscitation in ICU

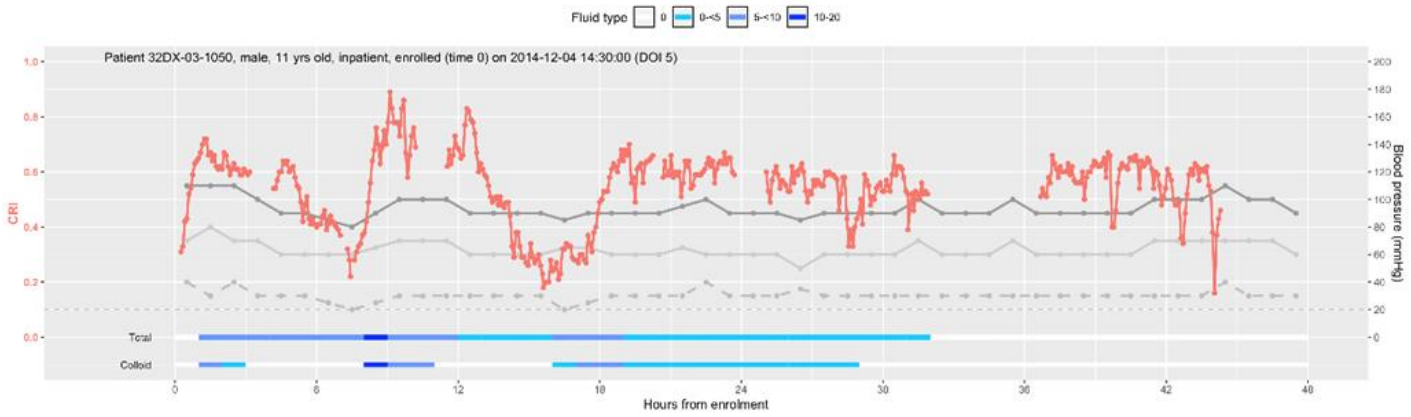
Figure 5-5 illustrates two representative cases with 48 hours of monitoring, which includes dynamic changes in CRI and BP and resuscitation fluid volumes. There were different trajectories of CRI over the first 48 hours of fluid resuscitation and the association between CRI with systolic blood pressure, diastolic blood pressure and pulse pressure. CRI values fluctuate but the overall trend of CRI reflects the changes in fluid resuscitation. The first case presented (Panel A) is data of a 9-year-old boy who was diagnosed with compensated shock and received only Ringers Lactate for the whole period of fluid management; the CRI gradually improved with fluid resuscitation and there were no subsequent episodes of reshock. The second case is that of an 11-year-old boy who presented with severe hypotensive shock at enrolment (unmeasurable blood pressure) and received Hydroxyethyl starch 6% 200/0.5 for initial fluid resuscitation. The CRI increased significantly after an hour of colloid infusion, reached the normal range of CRI, but decrease gradually after changing to crystalloid infusion then reshock occurred. Figure 5-6 had shown trajectories of CRI and pulse pressure of all 63 patients during the first 48 hours from enrolment.

Figure 5-5. Dynamic changes in the compensatory reserve index (CRI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) during the first 48 hours of fluid management.

Panel A shows the trajectories of CRI, SBP, DBP, and PP in a 9-year-old boy with DSS. Panel B shows the trajectories of CRI, SBP, DBP and PP in an 11-year-old boy with DSS and two reshock episodes. The red line is CRI, the dark grey line is SBP, the light grey line is DBP, the intermittent grey line is PP and the vertical blue line indicates episodes of clinical shock/reshock



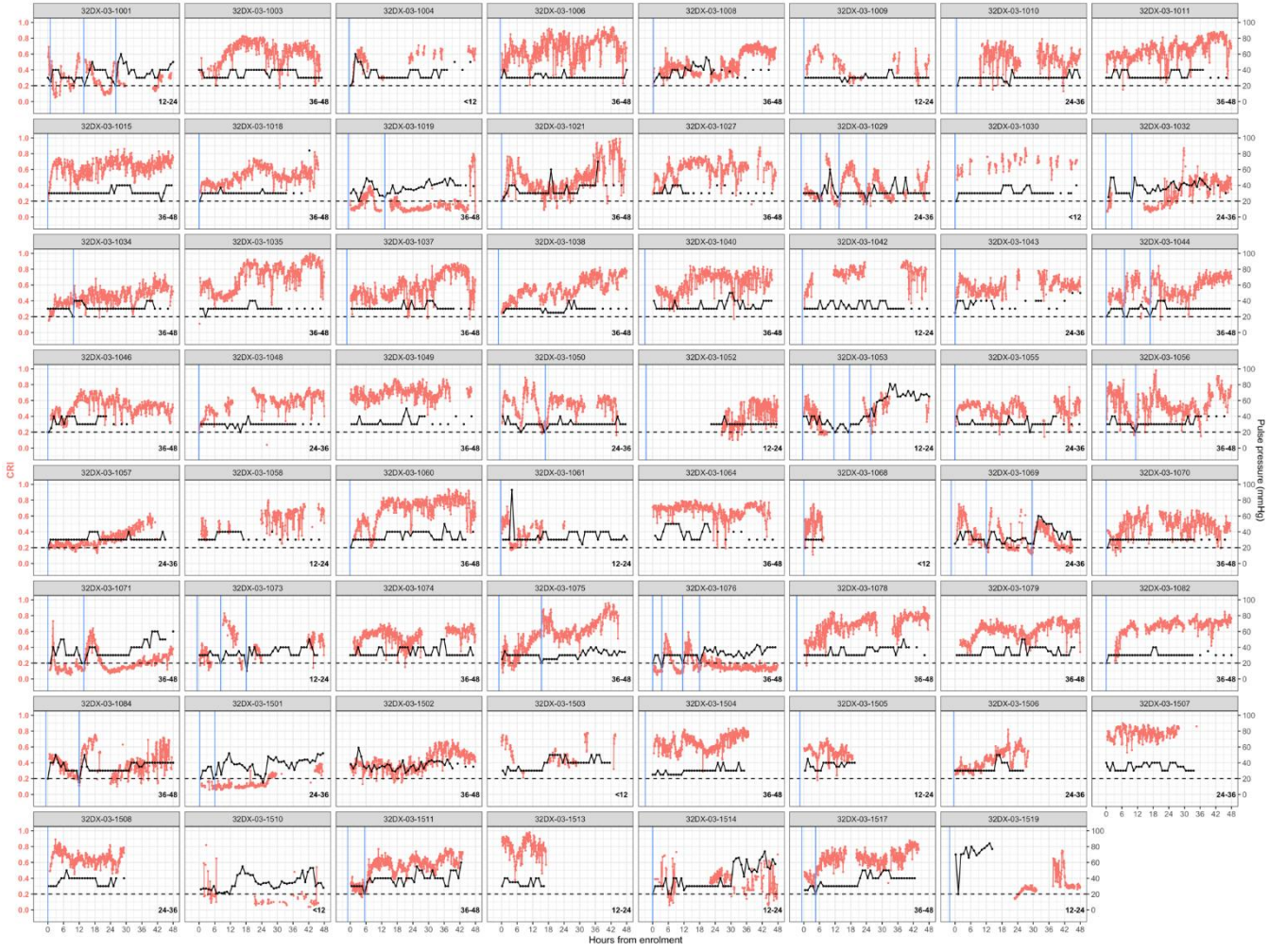
Panel A



Panel B

Figure 5-6. Trajectory of CRI (red line) and pulse pressure (black line) during 48 hours in paediatric intensive care unit form enrolment of 63 study patients with dengue shock syndrome (DSS).

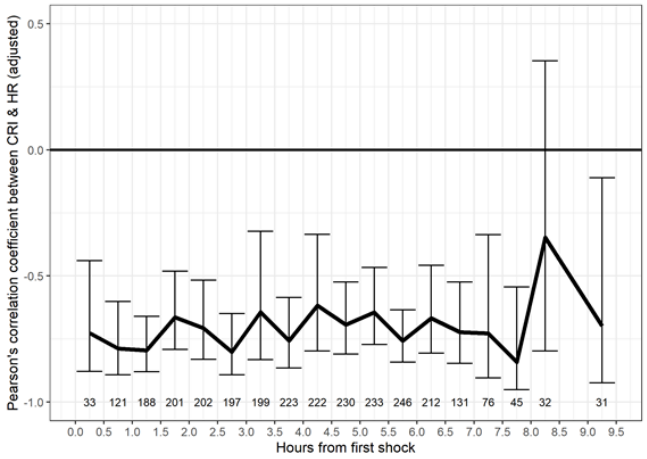
The intermittent black line is the indicator line for pulse pressure of 20 mmHg. The vertical blue line is the indicator of shock based on clinical decisions. A patient with DSS may have one or more episodes of shock during 48hours of fluid management. A clinical decision of shock is based on pulse pressure <=20 mmHg and signs of low peripheral perfusion with changes in fluid type (using colloids) and rates (≥ 10 ml/kg/h)



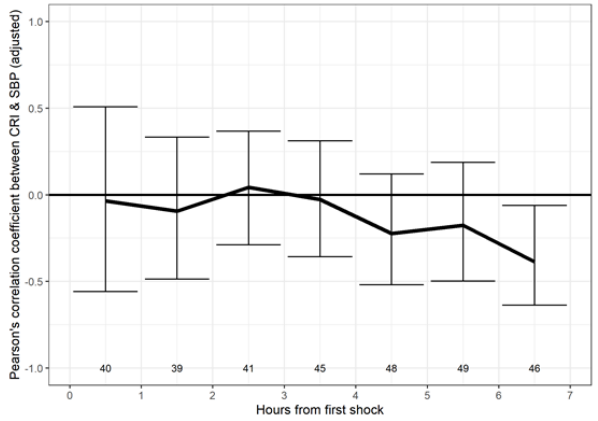
5.4.4 Correlation between CRI and heart rate and blood pressure

Figure 5-7 shows a negative association between CRI and heart rate, SBP and DBP within the first 8 hours from enrolment. CRI has a significant negative correlation with heart rate with an overall partial Pearson's correlation coefficient of -0.71 (95% CI -0.80, -0.60). This association is stable within the first nine hours from shock onset (Panel A, Fig. 4). CRI has a moderate negative association with SBP and DBP with overall partial Pearson's correlation coefficients of -0.12 (95% CI -0.31, 0.07) and -0.20 (95%CI -0.38, 0)

Panel A



Panel B



Panel C

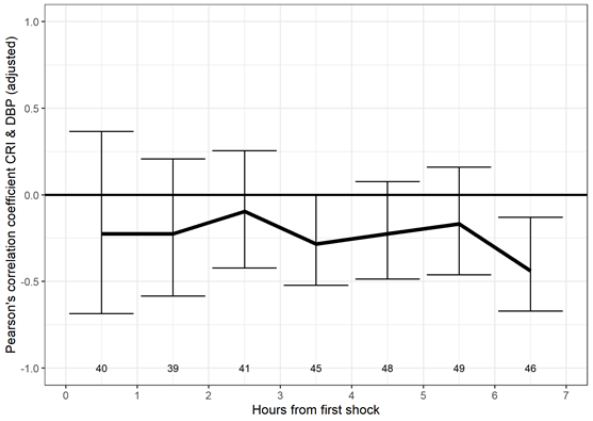


Figure 5-7. Pearson's correlation coefficient between compensatory reserve index (CRI) and heart rate (HR), systolic blood pressure (SBP) or diastolic blood pressure (DBP).

The three panels describe partial Pearson's correlation coefficients between CRI and heart rate (HR) (Panel A), systolic blood pressure (SBP) (Panel B) and diastolic blood pressure (DBP) (Panel C) for all 63 cases included in the analysis. Solid black lines represent the estimated partial Pearson's correlation coefficients between CRI values and the corresponding hemodynamic parameter at intervals from the onset of the first shock episode, after adjusting for age, gender and body weight. To perform these calculations, data within the first eight hours since the first shock were grouped every 30 minutes (for HR) or 1 hour (for SBP and DBP). Vertical lines are corresponding 95% confidence intervals of the estimated partial Pearson's correlation coefficient; repeated data was accounted for using bootstrap sampling. The number below each vertical line represents the number of hemodynamic parameter measurements in each group (HR in Panel A, SBP in Panel B and DBP in Panel C). Correlations based on small numbers of observations (<30) are unreliable and therefore they were excluded from these figures (>9.25 hours for HR, >6.5 hours for SBP and DBP).

5.4.5 CRI and prediction of reshock

CRI values could predict the first episode of reshock within 6 hours from the measurement with an odds ratio of 2.31 (95% CI 1.40 -3.81). The ability of CRI in predicting reshock within 12 hours from the measurement was still meaningful with odds ratio of 2.24 (95%CI 1.34-3.73) (Table 5-3).

Table 5-3. Prediction of the first episode of reshock using CRI measurement.

	Within 12 hours of CRI measurement		Within 6 hours of CRI measurement	
	Estimate (95%CI)	**p**	**Estimate (95%CI)**	**p**
First reshock				
OR adjusted	2.24 (1.34, 3.73)	0.002	2.31 (1.40, 3.81)	0.001
OR unadjusted	2.17 (1.42, 3.32)	<0.001	2.25 (1.55, 3.28)	<0.001

For outcome first reshock we considered two risk periods: within 6 hours from CRI measurement and within 12 hours from CRI measurement.

OR Odds Ratio of the first reshock for each decrease of 0.1 in CRI. These ORs and corresponding 95% confidence intervals (95%CI) and p values were estimated from logistic regression models that used cluster bootstrap with 1000 resamples of the original dataset to take into account repeated CRI measurements from the same participant.

OR adjusted corresponds to OR after adjusting for time of CRI measurement, age, gender and body weight.

OR unadjusted corresponds to OR from the univariate model with CRI as the only covariate.

Duration from initial CRI measurement to the first reshock

The median time from the shock onset to the first episode of reshock was 12 hours (IQR 11.5, 17.7). The median time from the first CRI measurement to the first reshock was 5.4 hours (IQR 2.9, 6.8) (Table 5-4).

Table 5-4. Duration from initial CRI measurement to first reshock

Variable	Median Number of Hours (Interquartile range) [min, max]	Number of Observations (n=13)
Hours from enrolment to the first reshock	12.0 (11.5, 13.7) [6.3, 16.8]	13
Hours from the first CRI measurement to the first reshock *	5.4 (2.9, 6.8) [1.3, 11.3]	13
Hours from any CRI measurement to the first reshock **	3.4 (1.8, 5.2) [0.0008, 11.3]	10668

3/63 patients were excluded from the analysis of first reshock prediction as there were no CRI measurements within the at-risk period (time period from hemodynamic stability to first reshock or end of the 48-hour period).

* First CRI measurement is taken from the at-risk period after haemodynamic stability (excluding measurements when patients were still unstable from the first shock). This duration corresponds to the maximum time from any CRI measurement to the first reshock per patient.

** This time period includes every CRI measurement within the at-risk period to the first reshock; this accounts for all CRI measurements per patient.

Mean CRI and heart rate at different time points prior to reshock

Figure 5-8 illustrates the mean values of heart rate and CRI at different time points before reshock. While the mean value of CRI was above 0.5 in the non-reshock group, the mean values of CRI were below 0.4 during the 7-hour-period before reshock.

Figure 5-9 illustrates the mean values of heart rate and CRI during 48 hours of monitoring patients who did not develop reshock. CRI values changed over time but all values were above 0.4 and the mean values of CRI were above 0.5.

Figure 5-8. Mean compensatory reserve index (CRI) and heart rate (HR) at different time-point prior to reshock

Dots and lines represent the means and corresponding 95% confidence intervals for CRI (in red) and heart rate (in blue) at different time points before the first reshock occurrence for the 13 patients with reshock, and the overall means and 95% confidence interval within the first 48 hours for the 47 patients without reshock. Data were grouped within sequential 1-hour windows leading up to the first reshock before calculation and 95% confidence intervals were estimated using the bootstrap standard errors based on 1000 resamples of the original dataset (the resampling accounts for repeated measurements from the same participant). Only CRI values measured after haemodynamic stability were included in this analysis. Numbers at the bottom present the number of CRI or HR measurements (upper) and the number of patients (lower) in each group.

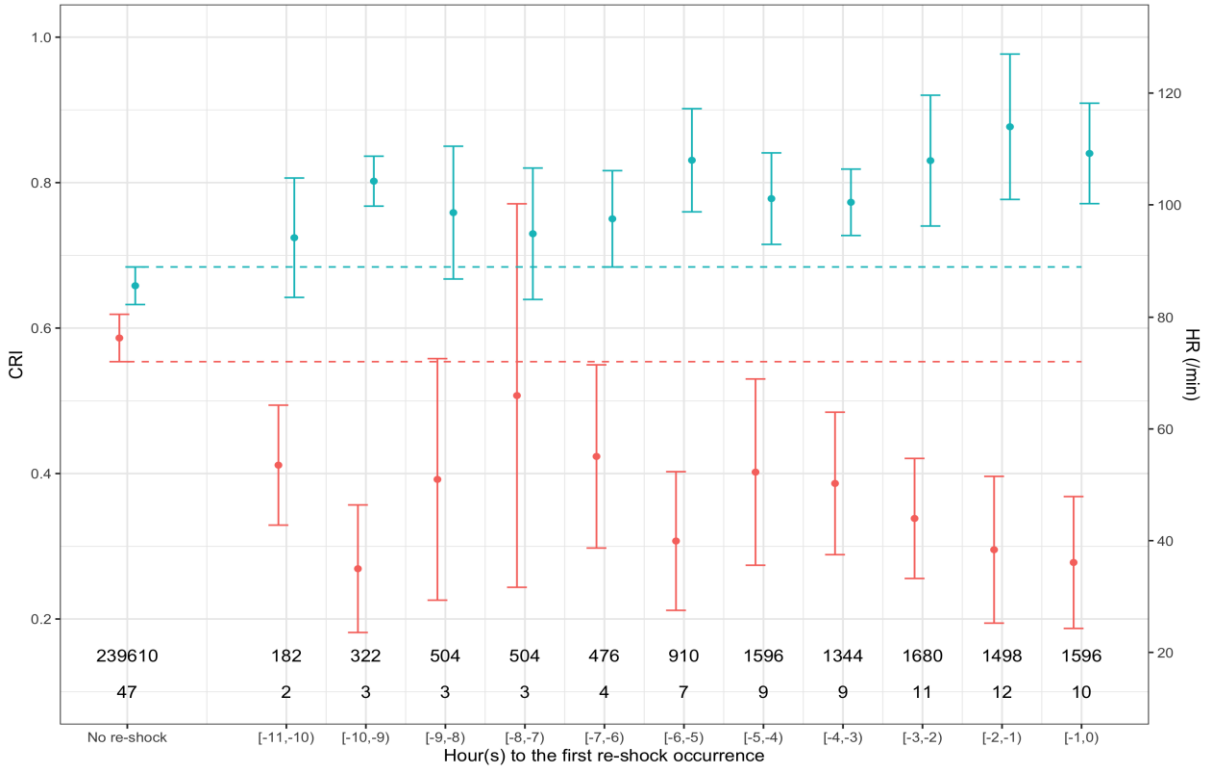
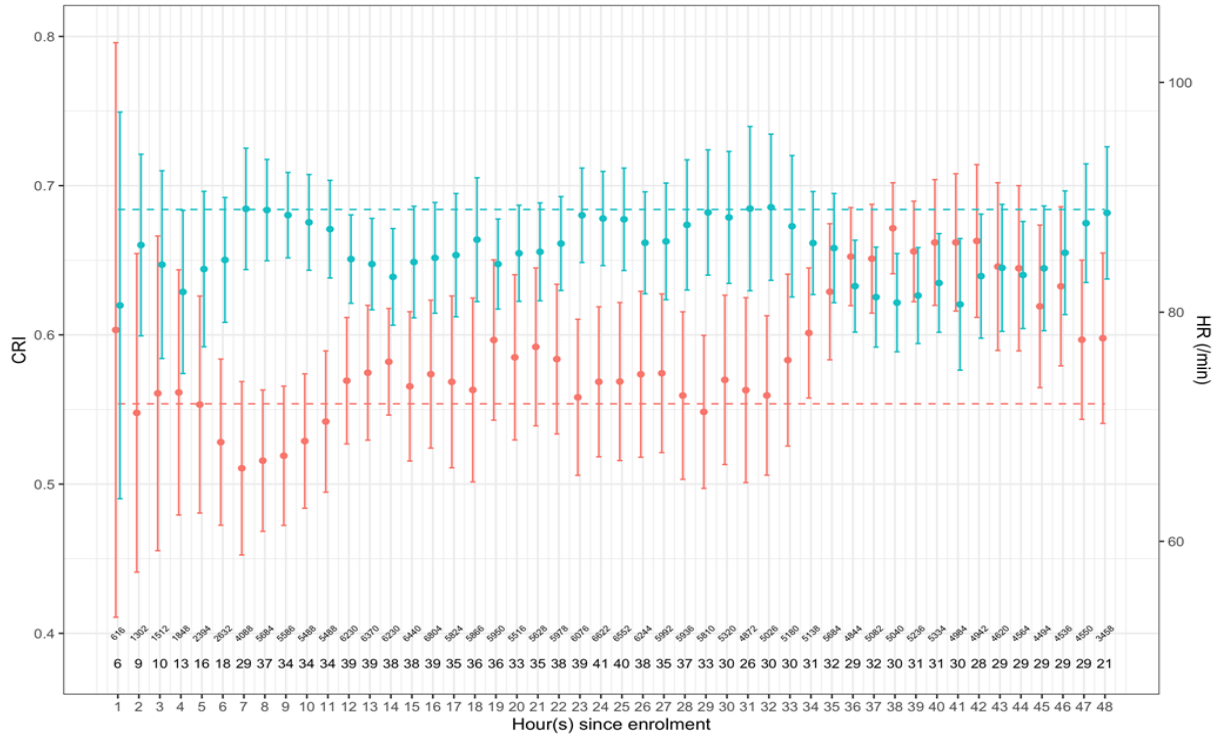


Figure 5-9. Mean compensatory reserve index (CRI) and heart rate (HR) at different time points from the onset of the initial shock episode among the 47 patients who never developed reshock.

Dots and lines represent the means and corresponding 95% confidence intervals for CRI (in red) and heart rate (in blue) at different time points from the onset of the initial shock at enrolment. Data were grouped within sequential 1-hour windows after the first shock onset before calculation and 95% confidence intervals were estimated using the bootstrap standard errors based on 1000 resamples of the original dataset (the resampling accounts for repeated measurements from the same participant). Numbers at the bottom present the number of CRI or HR measurements (upper) and the number of patients (lower) in each group.



Performance of different CRI cut-offs in predicting the first episode of reshock

CRI had a good predictive ability for reshock within 12 hours and within 6 hours before the event with AUCs of 0.84 and 0.86, respectively. The sensitivity and specificity for CRI at different cut-offs (0.2, 0.4 and 0.6) were performed. The cut-off of 0.4 showed the total highest sensitivity and specificity in predicting reshock (0.66 and 0.86, respectively) (Table 5-5). The cut-off of 0.2 had the highest specificity (0.98 (95% CI 0.97-0.99)) for predicting reshock.

Table 5-5. Performance of different CRI cut-offs in predicting the first episode of reshock

Measure	Within 12 hours of CRI measurement	Within 6 hours of CRI measurement
	Estimate (95%CI)	Estimate (95%CI)
AUC	0.84 (0.75, 0.93)	0.86 (0.77, 0.94)
CRI Cut-off 0.2		
Sensitivity	0.19 (0.02, 0.36)	0.22 (0.02, 0.42)
Specificity	0.98 (0.97, 0.99)	0.98 (0.97, 1.00)
CRI Cut-off 0.4		
Sensitivity	0.66 (0.47, 0.85)	0.68 (0.49, 0.88)
Specificity	0.86 (0.80, 0.92)	0.86 (0.80, 0.91)
CRI Cut-off 0.6		
Sensitivity	0.93 (0.84, 1.00)	0.95 (0.90, 1.00)
Specificity	0.51 (0.42, 0.59)	0.50 (0.42, 0.58)

AUC Area Under the ROC Curve
95%CI 95% confidence intervals were calculated using cluster bootstrap standard errors based on 1000 resamples of the original dataset
These results were based on CRI, without any adjustment and did not correct for optimism.

5.4.6 CRI and prediction of narrow pulse pressure

Narrow pulse pressure is the key criterion for reshock diagnosis. In practice, a narrow pulse pressure of 20 mmHg often occurs before the clinical diagnosis of reshock (which was diagnosed when a narrow pulse pressure accompanies with signs of poor peripheral perfusion).

CRI could predict the first episode of narrow pulse pressure within 6 hours before the event with an odds ratio of 1.33 but could not predict this event within 12 hours in advance (Table 5-6)

Table 5-6. Prediction of the first episode of narrow pulse pressure using CRI measurement.

	Within 12 hours of CRI measurement		**Within 6 hours of CRI measurement**	
	Estimate (95%CI)	**p**	**Estimate (95%CI)**	**p**
OR adjusted	1.24 (0.82, 1.86)	0.306	1.33 (0.96, 1.84)	0.083
OR unadjusted	0.98 (0.34, 2.77)	0.963	1.43 (1.01, 2.02)	**0.045**

For outcome narrow pulse pressure, we considered two risk periods: within 6 hours from CRI measurement and within 12 hours from CRI measurement.
OR Odds Ratio of the first reshock or narrow pulse pressure for each decrease of 0.1 in CRI. These ORs and corresponding 95% confidence intervals (95%CI) and p values were estimated from logistic regression models that used cluster bootstrap with 1000 resamples of the original dataset to take into account repeated CRI measurements from the same participant.
OR adjusted corresponds to OR after adjusting for time of CRI measurement, age, gender and body weight.
OR unadjusted corresponds to OR from the univariate model with CRI as the only covariate.

5.5 Discussion

We have shown that continuous CRI values derived from non-invasive algorithmic analysis of the pulse waveform in patients with severe dengue can predict the onset of shock. The CRI was able to predict an episode of reshock within 12 hours from measurement. CRI could predict a narrowed pulse pressure (PP $\leq$ 20 mmHg) within 6 hours from measurement. A CRI cut-off value of 0.4 had the best sensitivity (0.66 (95% CI 0.47-0.85)) and specificity (0.86 (95%CI 0.80-0.92)) to differ patients developing a recurrent shock from those did not. Of note, a CRI cut-off of 0.2 has the high specificity (0.98 [95% CI 0.97-0.99]) in predicting reshock. These results could significantly impact the management of dengue shock syndrome around the world when assessing intravascular volume remain challenged.

The CRI algorithm was developed using a simulated model of hypovolaemia, using lower limb negative pressure in young and healthy adults (assumed normal heart function and good vascular compliance), which we hypothesized would be applicable to dengue patients who tend to be children and young adults. However, we acknowledge that the vascular compliance of DSS patients would be different from other types of hypovolemic shock due to inflammatory changes and plasma leakage associated with dengue pathophysiology. The study patients were mainly children who would have different vessel compliance and shorter arterial length could affect the reflected waves, both of which could influence the CRI-algorithm. This is the first study using CRI for continuous monitoring DSS patients during 48-hour fluid resuscitation to observe how CRI changed with fluid infusion and with other vital signs. We were interested in investigating both the trend of changes and values of CRI for predicting reshock.

5.5.1 CRI versus traditional vital signs in monitoring severe patients

The difficulty of fluid management for DSS is providing just judicious fluid to balance the risk of hypovolemia and volume overload which results in respiratory distress, particularly in children (Wills B. A. et al. 2005). Accurate assessment of intravascular volume is clinically more challenging in dengue patients as hypovolemic status develops gradually due to plasma

leakage. The clinical diagnosis of shock relies on narrow pulse pressure in combination with poor peripheral perfusion. The latter is more subjective, thus, the narrow pulse pressure is the key diagnostic criterion for dengue shock. However, changes in blood pressure often present late and lack specificity in shock (Moulton S. L. et al. 2013). Additionally, invasive blood pressure monitoring techniques in severe dengue result in bleeding and many complications, therefore, they are not used routinely. The CRI operates similar to pulse oximetry which is non-invasive and based on pulse waveform analysis (Moulton S. L. et al. 2013). CRI has been clinically validated in blood donors which showed a significant decrease in CRI compared to changes in blood pressure (Nadler R. et al. 2014). In severe trauma patients, CRI also showed better ability over systolic blood pressure in predicting the need for life-saving interventions with ROC curves of 0.83 versus 0.62, $p< 0.05$ (Johnson M. C. et al. 2018). In assessing volume status, the continuous changes of CRI were accurate and specific for individual patients who were under acute blood loss. CRI values were significantly lower in symptomatic participants associated with acute blood loss compared to those without symptoms. There were 97% of symptomatic patients had CRI below 0.3 while 98% of asymptomatic participants had CRI above 0.3. (Moulton S. L. et al. 2017). In our study, we used reshock as our endpoint because it is a common clinical endpoint that is widely used in clinical practice. We also chose pulse pressure (PP) as a secondary endpoint, because a narrowed pulse pressure is a key criterion for reshock diagnosis, according to the WHO classification (World Health Organization 2012).

Although heart rate can be a good indicator of haemodynamic status, it can be influenced by many factors, including age, gender, temperature, stress, cardiac arrhythmias and pain. Furthermore, heart rate changes relatively late in shock (Lateef A. et al. 2007, World Health Organization 2012). In dengue patients, specifically, there was relative bradycardia observed which could mask deterioration of hypovolemic status (Yacoub S. et al. 2014). It is therefore required more effective ways of haemodynamic monitoring for dengue patients. There was more evidence of higher sensitivity and specificity of CRI for central blood volume status in

patients with injuries and blood loss when compared to heart rate (Moulton S. L. et al. 2013); blood pressure, and pulse pressure (Nadler R. et al. 2014, Benov A. et al. 2017, Johnson M. C. et al. 2018); peripheral perfusion index, pulse pressure variability (Janak J. C. et al. 2015), end-tidal CO2, respiratory rate, blood pH, blood lactate, and tissue oxygenation (Schiller A. M. et al. 2018). This evidence came from studies on healthy volunteers under lower body negative pressure models, blood donors or patients with trauma and acute blood loss.

5.5.2 Potential applications of CRI in dengue

In dengue-endemic countries, it is necessary to apply cost-effective monitoring techniques which are non-invasive and well tolerant to patients. This would not only help to reduce the healthcare workload for medical staff during dengue-endemic but also improve the quality of care. CRI is satisfy those criteria and CRI is useful to triage severe dengue patients who are at higher risk of reshock before clinical manifestations. This is important in clinical practice as these patients would be the ones who need more close monitoring for appropriate intervention and support. Currently, from my results, we can only recommend CRI be used for prompting more intensive monitoring rather than a change in fluid management.

In our study, we connected patients with finger probes and waveform data was transferred wirelessly to a laptop via Bluetooth. This is similar to pulse oximetry that is currently used for patients in ICU. If CRI could beintegrated into bedside monitors for multi-parameter monitoring, it would be more practical and helpful. In ICU settings, CRI can provide a continuous real-time assessment of cardiovascular status which could combine with other clinical parameters to guide individualised fluid resuscitation. In this study, when I observed remarkable changes in CRI after one hour of colloid bolus and gradual changes in CRI after one hour of a crystalloid bolus. I think CRI monitoring would be beneficial not only to identify shock and severity of shock but also to help evaluate the response to fluid therapy. Although our study was not powered to assess this association, future research will be required to answer this question. Furthermore, I think CRI could be used for dengue patients with warning signs,

out-patients or for patients in emergency or ambulatory settings with limited ability to assess diagnostics and other clinical tools. With a simple value from 0-1, CRI will become the simple parameter to quantify the intravascular volume status for a wide range of users. Regarding dengue patients with warning signs, it is possible to perform a prospective observational study using CRI to monitor these patients to investigate different levels of CRI associated with the progression of shock and to assess how rapidly changes in CRI are associated with developing shock. This study is expected to have a large sample size as the shock occurrence rate is low less than 5% for dengue patients with warning signs.

5.5.3 Strengths and limitations

The strengths of the study include its prospective design, the use of clinically-relevant endpoints and large patient sample size with the use of prolonged continuous CRI data for multiple days to construct prediction models. However, it has several limitations. Firstly, we only included 63 patients out of 103 cases and excluded many patients because of inadequate or incomplete CRI data. This reflects a disadvantage of PPG, which may be influenced by motion artefacts and poor skin perfusion which are commonly seen in patients in shock. Patients with dengue shock may have far more artefacts which affect the PPG including poor peripheral perfusion, excessive oedema, high level of blood concentration and other clinical factors that negatively affect the quality or completeness of measurement. The wireless connection between the CRI probe and the laptop is an advantage but also a weakness of this study as unstable signals often occurred due to the quality of Bluetooth. These issues became more important when we continuously monitored patients in ICU for a long period. The completion and quality of CRI data reduced significantly after 48 hours of the observation. We therefore suggest using line connection in future studies using the PPG-monitoring technique. Secondly, we used a clinical definition of reshock as a pragmatic endpoint which was decided by clinicians. The clinical assessment for this endpoint might have some subjective variation, however, it is still the most relevant outcome in dengue. We were also unable to compare CRI measurements with other

haemodynamic parameters at the time of this study such as stroke volume index (SVI) derived from invasive-arterial waveform analysis (LiDCO rapid) or SVI derived from cardiac ultrasound. Future research is warranted to investigate the association between CRI and other advanced haemodynamic parameters. Another limitation of this study is that we did not account for the effect of fluid type, fluid rate and fluid volume used in management. Fluid strategies for DSS patients varied very much depending on age range, severity at shock, initial fluid response, and fluid management before shock, therefore, we were not able to adjust for all these factors. Additionally, the study population include both adults and children but we could not stratify data by age due to the small number of adult cases. Finally, as an observational study, we could not assess the role of CRI in guiding patient management in dengue compared with standard practice. Interventional studies to compare outcomes of fluid management with or without CRI will be needed in future.

We recognised that CRI is the new parameter which is based on machine learning and arterial waveform analyses from healthy volunteer adults under mimic hemorrhagic model (Low Body Negative Pressure) and patients with trauma or under surgery. Although CRI was evaluated and validated in children and adults with different severity of bleeding and severe conditions, CRI can be further improved and specific for dengue patients. Future studies regarding analysing the morphology of PPG waveforms at different severities of dengue would help to generate a specific CRI algorithm for dengue.

5.6 Conclusion

CRI is a non-invasive and helpful method for assessing intravascular volume status in dengue shock patients. CRI can predict the onset of reshock within 12 hours of measurement and can predict narrow pulse pressure within 6 hours of measurement. The CRI cut-off of 0.4 can predict reshock with the best combination of sensitivity and specificity. The CRI cut-off of 0.2 has a specificity of 98% for reshock prediction. This study showed that CRI can support clinicians in detecting reshock earlier than clinical assessment. We also suggest a potential role

of CRI in guiding individualized fluid resuscitation for DSS patients in ICU. Future studies that include observational and interventional research are needed to investigate the benefits of using CRI in the ICU settings for guiding fluid therapy and comparing it with other hemodynamic parameters derived from different monitoring techniques (ultrasound, invasive arterial waveform analysis, bio-impedance measurement and so on). The application of CRI can be further explored in outpatients and dengue patients with warning signs.

CHAPTER 6

AN INVESTIGATION OF NOVEL MONITORING SYSTEMS FOR CHILDREN WITH DENGUE SHOCK SYNDROME

6.1 Introduction

Dengue infection, in the majority of cases, can progress to severe and potentially life-threatening manifestations including a systemic plasma leak leading to dengue shock syndrome (DSS). Although the vast majority of patients with dengue experience some evidence of plasma leak including pleural effusions and ascites, once a critical amount of plasma leaked out of blood vessels, haemodynamics ensues and eventually complete cardiovascular collapse. The increased capillary permeability and plasma leakage are transient and usually resolve around the end of the critical phase (day 6) when the extravasated fluid then gets reabsorbed through the lymphatic system. During this period, fluid overload in the form of massive pleural effusions or pulmonary oedema can occur (Yacoub S. et al. 2014). Various prognostic factors have been associated with severity of DSS, including day of illness, high haematocrit, low albumin, low platelets, evidence of fluid accumulation and high levels of venous lactate at shock were associated with recurrent shock (Yacoub S. et al. 2017). However, lactate clearance during fluid resuscitation has not been studied.

When managing patients with DSS, the balance of administering just sufficient intravenous fluid therapy to maintain hemodynamic stability while avoiding fluid overload and respiratory compromise is extremely difficult, especially where facilities for respiratory support may not be immediately available. The current method for monitoring patients with DSS relies on serial measurements of hematocrit (as a marker of hemoconcentration), together with frequent pulse and blood pressure measurements; however, these are insensitive markers of intravascular volume and the true relationships of these parameters to intravascular volume status remain unclear. For cases where recurrent shock occurs, central venous pressure monitoring is

sometimes used (World Health Organization 2012), but due to the associated coagulation disturbances in dengue, this procedure may result in significant bleeding complications. Better techniques are desperately needed for improved cardiovascular monitoring in DSS patients, especially utilizing non-invasive methods. Point-of-care ultrasound (POCUS) has been used in emergency and intensive care settings to assess intravascular volume, cardiac function and fluid leak. Ultrasound is a particularly helpful tool in DSS patients to assess fluid shifts from intravascular to extravascular space (particularly pleural and abdominal cavities. Other non-invasive haemodynamics monitoring systems are available (mentioned in chapter 1) and can provide accurate and continuous assessments of hemodynamics in patients with DSS (compensatory reserve index, bio-impedance, ultrasound cardiac output monitoring). In this chapter, I will focus on two monitoring devices, NICaS (bioimpedance device) and POCUS.

6.1.1 Intravascular volume status assessed by ultrasound

6.1.1.1 Cardiac parameters

Portable echocardiograms can help to assess volume status along with cardiac function. It has been used to assess haemodynamics for critically ill patients with different diseases and to guide fluid management both in adults and children (Cecconi M. et al. 2014, Singh Y. et al. 2020). Stroke volume index (SVI) and/or cardiac output index (CI) are commonly used by measuring cross-sectional area and velocity-time integral (VTI) at the left ventricular outflow tract in the apical-5-chamber view by pulse wave doppler. The CI derived from echocardiograms has a good correlation with CI measured by an invasive technique using thermodilution with $r = 0.943$, $p < 0.001$ (Aslan N. et al. 2020). A systematic review comparing CI derived from echocardiography versus thermodilution which included 68 studies and 1996 patients, found no significant differences between the two methods (Zhang Y. et al. 2019)

6.1.1.2 IVC diameter

The Inferior vena cava (IVC) is sensitive to intravascular volume changes and can be seen and measured easily on ultrasound. Minimum and maximum IVC diameters and IVC collapsibility

vary during the respiratory cycle and therefore can be used to estimate volume status. In spontaneous breathing patients with a normal right atrial pressure, IVC usually collapses 20 - 50% (Yildizdas D. and Aslan N. 2020, Kaptein M. J. and Kaptein E. M. 2021). When the IVC diameter is dilated and fixed during inspiration (collapsibility below 20%), this is an indicator of high right atrial pressures (ie above 10 mmHg). If IVC collapses by more than 50%, this suggests the patient is hypovolemic (Barbier C. et al. 2004, Feissel M. et al. 2004, Lang R. M. et al. 2005). IVC diameter can be also influenced by thoracic and abdominal pressure as well as high right atrial pressures, therefore, these need to be considered when assessing the IVC (Via G. et al. 2016).

In dengue, SVI, CI as well as IVC have been studied in Vietnamese adults and children with severe dengue using POCUS. The study included 103 patients, of which, 80 patients had DSS and 48/80 developed recurrent shock. The SVI at enrolment in patients with shock, recurrent shock was lower compared to patients without shock. However, there was no difference in the CI between patients with and without shock at admission. SVI and CI were still low on day 2 of ICU admission, increased slightly on day 3 and then increased to the normal range on day 4 and day 5 from ICU admission (Yacoub S. et al. 2017). Another observational study in 162 adults with dengue, of which, 17 developed shock, measured SVI and CI using a non-invasive device based on bioreactance technique (NICOM™). Measurements were made at three-time points during the day for 1-3 minutes. IVC diameters were also measured using ultrasound to estimate central venous pressure. The results showed that SVI was low from day 4 to day 15 of illness and CI was low from day 5 to day 8 of illness. In about 50% of patients with shock, their IVC diameters were less than 1.5 cm and IVC collapsibility was > 50% (Thanachartwet V. et al. 2016). There have not been any studies monitoring continuous changes of SVI or CI during fluid management in DSS patients or any describing the individual response to fluid therapy.

6.1.2 Bio-impedance technique and hemodynamic monitoring

6.1.2.1 Bio-impedance techniques

Bio-impedance analysis (BIA) is a technique using electrical properties to measure the resistance of a conductive material and then estimated its volume by applying mathematical algorithms. This method was discovered in 1871 but only applied for clinical applications in the 1990s (Kyle U. G. et al. 2004). The principle is that the resistance of a subject is proportional to their length and inversely proportional to the cross-sectional area. Each subject is not inhomogeneous in body shape and has different compartments (intracellular, extracellular, fat, bones, visceral proteins) which influence electrical conduction, therefore, the mathematical algorithm to calculate the subject's volume is complex. There are two types of resistance: 1/ reactance, which arises from the cell membrane; 2/ resistance, which arises from the intracellular and extracellular fluid. The term "impedance" describes the combination of both reactance and resistance. At zero or very low frequency, the current does not penetrate the cell membrane, allowing measurement of the extracellular fluid volume. Meanwhile, at high frequencies above 50 kHz, the current penetrates through the cells which allows measurement of total body resistance and reflects total body water.

There are several methods of bio-impedance analysis including single frequency, multi-frequency bioelectrical impedance analysis, bioelectrical spectroscopy, segmental and localized bioelectrical impedance analysis. The summary of these methods is presented in Table 6-1

Table 6-1. Methods of bioelectrical impedance analysis (Kyle U. G. et al. 2004)

Methods	Frequency	Equations	Applications	Limitations
Single frequency BIA	50 kHz	Mixture theories and empirical equations from healthy subjects	Estimate fat-free mass and TBW in healthy subjects	Not applicable for patients with dehydration
Multi-frequency BIA	Multiple frequencies (0-500kHz)	Empirical linear regression model	More accurate than SF-BIA for estimation of TBW in critically ill patients (Patel R. V. et al. 1996)	Not applicable for elderly patients
Bioelectrical spectroscopy (BIA)	Multiple frequencies from 4-100 kHz	Nonlinear mathematical modelling and prediction equations to fit impedance measurement and phase-angle data; generated in healthy populations	TBW, ICW, ECW	Accuracy of results needs further evaluation.
Segmental BIA	Multiple frequencies with electrodes put on different regions of the body	Measuring whole body BIA based on changes of FFM accounted for impedance of the trunk (about 10% of the whole body impedance)	Determine fluid shifts and fluid distribution of patients with ascites and renal failure to provide information on abdominal fluid accumulation	Accuracy needs more investigation; requires standardisation of electrodes and placement position. High error for arm and legs (Bracco D. et al. 1996)
Localised BIA	Single-frequency with electrodes put on limbs	Apply the anatomical model accounted for approximate distribution of body compartments	Focus on well-defined body areas, minimizing influences of hydration,	TWB measurement needs further validation

Methods	Frequency	Equations	Applications	Limitations
			fat and body shape → benefit in monitoring disease progression	
Bioelectrical impedance vector analysis (vector BIA)	Multiple frequencies (1-1000 kHz)	Direct measurement of the impedance vector (only influenced by impedance measurement error and biological variability)	Applicable for patients with renal diseases, critical illness or obesity	Results need further validation

BIA Bioimpedance Analysis; FFM Fat Free Mass; TBW Total Body Water

In summary, bioimpedance techniques use different frequencies and mathematical models to estimate the proportion of the different body compartments. Based on this principle, BIA will be influenced by several situations including levels of hydration, proportion of body fat, body shape or other geometrical factors. BIA can also differ by age, ethnicity and disease conditions. Therefore further studies on the different populations are needed to validate and improve the overall applicability of these techniques.

6.1.2.2 Applications of BIA in hemodynamic monitoring

During the 1960s, William Kubiceck and colleagues introduced impedance cardiography using thoracic electrical bioimpedance for astronauts (Blohm M. E. et al. 2014). They showed that changes in electrical resistance during systolic and diastolic periods reflected changes in aortic blood volume. High blood volume and high flow velocity during systole decrease impedance and vice versa. Therefore continuous measurement of bioimpedance changes every pulsatile flow could calculate SV, CO and fluid status.

There are three main approaches for using bioimpedance techniques for haemodynamic monitoring: 1/ the whole-body impedance cardiography using four pairs of sensors put on four limbs; 2/ the thoracic impedance cardiography (TIC) using two pairs of sensors put on the neck and the lower part of the chest; 3/ the regional impedance cardiography (RIC) using two pairs of sensors placed on the wrist and contralateral ankle. Some evidence suggests RIC can provide more accurate values of SV and CO over TIC (Cotter G. et al. 2006). Using patient limbs for sensor placement is preferable due to relatively uniform geometry, easier access and ease of modelling compared to other parts of the body.

6.1.2.3 NICaS

Principles

The NICaS (Non-Invasive Cardiac System, NI Medical, Petach Tikva, Israel) is a medical device using bio-impedance technique to assess and monitor cardiovascular, respiratory and fluid volume status. It provides real-time data on patients' cardiovascular functions.

NICaS has two pairs of electrodes placed on two limbs, one put on the wrist and the other put on the contra-lateral ankle. It operates using an alternating current of 1.4 mA and 32 kHz. The resistance signals are amplified and filtered then transmitted to a microprocessor where they are analysed via mathematical algorithms. The screen displays real-time waveforms and numerical patterns of ECG, body resistance and respiration. This type of electrical surveillance is called regional impedance cardiography (RIC). The principle is that the electrical conductance of blood is higher than other tissues and the conductance increases during the systolic period. NICaS measures the baseline body resistance named R, and its changes with each arterial pulsation named ΔR (Ohms). The stroke volume (SV) is calculated using the Tsogline and Frinerman formula

$$SV = \frac{\Delta R}{R} \times \rho \frac{L2}{Ri} \times \frac{(\alpha+\beta)}{\beta} \times Kw \times HF$$

ΔR is the change in impedance during the cardiac cycle (Ω), R is the basal resistance (Ω), ρ is the blood electrical resistivity, L is the patient's height, and Ri is the corrected basal resistance according to gender and age. KW is a correcting factor for body weight, HF is a hydration factor related to the body water composition, α + β is equal to the electrocardiogram R-R interval, and β is the diastolic time interval (Cotter G. et al. 2004, Yung G. L. et al. 2004, Germain M. J. et al. 2018)

Validation

NICaS has been validated in different studies in adult patients with various diseases: pulmonary hypertension, cesarean section, chronic heart failure, cardiac surgery, preeclamptic pregnancies, pheochromocytoma and acute pesticide poisoning. Taniguchi et al.(Taniguchi Y. et al. 2013) investigated 65 patients with pulmonary hypertension undergoing right heart catheterization and compared the CO derived from NICaS with CO derived from the gold standard methods of thermodilution and Fick method. The results showed a strong correlation between CO derived from NICaS and thermodilution ($r= 0.715$, $p<0.0001$) and Fick method

(r = 0.653, p<0.0001). Another study comparing CI derived from NICaS with thermodilution in a total of 122 cardiac patients during cardiac catheterization, coronary bypass surgery, and acute congestive heart failure exacerbation(Cotter G. et al. 2004). There were 418 paired CO measurements for final analyses. CI derived from NICaS had a food correlation with thermodilution CI (r = 0.886, p <0.001) and this association was similar between the different patient groups. The results of this study suggest that CO measurements from NICaS were accurate in cardiac patients. In a different cohort of 8 preoperative patients with pheochromocytoma CO was investigated with central blood volume measured by dilution method (Matsuda Y. et al. 2014). CO from NICaS correlated well with central blood volume (r = 0.906, p = 0.002). In a study using doppler echocardiography in 17 patients receiving hemodialysis, Michael et al. found that SV from NICaS had a strong correlation with SV from echocardiography during the first hours (r = 0.93, p<0.001) and last hours of treatments (r = 0.92, p < 0.001). The authors of this study suggested that NICaS may be a practical method to monitor SV during hemodialysis (Germain M. J. et al. 2018).

NICaS has been used to monitor haemodynamic parameters in a study of 17 pregnant women with severe preeclampsia and compared with 62- healthy-singleton pregnancies at term (Lavie A. et al. 2018). The results showed that CO was lower and total peripheral resistance was higher in the preeclampsia group. The authors concluded that NICaS may be helpful to monitor and optimise management for pregnant women. In another study by Orbach-Zinger et al., NICaS was used to monitor SV, CO of 137 parturients with either singleton or twin pregnancies at different time points during cesarean sections (Orbach-Zinger S. et al. 2019). There was no difference of haemodynamics during surgery between the two groups. However, women with twin pregnancies developed significantly lower CO after surgery, which needs to be investigated further in future studies. Recently, a study conducted in China investigated the clinical value of NICaS in haemodynamic monitoring guiding management of patients with acute pesticide poisoning (n= 68), compared with transpulmonary thermodilution method (PICCO) (n= 67) and empirical treatment (n= 65) (Gao X. et al. 2020). The results of this

study showed that patients monitored by NICaS or PICCO received lower daily average intravenous volume and had lower mortality compared to those with empirical treatment.

NICaS has also been validated in the paediatric population. Beck et al. conducted a study using NICaS and transesophageal Doppler (Car-dioQ ®) to monitor SVI and CI in 42 children undergoing cardiac, urologic and orthopaedic surgery (Beck R. et al. 2018). NICaS electrodes were positioned either on wrist-ankle or wrist–wrist and measured SVI, CI before surgery (T0) and during the first hour of surgery (T1-60). 10 ml/kg of crystalloid was infused intravenously during the monitoring period. The results showed a good Pearson correlation between the two techniques in measuring CI ($r= 0.85$, $p < 0.01$).

Overall, multiple studies have shown the relative accuracy of NICaS for haemodynamic monitoring in patients with different critical illnesses. However, to the best of our knowledge, there has been no study investigating this non-invasive technique for severe dengue patients.

6.1.3 Lactate and lactate clearance

Lactate is produced by multiple tissues with high levels found in muscle (van Hall G. 2010). The metabolism of lactate is complicated (James J. H. et al. 1999). Increased lactate levels are associated with different causes including accelerated glycolysis, hypoxemia, hypoperfusion, as well as reduced lactate removal due to increased sympathoadrenal activity, (increased adrenaline) and liver and renal dysfunction (Gladden L. B. 2004). Hyperlactatemia which often follows severe injury, haemorrhage or sepsis is thought to be due to anaerobic glycolysis from insufficient oxygen delivery and the result of an adrenaline surge which stimulates Na+--K+ - ATPase activity. In severe malaria, hyperlactatemia is a result of parasitized erythrocyte sequestration in the microcirculation rather than the reduced total oxygen delivery (Kingston H. W. et al. 2019). High lactates may also be secondary to the liver injury that often occurs in various severe infections, either due to direct cytotoxicity or from hypoperfusion resulting in both increased production and decreased clearance. Elevated levels of lactate are associated with multi-organ failure and mortality in septic shock (Shapiro N. I.

et al. 2005, Thomas-Rueddel D. O. et al. 2015) and therefore has been used as a biomarker in sepsis management. In the third international consensus definitions for sepsis and septic shock (sepsis-3), lactate levels greater than 2 mmol/L in the absence of hypovolemia and requirement of vasopressor are the criteria for the definition of septic shock. And a lactate level of more than 4 mmol/L is an indication for initiation of fluid resuscitation. Lactate clearance has been investigated as a target for therapeutic response. Lactate clearance achieved in the early phase of the resuscitation was an indicator of improved global tissue hypoxemia and was associated with better outcomes of septic shock (Nguyen H. B. et al. 2004, Nguyen H. B. et al. 2010). Research in this area suggested that lactate clearance of more than 10% after at least 2 hours from resuscitation initiation was helpful to assess early response for patients with severe sepsis (Jones A. E. 2013, Pan J. et al. 2019). Research in pediatric patients has also shown that lactate levels are an independent predictor of mortality in septic shock (Jat K. R. et al. 2011, Bai Z. et al. 2014). Lactate can be either measured in arterial blood or venous blood with good associations between them with a mean difference of 0.681 mmol/L (Mahmoodpoor A. et al. 2020). For clinical practice, venous lactate is easier and often used more commonly as an initial surrogate for further arterial blood gas assessment (Samaraweera S. A. et al. 2017, van Tienhoven A. J. et al. 2019).

Point of care (POC) lactate measurement from finger prick blood has been studied in emergency settings to allow rapid results. POC lactate has shown a good correlation with arterial lactate in healthy volunteers and intensive care patients (Raa A. et al. 2020). Another study demonstrated normal POC lactate levels were associated with subsequent normal serum lactate measurements with 100% accuracy (Swan K. L. et al. 2018). In addition, POC lactate can be used as a screening tool for pre-hospitalised patients (Swan K. L. et al. 2019). A 12-month-retrospective study in an Australian hospital showed that higher POC lactate levels measured prehospital admission were associated with mortality. However, there are only a few studies assessing the benefit of using POC lactate in ICUs so more work is needed in this setting.

In dengue, venous lactate has been investigated at ICU admission for predicting shock (Thanachartwet V. et al. 2016) and recurrent shock (Yacoub S. et al. 2017). Vipa Thanachartwet et al. investigated the predictive ability of venous lactate and procalcitonin for dengue shock and /or organ failure (Thanachartwet V. et al. 2016). Of 160 Thai patients recruited with dengue, 32 (20%) patients developed shock or organ failure. Peripheral venous lactate ≥ 2.5 mmol/L was associated with the outcome of shock/organ failure (OR 27.99, 95CI 8.47-92.53, p <0.002). A combination of procalcitonin ≥ 0.7 ng/ml and venous lactate ≥ 2.5 mmol/L at enrolment had a sensitivity of 81% and specificity of 84% for predicting shock and/or organ failure. The same study group assessed the utility of venous lactate at hospital admission of 125 dengue patients in predicting severe dengue, 20 patients developed severe dengue. The results showed that venous lactate ≥ 2.5 mmol/ L provided a sensitivity of 65% (95% CI 41%-84%) and specificity of 96% (95% CI 91%- 99%) for identifying severe dengue (Thanachartwet V. et al. 2016). Venous lactate was also an independent risk factor for predicting recurrent shock, according to the results of a study in Vietnam (Yacoub S. et al. 2017). S Yacoub et al. performed a prospective study in paediatric and adult ICUs. This study recruited 102 patients with dengue. Of 80 patients who developed shock, 32 patients subsequently developed two episodes of recurrent shock. Venous lactate level at ICU admission could predict recurrent shock with an OR of 1.46 (p = 0.004). However, to date, there have not been any studies assessing the utility of POC lactate and lactate clearance in predicting outcomes of severe dengue.

In summary, there are several knowledge gaps regarding intravascular volume changes during fluid management for DSS. In addition, lactate levels and lactate clearance during resuscitation of DSS patients have not been investigated. There were few studies assessing the use of non-invasive haemodynamic monitoring techniques for severe dengue. The overall aim of this study is to evaluate the utility of novel devices in assessing detailed intravascular volume changes and responses to fluid resuscitation.

Specific aims:

1. To describe changes in SVI during the first hour before and after a fluid bolus; SVI changes during 48 hours of fluid management,
2. To explore associations between SVI and traditional vital signs (pulse, blood pressure, hematocrit).
3. To investigate associations between echo-derived SVI and NICaS-derived SVI
4. To quantify the amount of fluid leakage, as assessed by ultrasound (pleural effusion size, thickness of gallbladder's wall).
5. To describe changes in serial point-of-care (POC) lactates during the first 6 hours of resuscitation and the association between venous lactate with POC lactate.
6. Investigate clinical and haemodynamic parameters in predicting clinical outcomes of patients with DSS (reshock and respiratory distress).

6.2 Methods

This is a prospective observational study conducted in the Paediatric Intensive Care Unit of the Hospital for Tropical Diseases (HTD) from December 2018 to December 2020. The full protocol is described in chapter 2. Briefly, children above 5 years of age with DSS, who were diagnosed at PICU admission and had not received fluid resuscitation elsewhere, were recruited. Following an initial consent process, enrolled patients were rapidly assessed by bedside cardiac ultrasound prior to fluid infusion, one hour later and then at multiple pre-defined time points during fluid resuscitation. The following parameters were measured, SVI, EF, inferior vena cava diameter, pleural effusion diameter, and gallbladder wall thickness. At the same time, the NICaS was connected to the patients to start recording the continuous haemodynamic parameters including heart rate (HR), stroke volume index (SVI), cardiac index (CI), and total body water (TBW). Patients were reviewed daily until discharge or for up to 4 days and at follow-up 1-2 weeks later. Detailed information on fluid volumes and hourly vital signs were documented in a case report form.

6.2.1 Portable echocardiography

Echocardiography was performed at the bedside at multiple time points (T0, T1, T3, T6, T12, T24 and repeated if reshock occurred) using a phased array transducer (C2-8, 2-5 MHz, Samsung Medison U6 My Sono, Korea) for assessing stroke volume and cardiac function; a convex transducer (P2-4, 2-6 MHz, Samsung Medison My Sono, Korea) for assessing IVC diameters, gallbladder wall thickness and pleural effusion diameter. Two-dimensional, M-mode and Doppler studies were performed. From the parasternal long-axis view, the following measurements were performed: aortic valve diameter at mid systole; left ventricular end-diastolic (LVEDD) and left ventricular end-systolic (LVESD) dimensions were measured below the tip of the mitral valve. From the apical five-chamber view, pulse wave Doppler was used with the sample volume positioned just below the aortic valve cusps to measure velocity time integrals (VTIs) at the left ventricular outflow tract (LVOT). From the subcostal view, the maximal and minimal diameters of IVC during expiration and inspiration were measured below the level of the hepatic veins, 5 cm from the inferior vena cava and right atrial junction, using M-mode recordings. Additionally, gallbladder wall thickness (GBW) was measured from the anterior abdominal wall in the right midclavicular line. Right and left pleural effusion size was also assessed at the same time points on lateral scans using the same position each time. All the images and/or 3-second video clips were stored digitally for further review. Each measurement was performed three times and an average was calculated. Stroke volume index, ejection fraction (EF) and IVC collapsibility index (IVCCI) were calculated using the following equations:

$$SV = crossectional\ area\ of\ the\ aortic\ valve \times VTI$$

$$= \pi \times (aortic\ diameter/2)^2 \times VTI$$

$$Body\ surface\ area\ (BSA) = \sqrt{\frac{height\ (cm) \times weight(kg)}{3600}}$$

$$SVI = SVI/BSA$$

$$EF\ \% = \frac{LVEDD^3 - LVESD^3}{LVEDD^3} \times 100$$

$$IVCCI = \frac{IVC\ max - IVC\ min}{IVC\ max}$$

6.2.2 Continuous hemodynamic monitoring using NICaS

The methods section for NICaS monitoring has already been described in chapter 2. Briefly, NICaS monitors haemodynamic parameters through two sensors which are fixed onto the patient's wrist and the opposite ankle. Data including age, gender, height, weight, blood pressure, haematocrit, and serum electrolyte levels were entered into the monitor. Output data is shown and updated every 20 seconds and stored in time-stamped excel files. The NICaS data was recorded from enrolment until discharge or up to 4 days.

6.2.3 Statistical analysis

6.2.3.1 Data cleaning & derivations

All clinical and echo data were entered into the secured database of OUCRU named Clires which was exported in an excel format. Continuous data from each patient were plotted in combination with 10 random checks with CRF to explore outliers and missing data. Each patient had between 5 to 350 NICaS excel files (depending on how long they were monitored for) that were then combined. Subsequently, each haemodynamic parameter for each patient was plotted. As this study was to describe the changes of haemodynamic parameters longitudinally, no outlier ranges were applied to exclude NICaS data. The mean value over 5 minutes was used to limit the variability and noise of the continuous data. Patients' BMI classification was performed using CDC 2000 references for boys and girls (from 2-20 years old): under-nourished was defined if BMI was below 5th percentile; overweight if BMI was from 85th -95 percentile; and obesity if BMI was equal to or more than 95th percentile. Lactate clearance was the difference between the lactate at the predefined time point and the lactate at the first shock (T0).

6.2.3.2 Variable and endpoint definitions

DSS patients were classified into DSS and severe DSS. DSS was defined as the patient has a pulse pressure of 15 to 20 mmHg at shock onset. Severe DSS was defined as the patient has a pulse pressure below or equal to 10 mmHg or unmeasurable blood pressure at shock onset.

The primary endpoint of this study is clinical fluid responsiveness to the fluid bolus in the first hour of resuscitation. The secondary endpoints are recurrent shock and respiratory distress. The clinical fluid response was defined as the decision to reduce the fluid rate in the second hour following the initial bolus in the first hour. Reshock was defined as another episode of shock occurring after 6 hours from the initial shock stability. Shock stability was the time point when systolic blood pressure was normal and pulse pressure was equal to or more than 25mmHg for at least 6 consecutive hours. Respiratory distress was defined if there was obvious efforts to use respiratory muscles or an increase in respiratory rate (age 3-5: more than 30 breath/minute; age 6-12: more thạn breaths/minute; age 12-16: more than 20 breaths/minute) or requirement of respiratory support (nasal continuous positive airway pressure NCPAP or mechanical ventilation)

6.2.3.3 Statistics

Descriptive results were presented in median (interquartile range) for continuous variables and proportion (percentage) for categorical variables. The trajectory of SVI for each patient was graphically described using a time plot of haemodynamic parameters of interest. The time-points T1 and T0 were used to evaluate fluid responsiveness (ie the first hour of resuscitation). For the continuous NICaS data, we used average of 5 minutes for SVI to limit noise and variability. The trajectory of SVI was plotted for the 48 hours of fluid resuscitation. Fisher exact test was used to compare values of categorical variables and the Wilcoxon rank-sum test to compare values of continuous variables. The association between venous lactate and point of care lactate was assessed using linear regression. Associations between SVI values derived from echo with SVI from NICaS, SBP, DBP, PP and haematocrit were assessed using partial Pearson's correlation coefficient. Overall correlation among subjects was

estimated based on Fisher transformation and the bootstrap standard errors using 1000 resamples to account for repeated values. Logistic regression was used to assess the association between clinical and haemodynamic parameters with outcomes with or without adjustment for potential confounders. All analyses were performed using R version 4.0.3 with the following packages: readxl, C306, tidyverse, ggplot2, ggplotgui, mosaic, arsenal, Hmisc.

6.3 Results

6.3.1 Characteristics of patients at enrollment

A total of 92 patients were enrolled into the study, of which 90 cases participated in both two monitoring periods. The median age was 12 years (IQR 10-13). The proportion of female patients was 38/92 (41%), and 37/92 (40%) of DSS patients were overweight/obese. There was no difference in the day of illness at shock, respiratory rate, haematocrit, platelets, creatinine or liver transaminase between DSS (pulse pressure > 10 mmHg) and severe DSS (pulse pressure ≤10 mmHg or unmeasurable blood pressure). Severe DSS patients had a higher heart rate (120 beats/min [IQR 110-127]) and higher level of Urea (5.07 mmol/L[IQR 3.9-9.1]). Median venous lactate was 3.2mmol/L (IQR 2.5-3.8) for all patients and there was no difference between the two severity groups (Table 6-2).

Table 6-2. Characteristics of study patients at enrolment

Characteristics at enrollment	N	All (n= 92)	DSS (n= 75)	Severe DSS* (n=17)	p
Age (years)	92	12 (10,13)	12 (10,13)	10 (8,13)	0.300
Female	92	38 (41)	29 (39)	9 (53)	0.292
Weight (kg)	92				
- Obese		22 (24)	18 (24)	4 (23)	
- Overweight		15 (16)	12 (16)	3 (18)	0.721
- Normal weight		45 (49)	38 (51)	7 (41)	
- Underweight		10 (11)	7 (9)	3 (18)	
Day of illness at shock	92	5 (5,6)	5 (5,6)	5 (5,6)	0.141
Pulse	92	110 (105, 121)	110 (104, 120)	120 (110, 127)	**0.03**
SBP	86**	100 (90,100)	100 (90, 100)	100 (90, 102)	0.884
DBP	86**	80 (75,85)	80 (70, 82)	90 (80, 95)	0.004
Pulse pressure	92	20 (15,20)	20 (20, 20)	10 (5, 10)	<0.001
Temperature (°C)	92	37.0 (37.0, 37.4)	37.1 (37.0, 37.5)	37.0 (37.0, 37.2)	0.208
Respiratory rate (breaths/min)	92	24 (22, 26)	24 (22, 26)	24 (22, 26)	0.591
Laboratory					
Haematocrit (%)	92	46 (43, 50)	46 (43, 49)	47(44, 51)	0.466
Platelets (×1,000 cells/µL)	92	30 (20,40)	32 (22, 40)	29 (17, 34)	0.277
WBC (k/µL)	92	4.51 (0.99, 2.40)	4.35 (3.37, 6.19)	5.36 (4.12, 6.45)	0.216
AST (IU/L)	84	135 (92, 240)	144 (91, 249)	107 (93, 169)	0.605
ALT (IU/L)	84	58 (35, 116)	65 (36, 122)	45 (32, 83)	0.533
Urea (mmol/L)	34	3.65 (2.9, 4.6)	3.50 (2.90, 4.45)	5.70 (3.90, 9.10)	**0.027**
Creatinine (umol/L)	81	54 (47, 62)	54 (47, 59)	59 (44, 76)	0.223
Na+ (mmol/L)	72	131 (129, 134)	132 (129, 133)	129 (125, 135)	0.297
K+ (mmol/L)	72	3.9 (3.5, 4.3)	3.9 (3.5, 4.3)	4.1 (3.8, 4.5)	0.230
Cl- (mmol/L)	72	95 (93, 99)	95 (93, 99)	95 (91, 97)	0.543
Ca++ (mmol/L)	49	1.95 (1.18, 2.12)	1.95 (1.18, 2.11)	1.95 (1.39, 2.09)	0.801
Mg++ (mmol/L)	46	0.81 (0.76, 0.88)	0.81 (0.76, 0.88)	0.81 (0.78, 0.89)	0.571

Albumin (g/L)	92	36.7 (33.0, 39.2)	36.6 (33.1, 39.2)	37.0 (33.0, 39.2)	>0.999
Venous Lactate (mmol/L)	92	3.2 (2.5, 3.8)	3.1 (2.4, 3.8)	3.2 (2.7, 3.8)	0.546
Glucose (mg/dl)	86	130 (112, 142)	130 (112, 143)	125 (117, 139)	0.724

DSS Dengue Shock Syndrome, SBP Systolic Blood Pressure, DBP Diastolic Blood Pressure, PP Pulse Pressure (PP= SBP-DBP). WBC White Blood Cell, AST Aspartate aminotransferase, ALT Alanine aminotransferase. Results are presented in median (IQR) for continuous data and absolute count (%) for categorical variables. N is the number of measurements. The percentages are calculated vertically.

* Severe DSS is defined if PP $\leq$ 10 or low systolic blood pressure for age.

** For immeasurable blood pressure at shock, PP was imputed equal to 5 mmHg for statistical report and analyses

6.3.2 Fluid management and outcomes of study patients

Outcome data from 90 patients with the complete dataset for all time points are shown in Table 6-3. The prolonged shock occurred in 7/73 patients in the DSS group but none occurred in the severe DSS group. Of the total cohort 16/90 (18%) cases developed reshock and among them, 4 developed a further episode of reshock.

The severe DSS group had a higher proportion of cases that had reshock compared to the DSS group (35% versus 14%, $p = 0.071$). The median time from initial shock to the first episode of reshock and the second reshock was 14 hours (IQR 11-17) and 25 hours (IQR 25-26), respectively. The median duration of fluid therapy for all study patients was 27 hours (IQR 25-31). The median total volume of fluid resuscitation for all study patients was 130 ml/kg (IQR 122-149). 10/90 patients had signs of respiratory distress but none required non-invasive or mechanical ventilatory support. The median length of stay in the ICU was 3 days (IQR 3-4 days). No patients had severe bleeding or acute kidney injury and there were no deaths.

Table 6-3. Patient outcomes

Characteristic	N	All	DSS n = 73[1]	Severe DSS n = 17[1]	p
Prolong shock	90	7	7 (10%)	0 (0%)	0.339
Reshock	90	16	10 (14)	6 (35)	0.071
Number of reshock 1		16 (18)	7 (10)	5 (29)	0.99
Number of reshock 2			3 (4)	1 (6)	
Time to reshock 1	16	14 (11,17)	14 (8,16)	16 (13, 17)	0.533
Time to reshock 2	4	25 (25, 26)	25 (25, 25)	29 (29,29)	0.500
Colloid (ml/kg)	34	35 (35, 47)	35 (33, 43)	36 (35, 58)	0.032
Crystalloid (ml/kg)	90	123 (117, 134)	125 (118, 136)	95 (89, 118)	<0 001
Total volume (ml/kg)	90	130 (122, 149)	129 (121, 144)	133 (125, 175)	0.089
Fluid duration (hours)	90	27 (25, 31)	27 (24, 30)	27 (27, 35)	0.123
Respiratory distress	90	10 (11)	6 (9)	4 (24)	0.090
NCPAP or ventilation	90	0	0	0	
PICU duration (days)	90	3 (3, 4)	3 (3, 4)	4 (3, 4)	0.033
Death	92	0	0	0	

DSS Dengue Shock Syndrome, Severe DSS is defined as patients with PP <= 10 or low systolic blood pressure for age. Prolonged shock is defined as patients with another episode of PP<= 20 mmHg within the first 6 hours from initial shock. Reshock is defined as another episode of shock occurring after 6 hours from the initial shock recovery time. Results are presented as median values (IQR) for continuous data and absolute count (%) for categorical variables. N is the number of measurements. The percentages are calculated vertically. Fisher's exact test was used to compare two values of categorical variables and Wilcoxon rank sum test to compare two values of continuous variables.

6.3.3 Fluid response

6.3.3.1 Characteristics and outcomes of patients by clinical fluid response

In the first hour of fluid resuscitation, patients with DSS received 15 ml/kg of crystalloid and patients with severe DSS received 15 ml/kg of HES 200/0.5 as per standard hospital guidelines. The clinical fluid response was assessed after one hour of resuscitation. Responders were defined as patients who received a reduced fluid rate in the second hour (ie a clinical decision had been made by the attending physician to reduce the rate of fluid guided by the status of the patient). Non-responders were defined as patients who received colloid with the rate of 15 ml/kg in the second hour. The characteristics and outcomes of responders vs. non-responders are presented in Table 6-4 and Table 6-5. At enrollment, there was no difference in baseline characteristics of responders vs non-responders (Table 6-4) or clinical outcomes of responders vs non-responders (Table 6-5).

Table 6-4. Characteristics of patients by clinical fluid response at one hour

Characteristics at enrollment	N	All (n= 92)	Non-response (n= 5)	Response (n=87)	p
Age (years)	92	12 (10,13)	10 (10, 12)	12 (10, 13)	0.380
Female	92	38 (41)	2	36	0.292
Weight (kg)	92				
- Obese		22 (24)	0	22	
- Overweight		15 (16)	2	13	0.220
- Normal weight		45 (49)	2	43	
- Underweight		10 (11)	1	9	
DOI at shock	92	5 (5,6)	5 (5,6)	5 (5,6)	0.889
Pulse	92	110 (105, 121)	120 (120, 120)	110 (105, 122)	0.192
SBP	86**	100 (90,100)	90 (90, 100)	100 (90, 100)	0.403
DBP	86**	80 (75,85)	75 (75, 80)	80 (75,85)	0.577
Pulse pressure	92	20 (15,20)	15 (15, 20)	20 (15, 20)	0.335
Temperature (°C)	92	37.0 (37.0, 37.4)	37 (37.0, 37.5)	37.0 (37.0, 37.4)	0.993
Respiratory rate (breaths/min)	92	24 (22, 26)	25 (25, 26)	24 (22, 26)	0.098
Laboratory					
Haematocrit (%)	92	46 (43, 50)	48.5 (47.3, 49.1)	45.8 (43.2, 49.7)	0.318

Platelets (×1,000 cells/µL)	92	30 (20,40)	37(34,43)	29 (20, 40)	0.502
WBC (k/µL)	92	4.51 (0.99, 2.40)	4.35 (3.37, 6.19)	5.36 (4.12, 6.45)	0.216
AST (IU/L)	84	135 (92, 240)	110 (92, 160)	139 (92, 241)	0.741
ALT (IU/L)	84	58 (35, 116)	51 (44, 54)	65 (34, 116)	0.806
Urea	34	3.65 (2.9, 4.6)	5 (4.4, 5.6)	3.55 (2.9, 4.5)	0.288
Creatinine (umol/L)	81	54 (47, 62)	52 (52, 53	54 (47, 62)	0.382
Na+ (mmol/L)	72	131 (129, 134)	130 (130, 135)	131 (129, 134)	0.816
K+ (mmol/L)	72	3.9 (3.5, 4.3)	4 (3.8, 4.2)	3.9 (3.5, 4.3)	0.658
Cl- (mmol/L)	72	95 (93, 99)	95 (94, 97)	95 (92, 98)	0.965
Ca++ (mmol/L)	49	1.95 (1.18, 2.12)	1.98 (1.61, 2.01)	1.94 (1.17, 2.12)	0.884
Mg++ (mmol/L)	46	0.81 (0.76, 0.88)	0.76 (0.71, 0.81)	0.81 (0.77, 0.88)	0.500
Albumin (g/L)	92	36.7 (33.0, 39.2)	36.8 (27.5, 37.2)	36.6 (33.1, 39.2)	0.454
Venous Lactate (mmol/L)	92	3.2 (2.5, 3.8)	3.17 (2.54, 3.36)	3.14 (2.46, 3.78)	0.843
Glucose (mg/dl)	86	130 (112, 142)	129 (126, 167)	130 (112, 140)	0.631

Table 6-5. Patient outcomes by clinical fluid response at one hour.

Characteristic	N	All	Non response n = 5	Response n = 85	p
Prolong shock	90	8	5(100)	3 (4)	
Reshock	90	16	1 (20)	15 (18)	>0.999
Number of reshock					0.250
1			0	12 (80)	
2			1 (100)	3 (20)	
Colloid (ml/kg)	34	35 (35, 47)	43 (39, 43)	35 (34,48)	0.180
Crystalloid (ml/kg)	90	123 (117, 134)	101 (98, 118)	123 (117, 134)	0.101
Total volume (ml/kg)	90	130 (122, 149)	144 (141, 153)	127 (121, 149)	0.037
Fluid duration (hours)	90	27 (25, 31)	29 (28, 32)	27 (25, 31)	0.355
Respiratory distress	90	10 (11)	1 (20)	9 (11%)	0.450
PICU duration (days)	90	3 (3, 4)	3 (3, 4)	3 (3, 4)	0.667

Clinical fluid response was defined as the decision to reduce the fluid rate in the second hour following the initial bolus in the first hour.

Prolonged shock is defined as patients who experienced another episode of PP<= 20 mmHg within the first 6 hours from initial shock. Reshock is defined as another episode of shock occurring after 6 hours from the initial shock recovery time. Results are presented as median (IQR) for continuous data and absolute count (%) for categorical variables. N is the number of measurements. The percentages are calculated vertically. Fisher's exact test is used to compare two values of categorical variables and Wilcoxon rank-sum test is used to compare two values of continuous variables.

6.3.3.2 Changes in stroke volume index and haematocrit of patients by clinical fluid response

In clinical practice DSS and severe DSS are managed differently, therefore the difference in clinical fluid response was compared between the two severity groups. All 17 severe DSS patients receiving colloid achieved clinical fluid response, whereas 70/75 (93%) of DSS patients receiving crystalloid fluid achieved clinical response (5/70, 7% did not respond). All patients had normal ejection fraction with a median of 64% (IQR 60-69). The median value of SVI at initial shock of patients with severe DSS was significantly lower than those with DSS (14 ml/m2 (IQR 12 -19) versus 17 ml/m2 (IQR 14, 21), p = 0.007). A bolus of 15 ml/kg/h of HES 200/0.5 increased SVI by 84% (IQR 58-109) in severe DSS while a bolus of 15 ml/kg/h of crystalloid in DSS increased SVI by 33% (IQR 19-47). After one hour of a fluid bolus using colloid and crystalloid, the median SVI measured by echo increased to 27 ml/m2 in severe DSS (IQR 22-35) and 24 ml/m2 in DSS (IQR 20-28), respectively. There was no difference between the median haematocrit (Hct) measured at initial shock between patients with severe DSS and DSS (52% (IQR 50-55) versus 50% (47-52), p = 0.1). There was however a difference in the median Hct at one hour with severe DSS patients who received colloid having a decrease in Hct on average by 9% to 42% (IQR 40-44) while DSS patients who received crystalloid decreased their Hct on average by 4 % to 45% (IQR 42-48), p = 0.028. Both the SVI and Hct at enrollment (T0) were similar in patients with severe DSS and DSS (Table 6-6).

Table 6-6. Stroke volume index and haematocrit stratified by fluid types and clinical fluid response

	Severe DSS (Colloid 15 ml/kg/h) n= 17	DSS (Crystalloid 15ml/kg/h) n=75		p*
	Response, N = 17	Non-response, N = 5	Response, N = 70	
SVI T0	14(12, 19)	14 (14, 17)	18 (15, 21)	0.007
SVI T1	27 (22, 35)	20.9 (17, 25)	24 (21, 28)	0.165
%SVI change	84 (58, 109)	33 (21, 37)	33 (19, 47)	<0.001
Hct T0	52 (50, 55)	52(50, 53)	50 (47, 52)	0.10
Hct T1	42(40, 44)	48 (45, 48)	45 (42, 48)	0.028
Hct change	9 (8,11)	6 (5, 6)	4 (3, 6)	<0.001

SVI Stroke Volume Index

Results are presented as median (IQR). T0 is the initial shock time. T1 is one hour post shock.

*p-value represents comparisons between DSS and severe DSS using Wilcoxon rank sum test

Clinical fluid response was defined as clinical improvement and the decision to reduce the fluid rate in the second hour following the initial bolus in the first hour. Non-responder was defined as a patient who did not improve clinically and received a colloid bolus of 15ml/kg/h in the second hour

6.3.3.3 Performance of different SVI cutoffs at T1 to detect fluid response

We used different cutoffs of SVI at T1, based on the range of SVI from the lowest to the normal physiological values. The SVI cutoff of 30 had the highest specificity of 100% and zero false-positive rates to detect fluid response at T1 (Table 6-7).

Table 6-7. Performance of different SVI cutoffs in detecting clinical fluid response

SVI cutoff	Sensitivity	Specificity	False positive rate	False negative rate
14	0.97	0.00	1.00	0.02
16	0.94	0.20	1.00	0.06
18	0.90	0.40	0.60	0.09
20	0.79	0.40	0.60	0.21
22	0.64	0.60	0.40	0.36
24	0.54	0.60	0.40	0.46
26	0.40	0.80	0.20	0.60
28	0.26	0.80	0.20	0.74
30	0.21	1.00	0.00	0.78

6.3.3.4 Fluid response at reshock

All patients who experienced reshock achieved a clinical fluid response with a 10 ml/kg/hour of colloid. The median SVI of patients at the first and the second episode of reshock were 18 ml/ m2 (IQR 16-21) and 16 ml/m2 (IQR 15, 17), respectively. The use of 10 ml/kg/h HES 200/0.5 for resuscitation of reshock episodes increased the median SVI by 53 % at the first reshock and by 52 % at the second reshock. Results were shown in Table 6-8.

Table 6-8. SVI changes at reshock after one hour of 10 ml/kg of colloid bolus

SVI (ml/m2)	1st Reshock 1 (n=16)	2nd Reshock (n=4)
T0	18 (16, 21)	16 (15, 17)
T1	29 (23,33)	25 (24, 26)
%SVI change	53 (41,62)	52 (49,59)

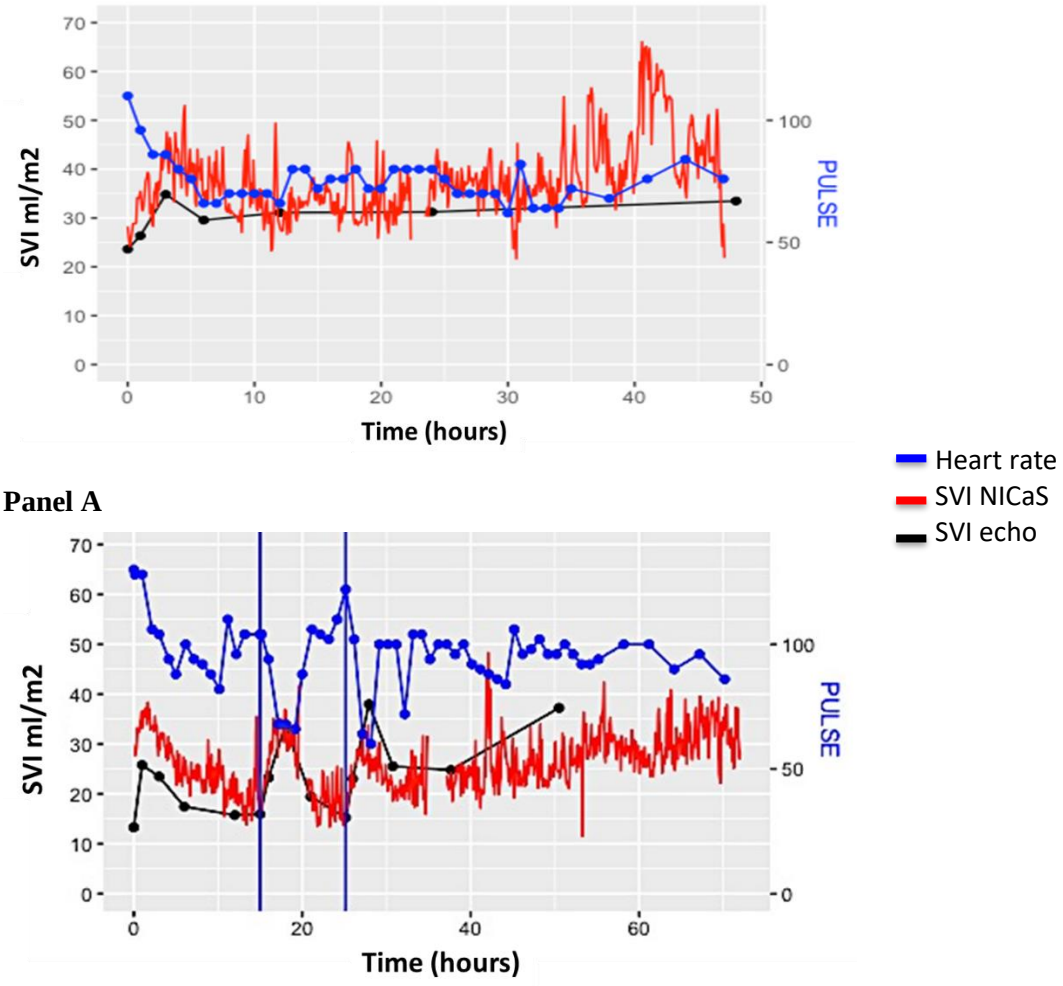
SVI Stroke Volume Index. Results are presented as median (IQR). T0 is the first shock time. T1 is one hour from shock

6.3.4 Dynamic changes in intravascular volume during fluid management

6.3.4.1 SVI and HR changes

Echo and NICaS-derived SVI trajectories during fluid management of two patients are presented in Figure 6-1. The graphical representation of all 92 cases are shown in Figure 6-2. DSS patients with prolonged shock and/or reshock were managed with different fluid strategies, therefore they were excluded from the graphical description of SVI within 6 hours and 48 hours (Figure 6-3). SVI was lowest at T0, peaking around T3. At T6, SVI decreases slightly but rises again to within the normal levels there after.

Figure 6-1. Dynamic changes of echo and NICaS-derived Stroke Volume Index (SVI) and heart rate (HR) during ICU stay.



Panel A

Panel B

Panel A shows trajectories of echo-derived SVI (in black), NICaS-derived SVI (in red) and HR (in blue) of a DSS case without reshock. Panel B shows trajectories of those parameters in a patient with two episodes of reshock

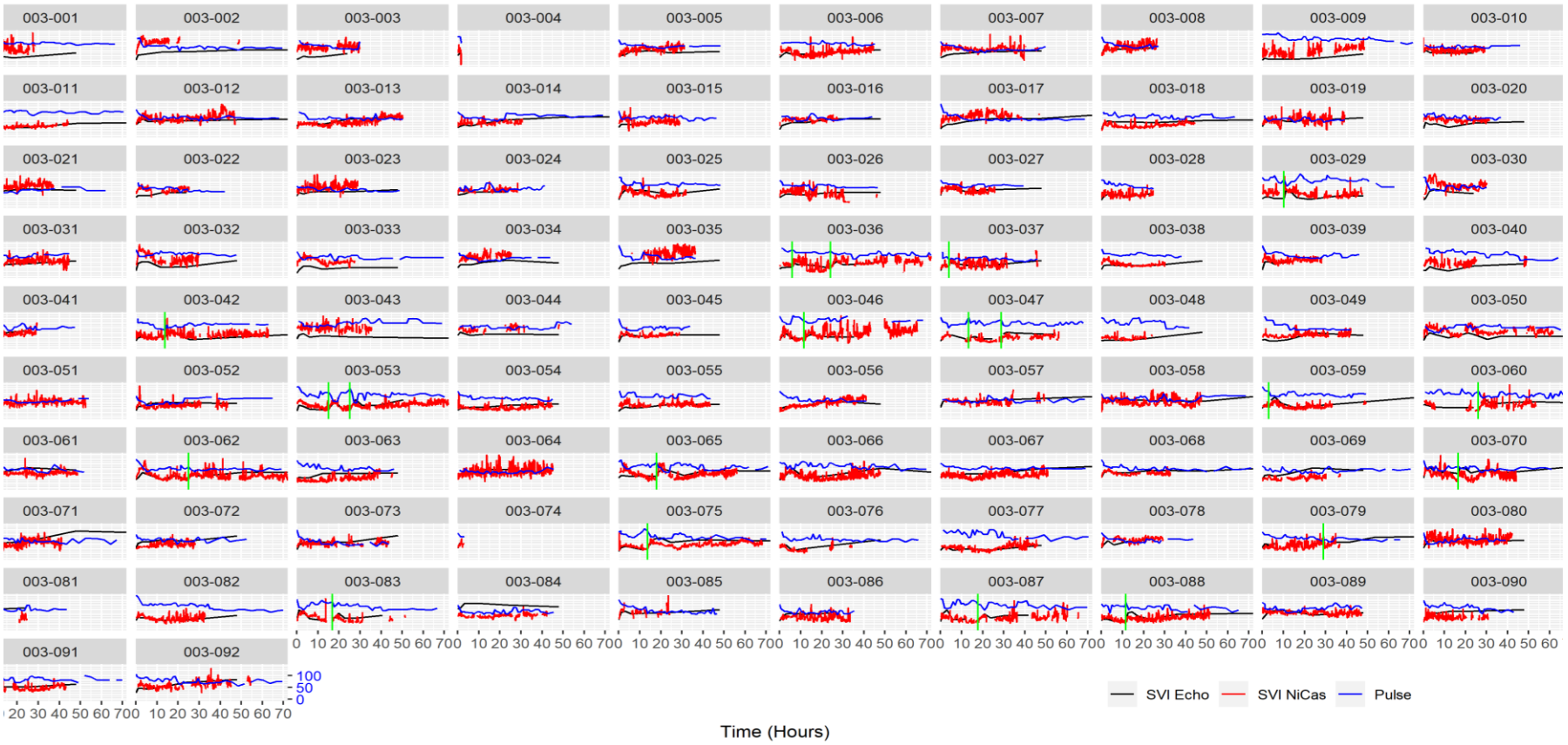
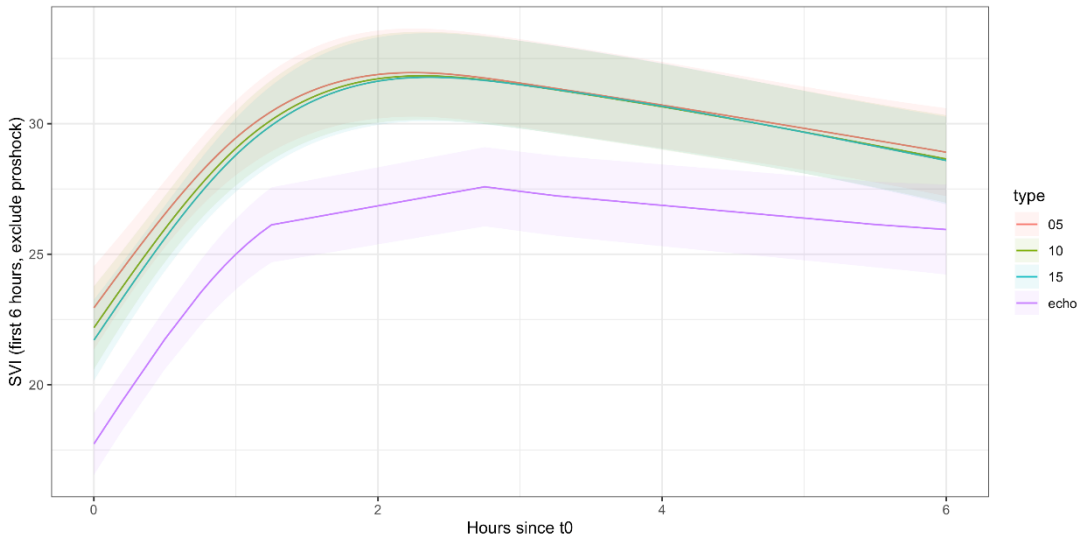


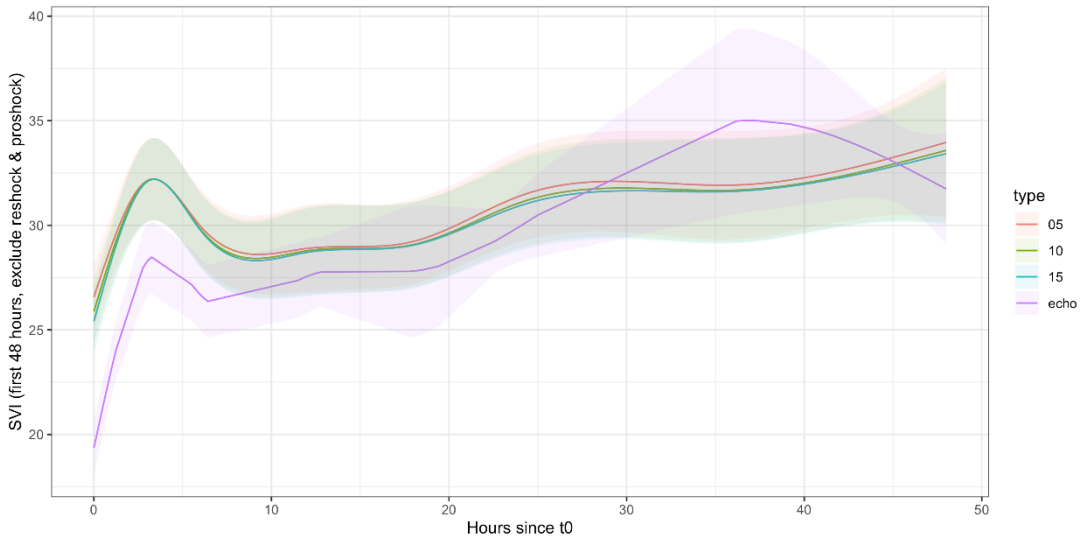
Figure 6-2. Trajectories of stroke volume index (SVI) from echo (in black), SVI from NICaS (in red) and heart rate (in blue) during the ICU stay of 92 study patients.

The vertical green lines indicate time of reshock. A clinical definition of reshock is based on pulse pressure <= 20 mmHg and poor peripheral perfusion occurring at least 6 hours from the initial cardiovascular stability.

Figure 6-3. Dynamic changes of echo-derived SVI (purple line) and NICaS-derived SVI (red line for 5-minute average; green line for 10-minute average and blue line for 15 minutes average).



Panel A



Panel B

Panel A shows trajectories of SVI from echo and NICaS within the first 6 hours for all patients without prolonged shock. Panel B shows trajectories of SVI from echo and NICaS during 48 hours of all study cases without reshock and prolonged shock. Prolonged shock is defined as shock occurring within the first 6 hours of initial shock recovery. Reshock is defined as shock occurring after 6 hours from the initial cardiovascular stability.

6.3.4.2 Stroke Volume Index (SVI) and Inferior Vena Cava (IVC) changes

Assessing IVC diameter is another way of measuring volume status. The IVC diameter is influenced by pressure changes during respiration, with a maximal diameter at the end of expiration and a minimum at the end of inspiration. The trajectories of IVC diameter and SVI values from a DSS case are presented in Figure 6-4. The IVC diameters displayed a similar trend over time with the SVI. IVC collapsibility index (IVCCI), which represents intravascular volume status (IVCCI increases with hypovolemia and decreases with normovolemia or hypervolemia) showed an inverse relationship with SVI.

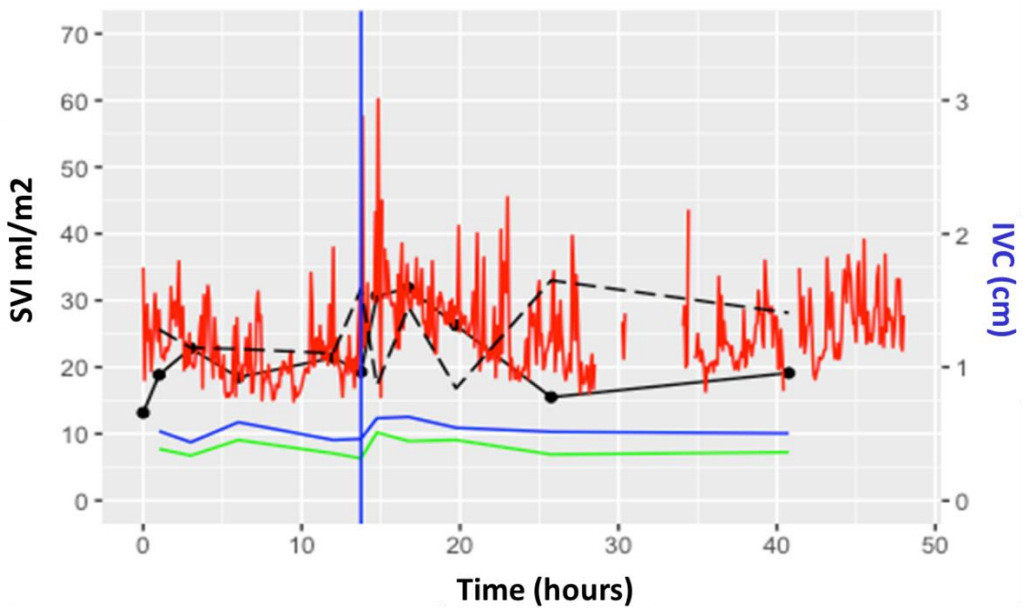


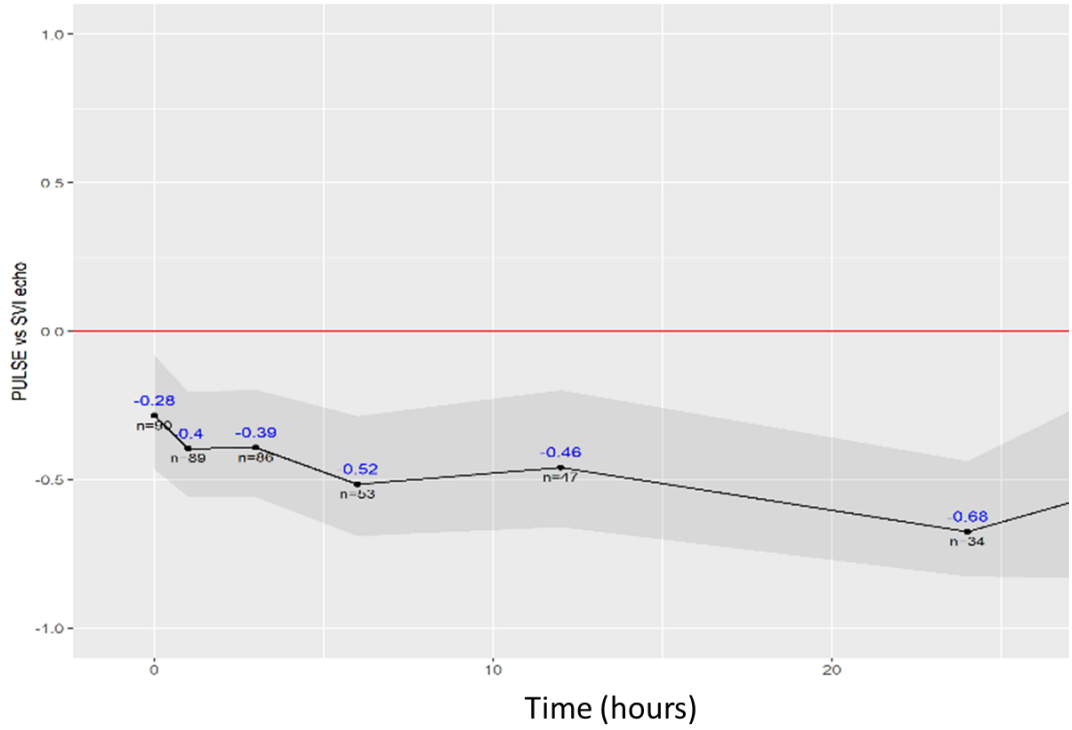
Figure 6-4. Trajectories of SVI from echo (black line), SVI from NICaS (red line), maximum IVC diameter (blue line), minimum IVC diameter (green line), IVCCI (intermittent black line) of one study patient. The vertical blue line is the indicator of reshock.

6.3.5 Associations between Echo-derived SVI and HR, SBP, DBP, PP, Hct and SVI from NICaS.

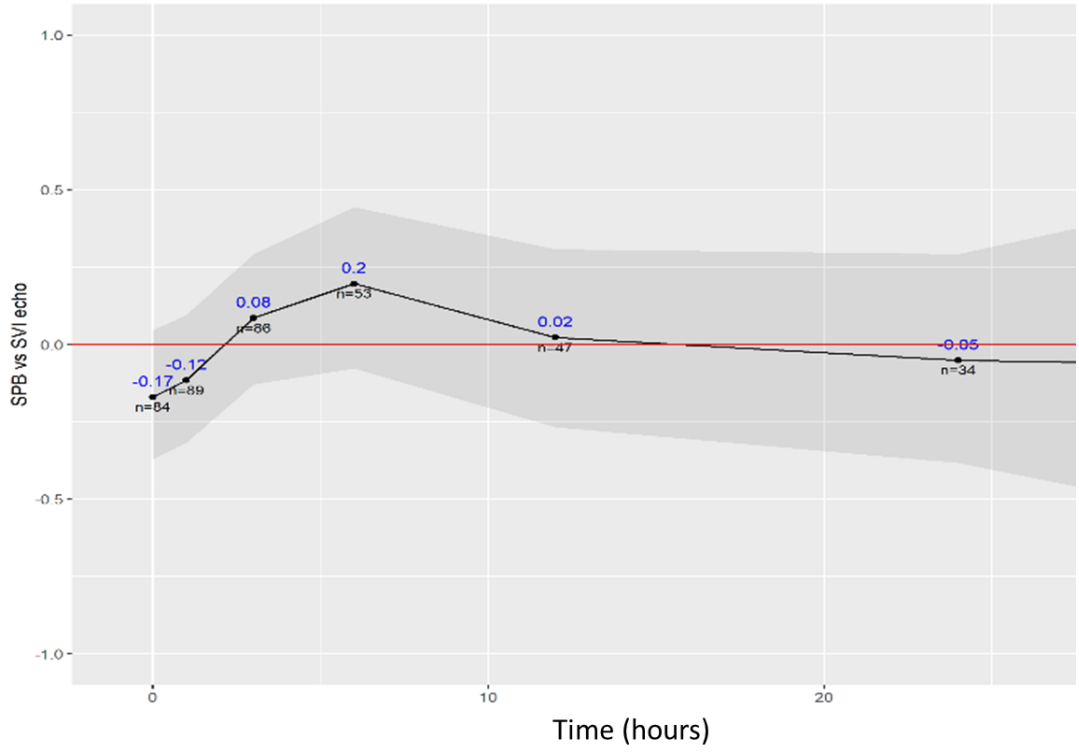
Associations between echo-derived SVI and HR, SBP, DBP, PP, Hct and NICaS-derived SVI over 48 hours of fluid resuscitation are shown in Figure 6-5. Echo-derived SVI had a negative correlation with HR and Pearson's correlation coefficient was -0.57 ([95% CI -0.67, -0.45], p <0.001). However echo-derived SVI did not have a significant correlation with SBP (r = -0.07, [95%CI -0.25, 0.11], p = 0.45) or DBP (r = -0.07, [95%CI -0.23, 0.09], p=0.40). Echo-derived SVI had a strong negative correlation with Hct and a positive correlation with NICaS-derived SVI.

Figure 6-5. Associations between Echo-derived SVI, HR (panel A), SBP (panel B), DBP (panel C), PP (panel D), Hct (panel E) and SVI from NICaS (panel F).

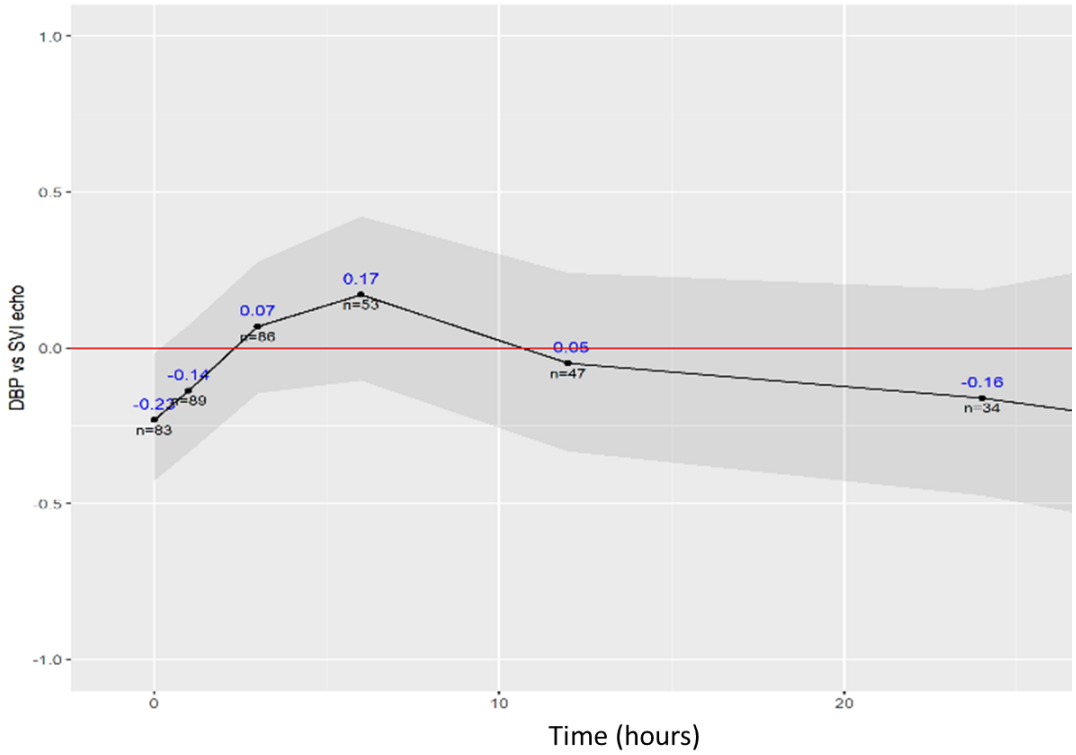
The panels describe partial Pearson's correlation coefficients between Echo-derived SVI with HR, SBP, DBP, PP, Hct and NICaS-derived SVI during 48 hours in all the study patients. Available data within 48 hours from initial shock was grouped every hour (for HR, SBP, DBP, PP, Hct) or 5 minutes (for NICaS-derived SVI). Grey areas represent the 95% confidence intervals of the estimated partial Pearson's correlation coefficients. Numbers in blue are the correlation coefficient and numbers in black are the number of observations.



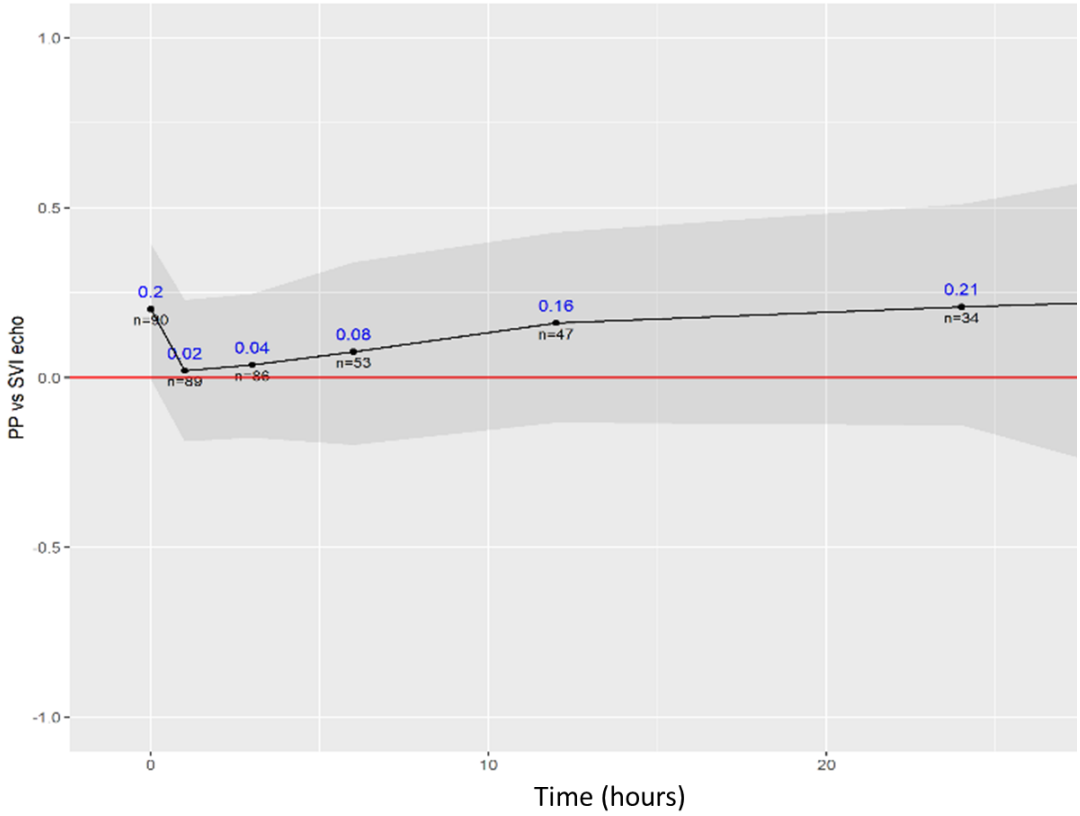
Panel A



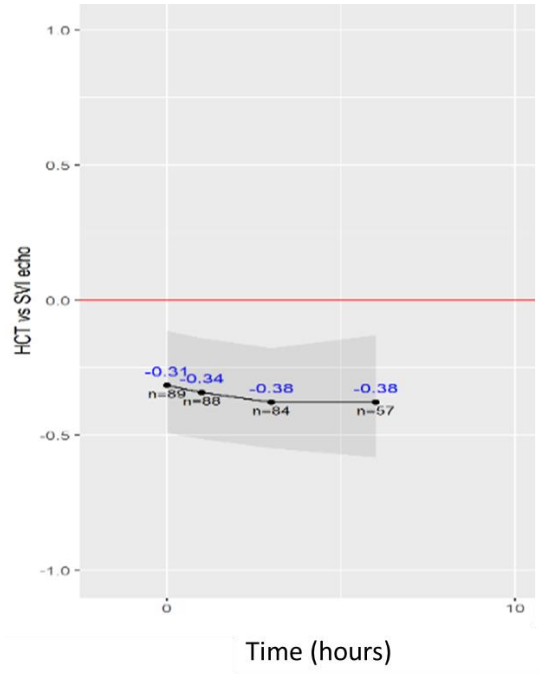
Panel B



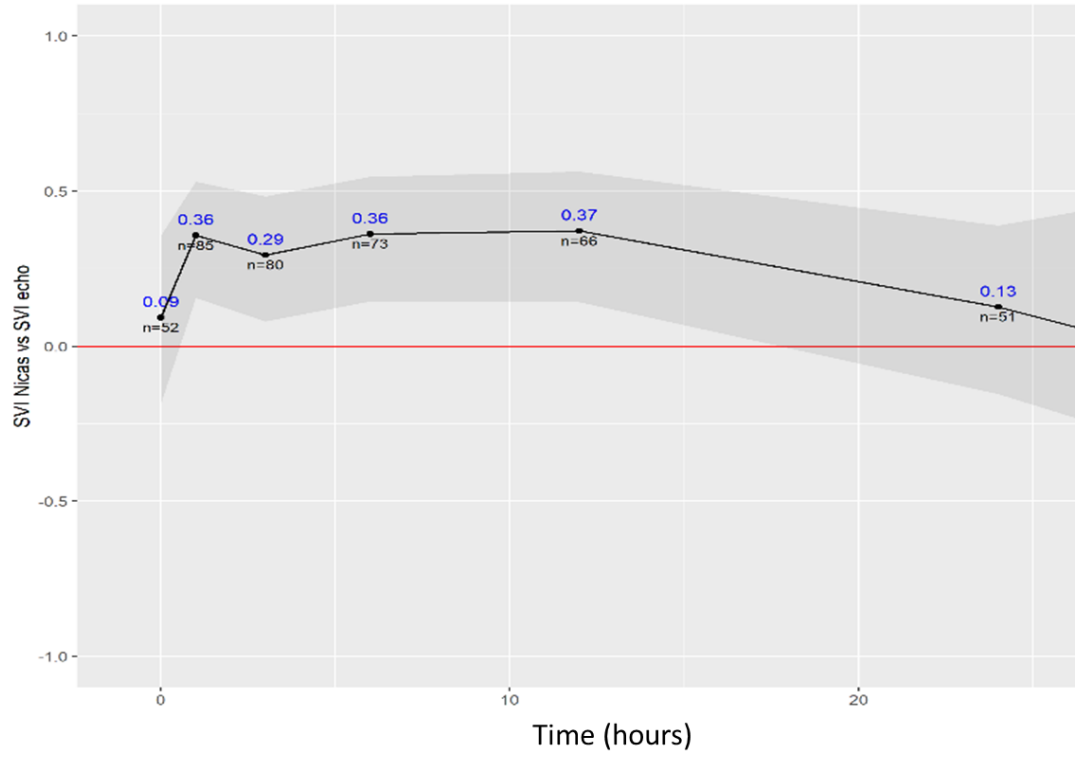
Panel C



Panel D



Panel E



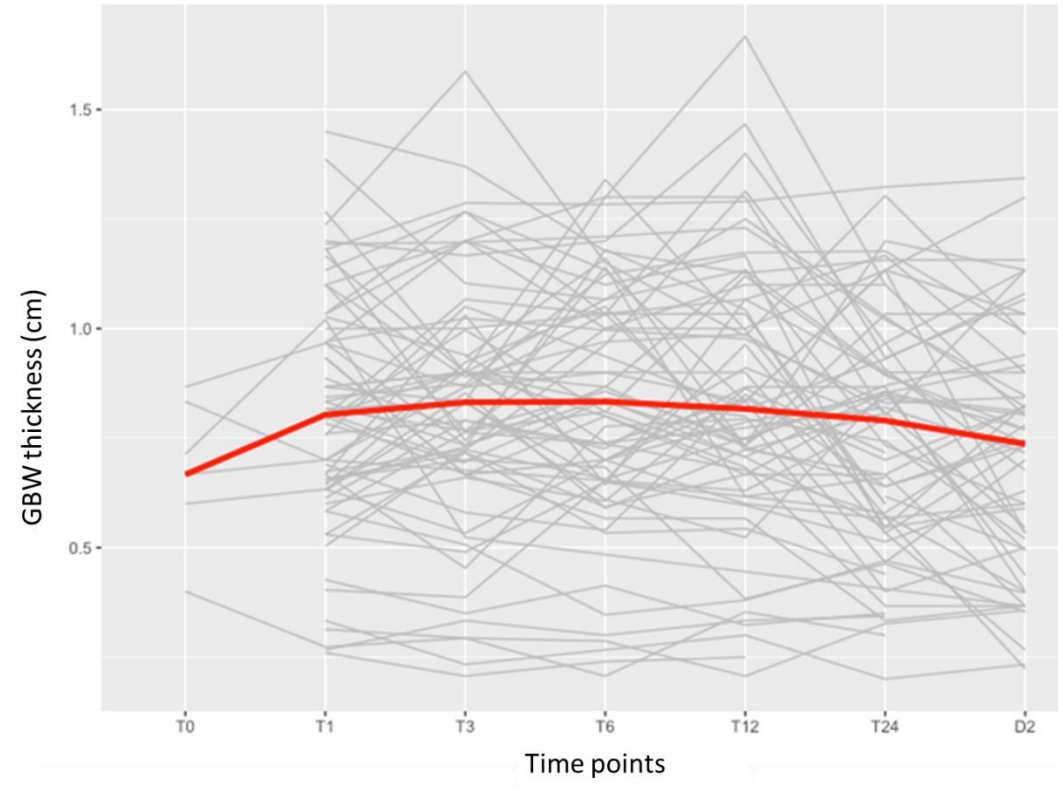
Panel F

6.3.6 Assessment of plasma leakage parameters over time

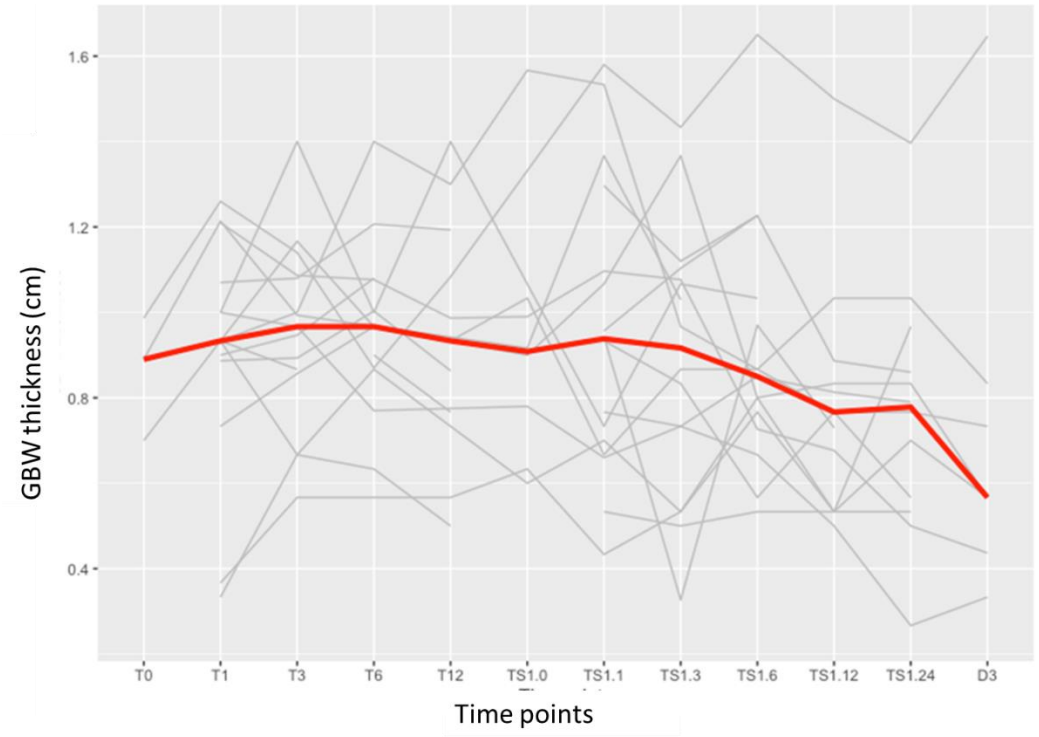
The pleural effusion depth and gallbladder wall (GBW) thickness were assessed at serial time points as surrogates of plasma leakage severity. Figure 6-6 shows data for pleural effusion (combined right and left effusions) and GBW thickness for 48 hours for non-reshock cases and 72 hours for reshock cases. There was a steady increase in pleural effusion thickness over the 72 hours of the observation period, while the GBW thickness decreased after 24 hours from initial shock. The total body water increased gradually over 36 hours then decreased.

Figure 6-6. Changes in pleural effusion thickness, gallbladder wall (GBW) thicknesses and total body water (TBW) over time.

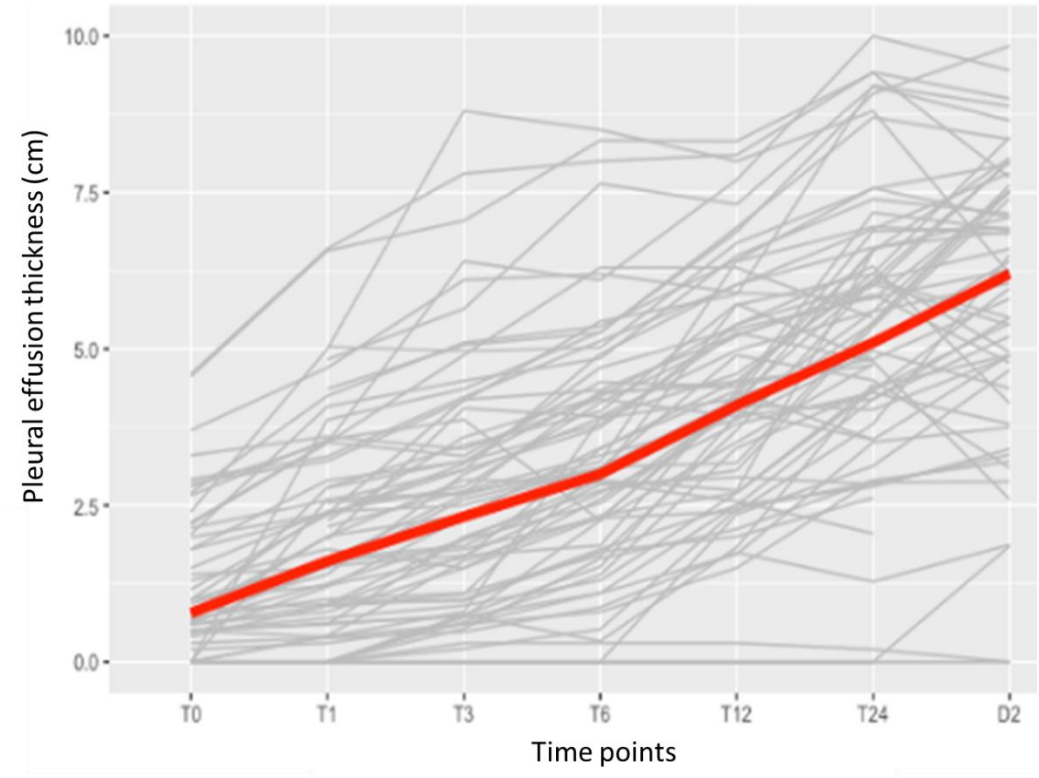
Panel A shows GBW thicknesses from 74 patients without reshock. Panel B shows GBW thickness from 16 patients with reshock. Panel C shows the total pleural effusion thicknesses (combined right and left) from patients without reshock. Panel D shows the total pleural effusion thicknesses of patients with reshock. Panel E shows the total body water of patients without prolonged shock and reshock. The red line represents the median.



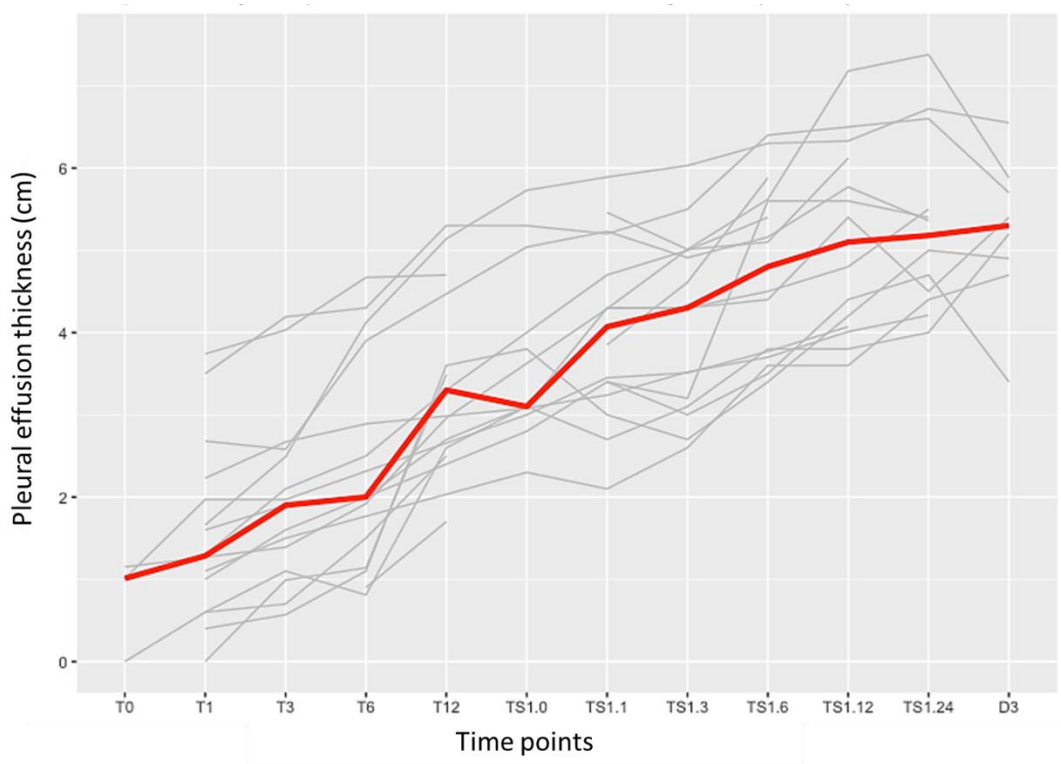
Panel A



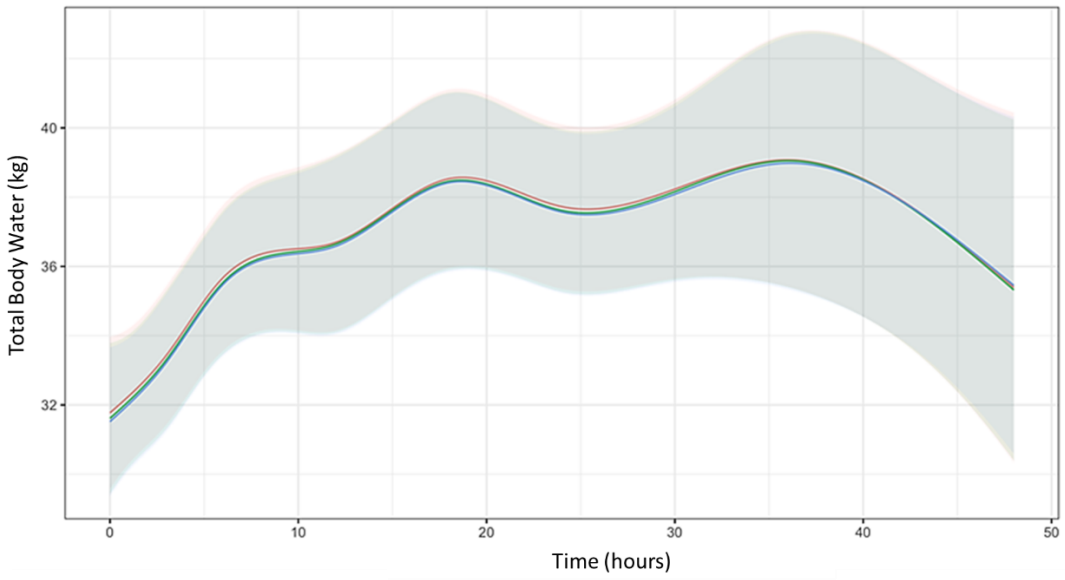
Panel B



Panel C



Panel D



Panel E

6.3.7 Point of care (POC) lactate

6.3.7.1 Association between venous lactate and POC lactate.

Venous Lactate and POC lactate measured at T0 had a significant correlation with r square of 0.444 and p value < 0.0001 (Figure 6-7).

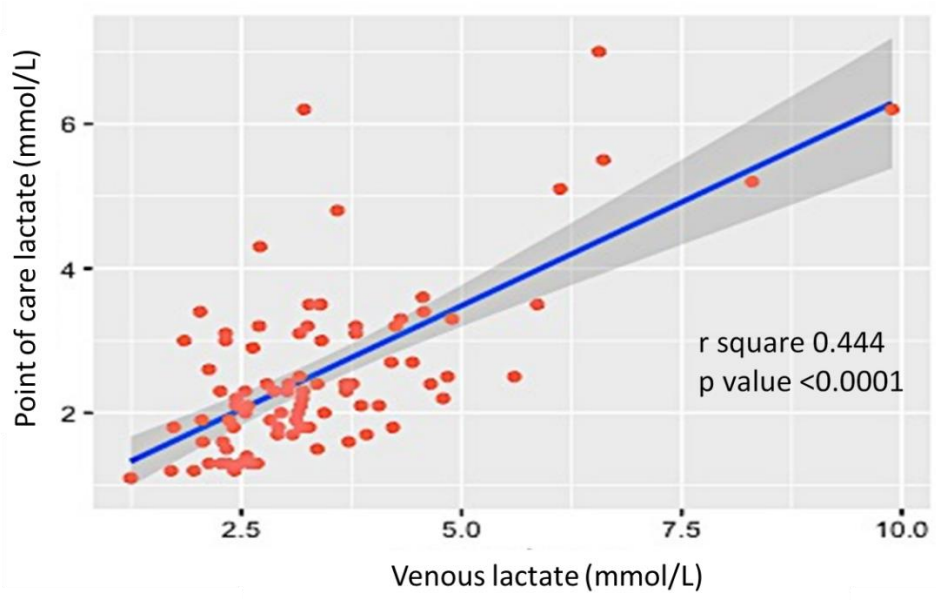


Figure 6-7. Association between venous lactate and point of care lactate at the first shock.

The red dots represent lactate values for all subjects. Linear regression was used for the analysis.

6.3.7.2 Dynamic changes of POC lactate during the first 6 hours of shock management

POC Lactate decreased over the first 6 hours from the initial shock (Figure 6-8). Median lactate levels appear to be stable from T0-T1, after which time they start to decrease.

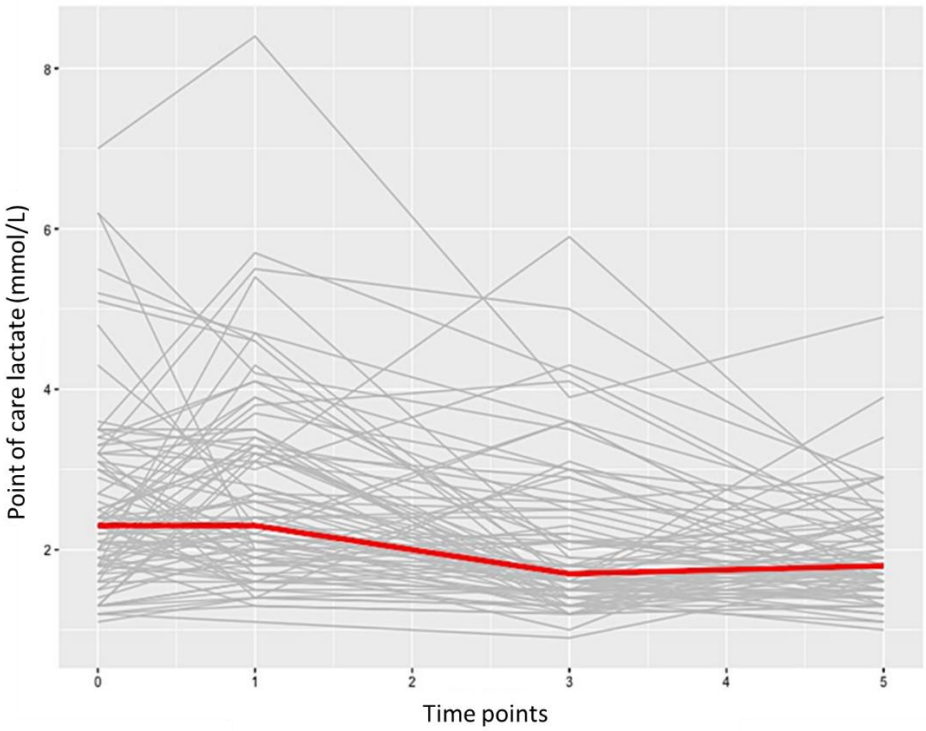


Figure 6-8. Dynamic changes of point of care (POC) lactate during the first six hours from shock.

Each grey line presents the trajectory of POC lactate from each patient. The red line represents the median lactate.

6.3.7.3 Association between lactate clearance and outcomes

Logistic regression was used to assess the association of lactate clearance with reshock and with respiratory distress. There was no association between lactate clearance and reshock or respiratory distress at 3 and 5 hours post initial shock. The results were shown in Table 6-9.

Table 6-9. Association between lactate clearance at T3 and T5 with outcomes

Lactate clearance	Reshock		Respiratory distress	
	OR (95% CI)	p	OR (95% CI)	p
At T3	1.3 (0.5, 3.3)	0.615	1.55 (0.5, 4.4)	0.398
At T5	1.0 (0.4, 2.5)	0.993	0.9 (0.2, 2.5)	0.834

6.3.8 Clinical factors associated with reshock and respiratory distress.

Univariable logistic regression was used to assess the association between baseline characteristics, haemodynamic parameters and SVI with reshock and respiratory distress. Day of illness of first shock and severity of the first shock were both associated with reshock (OR = 0.35, p = 0,001 and OR = 3.49, p= 0.041, respectively) (Table 6-10). Multivariable regression was used to assess the association between clinical factors and reshock or respiratory distress. Only the day of illness at first shock was associated with reshock (OR= 0.4, p= 0.022). Logistic regression with adjustment for day of illness and severity at the first shock was used to investigate echo-derived SVI with reshock. SVI at T6 and the difference of SVI over 6 hours were associated with reshock (OR = 0.9, p =0.029 and OR = 0.8, p = 0.004, respectively) (Table 6-11). No clinical parameters were associated with respiratory distress (Table 6-12).

Table 6-10. Associations between clinical factors and reshock using univariable logistic regression model

Factors	**Reshock (n=16)**			
	OR (95% CI)	p value	Adjusted OR* (95% CI)	p value
Overweight	1.58 (0.53, 4.77)	0.404	1.6 (0.5, 5.3)	0.427
DOI at first shock	**0.35 (0.15, 0.74)**	**0.010**	**0.4(0.2, 0.8)**	**0.022**
Severe DSS	**3.49 (1.02, 11.5)**	**0.041**	2.7 (0.6, 8.9)	0.120
HR	1.04 (1.00, 1.08)	0.051	1.0 (1.0, 1.08)	0.085
SBP	0.98 (0.92, 1.04)	0.6	1.0 (0.9, 1.0)	0.487
DBP	1.00 (0.94, 1.96)	0.9	1.0 (0.9, 1.0)	0.354
Venous lactate	0.81 (0.46, 1.24)	0.4	0.9 (0.5, 1.4)	0.676

DOI Day of Illness, HR Heart Rate, SBP Systolic Blood Pressure, DBP Diastolic Blood Pressure. Overweight was defined if the patient's BMI was higher than the 85th percentile.

Table 6-11. Associations between echo-derived SVI and reshock

SVI	**Reshock (n=16)**	
	OR (95% CI)	P value
At T0	1.0(0.9, 1.2)	0.603
At T1	1.0 (0.9, 1.1)	0.953
At T3	1.0 (0.9, 1.1)	0.736
At T6	**0.9 (0.8, 0.9)**	**0.029**
SVI change after 1 hour	0.9 (0.9, 1.2)	0.614
SVI change after 3 hours	0.9 (0.8, 1.1)	0.345
SVI change after 6 hours	**0.8 (0.7, 0.9)**	**0.004**

SVI Stroke Volume Index. Logistic regression was used to assess the association between SVI and reshock adjusted for day of illness at first shock and DSS severity.

The 6-hour time point was chosen to assess the association with reshock due to the clinical application of assessing intravascular volume in current practice (checking Hct at T0, T1, T3, T6- according to WHO guidelines)

Table 6-12. Associations between clinical factors and respiratory distress

Factors	**Respiratory distress (n=10)**			
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Overweight*	4.0 (1.0,19.5)	0.058	4.06 (0.92, 22.41)	0.0766
DOI at onset of the first shock**	0.7 (0.3, 1.56)	0.3	0.651(0.270, 1.531)	0.325
Severe DSS***	3.49 ((0.80, 14)	0.080	3.74 (0.8433, 15,57)	0.069

*DOI Day of Illness. Association between clinical factors with respiratory distress was assessed using a logistic regression model. *Association between overweight and respiratory distress was adjusted for severe DSS and reshock. **Association between DOI at first shock and respiratory distress was adjusted for overweight and severe DSS. ***Association between severe DSS and respiratory distress was adjusted for overweight and reshock.*

6.4 Discussion

Overall, the results of this study have shown that the current fluid management protocol at HTD with a bolus of 15ml/kg/h of crystalloid for DSS or 15 ml/kg/h of colloid for severe DSS achieved a good clinical fluid response. For patients who achieved a fluid response, the SVI continued to increase after a further 2 hours of fluids with the rate of 10 ml/kg/h. Those who did not respond then received a colloid bolus of 15 ml/kg/h for 1 hour, followed by 2 hours of 10 ml/kg/h and all of them responded. Earlier illness day at first shock and low SVI at 6 hours from shock were associated with reshock. We also demonstrated that NICaS provided reliable continuous data on SVI as compared with echocardiograms (the gold standard) and therefore could be a helpful additional tool to monitor DSS patients, predict recurrent shock and guide fluid management. POC lactate had a good correlation with venous lactate, however, lactate clearance in the first 6 hours was not found to be associated with reshock or respiratory distress in this study.

6.4.1 Characteristics and outcomes of study patients

We recruited only patients who were diagnosed DSS and received fluid resuscitation in the PICU of HTD. The overall outcomes of the patients were generally good with only 16 recurrent shock cases (18%), and 10 patients developing respiratory distress (11%) but none requiring organ support and no deaths. The reshock rate was lower than reported by other studies in the same setting (24%) (Trieu H. T. et al. 2022). It is likely that the patients recruited in this current study were not representative of the general DSS patients at our hospital due to the study selection bias. We just enrolled 92 out of 735 patients admitted over the 2-year- study period. Due to the intensity of the protocol, it was only possible to enroll the first DSS patient admitted to PICU during the day (normal working hours of 9 am-5 pm). This may have resulted in milder patients being selected as the more severe patients tend to present outside normal working hours. Further studies are required to explore the influence of nighttime or weekend admission on outcomes of DSS patients. Even so, this study included a wide range of severities at

presentation with similar baseline characteristics, and total volume of fluid compared to other previous studies at the same hospital (Wills B. A. et al. 2005, Lam P. K. et al. 2013).

6.4.2 Clinical fluid response and stroke volume index (SVI) changes

We chose SVI to describe the changes of intravascular volume because it is not influenced by heart rate. During the compensatory phase of DSS, the pulse pressure narrows and the heart rate increases which can initially keep the CI stable. In one of our previous studies using echocardiograms to assess SVI and CI in dengue patients with and without shock, we found SVI was lower in shock patients compared to non-shock patients while CI was similar between these two patient groups (Yacoub S. et al. 2017). SVI at the time of shock in the severe DSS patients was significantly lower compared to DSS patients as expected. Interestingly, we found that five DSS patients (who had pulse pressures of 20 mmHg) who did not achieve clinical fluid response after one hour of a crystalloid bolus had the lowest SVI equivalent to the severe DSS patients (pulse pressure below 10 mmHg or unmeasurable blood pressure). This suggests three important things in assessing DSS patients: first, blood pressure is not a perfect indicator for shock or shock classification; second, a crystalloid bolus of 15ml/kg/hour is likely to be inadequate for DSS patients with SVIs< 14 ml/m2 as all did not achieve clinical response; and third, POCUS is an important skill for intensivists to assess the severity of hypovolemia for more tailored and individual interventions. Systolic and diastolic blood pressure can be influenced by multiple factors including circulating intravascular volume, cardiac function, vascular resistance, the viscosity of the blood, vascular elasticity, and sympathetic, parasympathetic activities as well as the endocrine system, which all vary by individual. Therefore a classification of shock severity based purely on blood pressure could miss the compensatory phase when early intervention could prevent further cardiovascular compromise and potentially better patient outcomes. Not using one single haemodynamic parameter to diagnose shock also agrees with the consensus of the European Intensive Care expert panels on haemodynamic monitoring for patients with circulatory shock (Cecconi M. et al. 2014). The

consensus recommendation using multiple variables for the diagnosis and /or management of shock and blood pressure needs to be in addition to individualised haemodynamic targets during shock management. In western countries, the majority of intensivists are trained to use POCUS, however, this is still a new skill for doctors working in low middle-income countries (LMICs). There is need for more POCUS training in LMICs, especially for clinicians working in emergency or intensive care settings.

When we investigated changes in SVI and in Hct (a current standard of intravascular volume monitoring in DSS), and the severity of shock, we found that SVI could discriminate between DSS and severe DSS while Hct could not. This likely reflects that Hct is insensitive and is a surrogate for intravascular volume (just represents haemoconcentration) unlike more accurate haemodynamic parameters like SVI or CI. However, Hct and SVI during the monitoring period of fluid management showed a good correlation. For clinical practice, especially in LMICs, the use of Hct monitoring still plays an important role in tracking hypovolemic status, due to the ease and low cost of POC hct monitors, as well as the long track record of using HCT in dengue management.

I have presented the sensitivity and specificity of SVI at T1 to suggest some target levels for the clinical response after one hour of a fluid bolus. The SVI cut-off of 30 ml/m2 has 100% specificity for clinical response and therefore should be the target for one-hour resuscitation in future dengue guidelines. This cut-off is the lower limit of normal for SVI in children (the normal range of SVI is 30-70ml/m2). My data suggest that clinical response occurs with a low normal level SVI, which may be adequate. Patient variability occurs with respect to cardiac function, vascular tone and compensatory ability. Therefore, a range for the SVI target at T1 could be from 26-30 ml/m2 as the specificity for clinical response with this range is above 80%. A higher SVI target above 30 ml/m2 may not be necessary and the potential damage of a fast bolus needs to be considered which could further disrupt the endothelial layer of the microvessels which are already compromised in dengue ((Lam P. K. et al. 2020, McBride A.

et al. 2020). The harm of fast fluid boluses was demonstrated in other infections in the FEAST (Fluid Expansion as Supportive Therapy) trial. In this trial, the mortality of patients receiving 20 ml/kg/hour of normal saline or 5% albumin (10.5% and 10.6%, respectively) had 3.3% higher mortality compared to the non-bolus group (7.3%). The higher bolus volume of 40 ml/kg/h of either normal saline or 5% albumin for children with severe hypotension had very high mortality (56% and 69%, respectively) (Maitland K. et al. 2011). The monitoring for haemodynamic changes and complications in this trial was solely based on clinical signs but clinical examination correlates poorly with true volume status (Hanson J. et al. 2013), Therefore, advanced haemodynamic parameters such as SVI, CI and cardiac function need to be added into the assessment of any fluid intervention study to understand the effect of fluid bolus on both clinical response and haemodynamic parameters. Fluid intervention trials in dengue using SVI as a resuscitation target are now required to confirm the clinical effectiveness of using the target range suggested from this study and explore whether a higher or lower target of SVI at T1 would provide better outcomes (i.e. SVI target of 26-30 ml/m2 versus SVI target above 30 ml/m2).

6.4.3 Stroke volume index (SVI) changes and inferior vena cava (IVC) diameters changes during fluid management

We have shown detailed SVI trajectories during fluid management in the ICU of 92 DSS patients (Figure 6-2). Each patient responded differently with the same fluid protocol, demonstrating the potential need to individualize fluid management in DSS patients. Patients receiving colloid (HES 6% 200/0.5) increased their SVI by 84% after a 1-hour bolus of 15 ml/kg/h, after which the SVI continued increasing to reach the peak at T3. At HTD, the protocol includes using 3 hours of colloid for severe DSS, then switching to lower rate crystalloid. Therefore as expected after 3 hours of colloid use, the SVI gradually decreased with the lower rate of crystalloid. The patients who likely had continued severe vascular leakage developed reshock when the SVI decreased to the level of the first shock. Tracking SVI and intervening prior to the reaching this critical level could therefore prevent further episodes of reshock. The

initial increase of SVI was also seen in DSS patients receiving crystalloid but SVI only rose by 33% with a bolus of 15ml/kg/h. By monitoring SVI, we can predict who is more at risk or imminently about to develop reshock. A weakness of echocardiography is that it can only be performed intermittently, however from my results, the most useful POCUS time-point to predict patients developing reshock is at T6. Patients with higher SVI at T6 had a lower risk of reshock (OR = 0.9, 95% CI 0.8-0.9, p = 0.029) and patients with higher SVI change at T6 compare to T0 also had lower risk of reshock (OR = 0.8, 95% CI 0.7- 0.9, p = 0.004).

At reshock, the use of a colloid bolus of 10 ml/kg/hour provided a clinical response in 100% of the cases, with a median SVI rise of 53%. This suggests a lower fluid bolus volume for shock is also effective. The WHO guideline gives a range of 10-20 ml/kg/h for initial fluid bolus and different countries decide what to include in their national guidelines. In Thailand, 10 ml/kg/h and Dextran 70 or Dextran 40 are chosen. In Myanmar, the fluid choice is gelatin and fluid bolus rate is 10 ml/kg/h. In Vietnam and the Philippines, the guidelines include a dose of 15-20 ml/kg/h and the colloid use are hydroxyethyl starch (SEARO WHO 2011, Malaysia Ministry of Health 2015, Vietnam Ministry of Health 2019). There was no report on how these fluids types and different bolus doses increase stroke volume in DSS patients. There are also no guidelines for the fluid volume that should be used for patients with reshock. The case management for severe dengue cases relies on expert opinions rather than research evidence

Overall SVI measured by echocardiograms were low compared to the normal range (30-70 ml/m2) even after patients recovered from shock. This is in accordance with results of studies comparing cardiac output (CO) derived from transthoracic echocardiography (TTE) and thermodilution technique-standard technique of CO measurement. A study in 15 patients after cardiac arrest, CO measurements were lower using TTE with a mean difference of -0.426 L/min (Souto Moura T. et al. 2018). Another study in 43 patients with heart failure, CO measured by echocardiography was lower compared to CO measured by Swan-Ganz catheter with a mean difference of -0.81 L/min (Temporelli P. L. et al. 2010). A systematic review and meta-analysis

comparing cardiac index (CI) derived from echo and thermodilution showed echo-derived CI was lower with mean difference – 0.28 L/min/m2 (Zhang Y. et al. 2019). I chose echocardiography as the standard method in this study to measure SVI as although not equivalent, invasive monitoring is contraindicated in dengue patients due to the high risk of bleeding complications In addition the multiple time-points of POCUS allowed dynamic assessments of SVI. This provides more actionable information than static values as intravascular volume changes over time during fluid intervention, particularly in DSS patients who often experience ongoing plasma leakage. A single echo operator who was not part of the routine clinical care was used for this study to reduce operation bias and reduce inter-operator variability thus increasing the reliability of the results.

In this study, I have also described the IVC changes during fluid resuscitation. The IVC diameters during expiration (IVC max) changed with similar trends to SVI-echo. As measuring IVC diameter is easier compared to using Doppler for SVI measurement, this parameter could be used as a rapid tool for assessing fluid status. However, in patients with DSS at later stages when ascites and pleural effusions become larger, patients had increased respiratory effort, which influenced the echo views, making the collapsibility index harder to assess. Therefore the overall benefit of using IVC and IVC collapsibility needs to be considered in the later stages of admission. In our previous study assessing IVC in patients with and without shock, we could not find a significant difference in IVC diameter and IVC collapsibility between the two patient groups (Yacoub S. et al. 2017). The multiple time-points and detailed description of the IVC in my current study support the importance of repeated IVC measurement to assess intravascular volume during initial resuscitation (but with limited utility in the later stage of the disease). A limitation of this part of the study is the use of IVC measurements in children is hindered due to the fact IVC diameters vary by age. Some investigators used IVC diameter as compared to their aorta diameters (Modi P. et al. 2016, Mannarino S. et al. 2019). This technique has not been validated and was not used in my study. It is therefore difficult to provide a universal cut-off of IVC diameter as a target for fluid response or when assessing fluid status. Future studies

could assess the ratio of IVC maximal diameters and aorta diameters, reference values of IVC max and IVCCI stratified by age to achieve fluid response and influence of fluid accumulation status on IVC diameters and IVCCI.

6.4.4 Association between SVI derived from echocardiograms (SVI-echo) and traditional vital signs

The following vital signs were monitored in all patients: hourly heart rate, BP for 24-48 hours of fluid management and Hct every two hours in the first 6 hours. SVI had a strong negative correlation with both heart rate and Hct. However, there was no clear association between SVI and SBP, SVI and DBP. These results are similar to the CRI results in chapter 5 where CRI correlated well with heart rate but not systolic or diastolic blood pressure. This could be explained considering the physiological changes that occur in the compensatory phase of dengue shock, where the SV starts to decrease, which is followed by an increase in HR by activation of the sympathetic system to maintain a normal cardiac output. The decrease of SV also activates vasoconstriction, which results in the elevation of diastolic BP. When the circulating volume is restored, these compensatory mechanisms cease and the vital signs stabilise (van Genderen M. E. et al. 2013, Cecconi M. et al. 2014). The majority of study patients were enrolled during the compensatory period prior to hypotensive shock which could explain the weak association between SVI and blood pressure.

This study provides novel data regarding the association between SVI and vital signs at multiple time points during fluid intervention. This allows a more detailed and better understanding of how individuals respond to fluid resuscitation in dengue. It also confirms the importance of combining multiple haemodynamic variables to assess patients during shock management as recommended by international guidelines (Antonelli M. et al. 2007, Cecconi M. et al. 2014).

6.4.5 Association between echo-derived SVI and NICaS-derived SVI

At the first episode of shock, all patients were assessed for SVI by echocardiography and connected to a NICaS device. However, due to the delays in connecting the device, only 50 %

of patients had SVI from NICaS recorded within the first 10 minutes of shock (whereas all patients had SVI echo at T0). All patients had SVI-NICaS data after 30 minutes from enrollment. Despite some initial missing data, NICaS still provided good continuous data on SVI throughout the rest of the monitoring period. These lower case numbers may explain the relatively weak association between SVI- NICaS and SVI-echo at T0, after which the association became stronger. We also have fewer data from NICaS after 24 hours when patients had started to recover and were more ambulatory, and regularly disconnected the electrodes. So with fewer data for comparison at the later phase of shock management, there was a weaker correlation between the two monitoring techniques at this time point. NICaS has been validated with an invasive monitoring technique and has shown a good correlation with thermodilution technique in measuring CO and SV (Cotter G. et al. 2004, Cotter G. et al. 2006, Taniguchi Y. et al. 2013, Matsuda Y. et al. 2014). A study in adults patient during hemodialysis showed that SV derived from NICaS had a strong correlation with Doppler echo during the first hours ($r = 0.93$, $p<0.001$) and last hours of treatments ($r = 0.92$, $p < 0.001$) (Germain M. J. et al. 2018). Previous NICaS studies all used intermittent measurements. Our study is unique as we present for the first time continuous data of NICaS-derived SVI for more than 48- hour monitoring. Results from this study have contributed more evidence for the reliability of NICaS in continuous haemodynamic monitoring and suggest a beneficial role of NICaS in the continuous monitoring of DSS patients during fluid resuscitation. NICaS has advantages over POCUS in that it does not require skilled technicians, and is able to provide automatic, continuous real-time haemodynamic measurements.

6.4.6 Assessing plasma leakage severity during fluid management

All dengue patients experience some levels of plasma leakage and dynamic measurements of the leak are extremely important in dengue management. Clinical fluid accumulation in dengue patients manifests as gallbladder wall oedema, ascites, pleural effusions and peripheral oedema. Multiple studies have shown that gallbladder wall thickening is one of the earliest signs of

vascular leak and is associated with progression to severe dengue (Tavares M. A. et al. 2019, Adil B. et al. 2020). Pleural effusion and ascites also develop but usually are only detectable later on in the disease and are prominent in patients with a significant vascular leak. In my study, all the DSS patients had evidence of fluid accumulation at enrolment and the amount of leakage increased during fluid resuscitation as demonstrated by the rapid increase in the size of the pleural effusions. To date, there have not been any studies describing how gallbladder wall thickness (GBWT) and pleural effusion (PE) depth change during fluid resuscitation and recovery period. My results demonstrate a steady increase of GBWT in the 24 hours of fluid management and start to decrease after that. However, the PE thickness continued increasing during 48 hours of monitoring. At follow-up (7-14 days after hospital discharge), there was no pleural effusion or ascites on ultrasound. The gallbladder also was normal. The limitation of measuring GBWT is that its shape was not symmetrical across its length which made accurate thickness measurement difficult. Also, the relative small measurements and changes (millimetres) can be prone to errors. From these detailed descriptions, I suggest that the role of measuring GBWT may be important in the early stages of dengue illness but may not be helpful to discriminate the ongoing vascular leak or reabsorption period. Pleural effusions are easy to measure on ultrasound and can change significantly, but similar to GBWT, cannot be used to identify when plasma leakage stops but could be useful to estimate overall clinical fluid accumulation and massive effusion, which could warn for the potential of respiratory compromise. Future research in this area should focus on pleural effusion changes and their association with respiratory distress, which I could not analyse in this study due to few patients with respiratory distress.

6.4.7 Lactate POC and venous lactate

This study has shown a relatively good association between venous lactate and POC lactate in dengue patients at the time of shock. These results were comparable with other studies on POC lactate for pre-hospitalised patients (Swan K. L. et al. 2019, Raa A. et al. 2020). POC lactate

clearance has not been studied in DSS patients before. This study has shown POC lactate clearance was not associated with clinical outcomes. One of the reasons may be that study patients were not so severe and most of them were in the compensated stage of shock. A relatively small change in lactate may not reflect a significant improvement. Another reason could be that lactate levels increase in severe dengue due to multiple factors involving injury to microvascular, liver, kidney, myocardium, muscles as well as influences of sympathoadrenal activity, therefore improvement of hypovolemia may not result in significant lactate reduction. The results should be validated in future studies with other lab techniques for measuring lactate as well as investigating the aetiology of the increased lactate.

6.4.8 Strength and limitations

This study is the first study providing in-depth details of SVI changes during the fluid management of DSS patients in the ICU using different non-invasive techniques. I assessed the association between SVI with other traditional vital signs and techniques for estimating intravascular volume in patients with DSS. Results of this study provide a detailed picture of how an individual patient responds to fluid intervention and suggest outcome predictors using dynamic values. This study is also the first study using continuous data to explore the use of NICaS in haemodynamic monitoring of severe dengue patients in an ICU setting. Results of this study have also provided evidence demonstrating the potential benefits of using POCUS as well as NICaS for DSS patients during resuscitation.

However, this study also has some limitations. The limited number of patients enrolled meant I could not explore the association between some baseline characteristics (obesity, severity of shock, venous lactate level) and outcomes. I recorded multiple images and video clips of echocardiograms, however, I have been unable to analyse all the parameters derived from echo, including diastolic and systolic cardiac function and also right heart function due to time limits. Another limitation is that the results of this study can only apply to children and may not be generalised to adults, due to the differences in physiology and compensatory mechanisms in

different age groups and the different clinical phenotype in adults with severe dengue. There is a need to investigate the cardiovascular changes during the critical phase of severe dengue in adults.

Future work includes further analyses on cardiac function and their influence on outcomes; changes in biomarkers for endothelial damage and cardiac impairment during fluid resuscitation; the use of LVOT VTI cut-offs as a resuscitation target instead of using SVI, as this parameter is measured directly from echo without further calculations (VTI is less affected by age and gender and has narrower normal range (17-23 cm/sec)). The values of IVC diameter stratified by age also need further analyses. The obese patient group seem to increment their SVI with fluid less than other age-groups, which needs to be explored in more detail as body weight is used for fluid volume calculation and may not be appropriate.

Results of this study can also be used for future clinical intervention trials to evaluate whether the use of POCUS or NICaS to guide individualised fluid resuscitation improves outcomes (with potential endpoints being: fluid response, total fluid use, occurrence of reshock, respiratory distress, length of stay in ICU). This trial could assess fluid resuscitation strategies using different target range of SVI, eg 30 ml/m2 versus higher target SVI of more than 30 ml/m2 and the association with outcomes. Further trials should also focus on cardiovascular changes during the critical phase of severe dengue in adults, particularly the population with comorbidities (due to lack of data in this group) to provide reference values and recommendations of fluid type and fluid volume for shock resuscitation.

6.5 Conclusion

The current protocol of fluid management in paediatric DSS at HTD results in a good clinical fluid response in the majority of patients. The use of cardiac echo was able to identify severe hypovolemia through measurement of SVI at ICU admission. My results suggest that the target SVI after one-hour fluid resuscitation should be greater than 26 ml/m2. SVI measured again at T6 was able to predict the occurrence of subsequent reshock. This study confirms the

importance of continuous vital sign monitoring together with serial Hct testing as both are strongly associated with SVI. This study also demonstrates that NICaS can be a helpful additional tool for continuous monitoring of DSS patients, and as this is less labour intensive and does not require specific expertise like POCUS can be more applicable to LMIC settings. Taken together the results from this study have shown SVI to be a useful parameter whether derived from echo or NiCaS and can identify the early progression of reshock allowing appropriate intervention. Future clinical trials are now required to investigate whether using POCUS and NICaS can improve outcomes for DSS patients.

CHAPTER 7

SUMMARY AND FUTURE DIRECTIONS

7.1 Summary

In this thesis, I have answered several important questions regarding fluid therapy for children with DSS. Firstly, a systematic review of fluid resuscitation for children with dengue shock syndrome or septic shock found a small number of RCTs with high heterogeneity. The use of synthetic colloids versus crystalloids for compensated dengue shock as initial fluid resuscitation had similar outcomes. The benefits of using colloids versus crystalloids for patients with decompensated shock have not been studied. For children with septic shock, the bolus of fluid of 20-40 ml/kg/hour increased mortality in settings without advanced ventilatory support. With the current situation, where the global production of synthetic colloids is restricted, there are limited choices for fluid therapy in DSS. Therefore individualized clinical trials of targeted fluid therapies are needed to improve the management of DSS patients. The limitations of fluid management in dengue are the lack of multi-centre RCTs, and studies with high clinical and methodological heterogeneity, so the current data could not provide strong recommendations. Future clinical trials should utilize an adaptive approach, which would allow more intervention arms, increased numbers of participants, reduced time and cost as well as a reduction in the overestimation effects. Ethical issues are also important as research studies should protect study participants, which can be challenging especially in paediatric studies and those in critically ill patients in the ICU.

Secondly, I have described a wide range of fluid management strategies for children with DSS across seven centres in Asia, through a large retrospective study. We have shown that the use of colloid fluid in the first two hours of resuscitation provided similar outcomes compared to the use of crystalloid only; the prolonged use of colloids for more than 4 hours in the first 6 hours of resuscitation, reduced reshock occurrence and the number of reshock episodes but prolonged time to achieve final cardiovascular stability and also increased the risk for

respiratory compromise. These results suggest a cautious approach is necessary when using colloids for children with DSS, especially in settings without respiratory support.

I have also shown the utility of different non-invasive haemodynamic monitoring techniques for DSS patients during fluid resuscitation and highlighted their potential to guide fluid therapy. I investigated the utility of the compensatory reserve index (CRI), a new physiological parameter analysing PPG signals through machine learning. CRI values reflected real-time changes in intravascular volume of DSS patients who were receiving fluid resuscitation and were able to predict reshock within 12 hours from measurement. The cut-off value of 0.4 provided the best sensitivity and specificity to distinguish patients who would progress to reshock. I also demonstrated the utility of another novel haemodynamic monitoring method based on bioimpedance analysis (NICaS) in monitoring DSS patients during fluid resuscitation. NICaS-derived SVI had a strong association with echo-derived SVI and provided continuous information that could identify hypovolaemia before the clinical manifestations of reshock. Results of my study also demonstrate the potential role of POCUS in identifying patients with hypovolemia and therefore ability to guide fluid therapy; with a target range of SVI 26-30 ml/m2 after one hour of fluid, and re-examination of SVI at T6 to assess the risk of reshock. My study also highlights the issue of relying on a single value of blood pressure to classify the severity of shock. Future research should explore a combination of blood pressure with heart rate, haematocrit and/or SVI measurement to identify the severity of hypovolemic status to guide fluid management. Fluid type or fluid volume should not be the same for all patients with DSS and should be tailored depending on the level of volume depletion of each patient. To apply individualised fluid management protocols we need further intervention studies to confirm the utility of non-invasive haemodynamic monitoring methods, set target SVIs and assess improvement in clinical outcomes.

7.2 Future direction

For future research, I think there are several important areas that we need to focus on.

7.2.1 Future trials for fluid management

First, we need a study to demonstrate improved clinical outcomes with a targeted and individualised fluid regimen in DSS management. For example, an interventional study using POCUS and other non-invasive hemodynamic monitoring (either NICaS or CRI) to guide fluid management, comparing a high-normal SVI target versus a low-normal SVI target. However, the design of such a trial would need to take into account ethical considerations and address safety issues including lessons around fluid rate learned from the FEAST trial. Another approach that could be considered, as different hospitals apply different fluid protocols, is conducting a prospective observational study at multiple hospitals, assessing POCUS/ NICaS –derived SVI changes in response to the different fluid strategies. Thus allowing a comparison of clinical outcomes between the different hospital fluid regimens. Although not as robust as a clinical trial, the detailed description of SVI changes from cardiac echo or non-invasive haemodynamic monitoring with these different fluid protocols would provide further evidence of best fluid practice. Other parameters that should be included in any future trials should be the total body water, the patient's weight, total fluid volume given, presence of clinical fluid accumulation and the association with respiratory compromise and other organ impairments needs to be explored.

Second, we need an interventional study to investigate the impact of different fluid types and fluid strategies on glycocalyx integrity and endothelial function. Rapid infusion or a high volume fluid bolus may further damage the endothelial layer whereas continuous low volume infusion of plasma, starch, or dextran for 12-24 hours may actually restore the glycocalyx layer (Nguyen-Pouplin J. et al. 2011, Lam P. K. et al. 2020, McBride A. et al. 2020, Trung D. T. et al. 2020). Ethical concerns using synthetic colloids (which have shown worse outcomes in adult sepsis) may not allow this kind of clinical trial to be performed. However, in dengue-endemic countries where colloids are still being used for patients with decompensated shock or severe DSS and in a patient population where there have been no safety concerns, it may be possible to do this trial. A multi-centre randomised control trial with an adaptive

platform design using a single master protocol could be an ideal option. RECOVERY trial has been a successful example of how a platform trial can provide evidence in an efficient way for many different interventions during the COVID-19 pandemic (Group Recovery Collaborative 2021, Group Recovery Collaborative et al. 2021). A similar platform as RECOVERY trial would be a model protocol for future research in dengue whereby multiple fluid strategies with different fluid types and volumes could be investigated simultaneously. As I have shown in chapter 4, the fluid management for DSS in different centres varied considerably and fluid choice also depended on availability in each country. The results of such a clinical trial would also bring more flexibility and benefits for specific study sites.

7.2.2 Novel host-directed therapeutics for severe dengue.

By using a platform trial approach, future trials could also evaluate multiple therapeutics in dengue targeting specific host pathways as well as anti-virals. Re-purposing drugs licensed for other viral-induced hyperinflammatory syndromes, like high-dose steroids, baricitinib (Janus kinase inhibitor), anakinra (interleukin-1 receptor inhibitor), tocilizumab (interleukin 6 receptor inhibitor) might be possible, especially for those drugs that have shown efficacy in severe COVID-19 patients, where some pathways may be shared with dengue. The severe dengue patients who could benefit from these drugs need to be carefully selected using both clinical and biomarker profiles (Lam P. K. et al. 2015, Vuong N. L. et al. 2021).

Other adjunctive therapies that may reduce the hyper-inflammation as well as the vascular hyperpermeability include antihistamine agents (chlorpheniramine, diphenhydramine, alimemazine) which act by blocking H1 receptors situated on endothelial cells. Mast cells and their products like histamine may play a role in the pathogenesis of vascular leak and vascular dilation (Kunder C. A. et al. 2011), therefore, inhibition of mast cells or their products could impact dengue patients clinically. Another suggestion is melatonin. This drug has been investigated for improving the outcome of ICU patients with other infections (Huang H. et al. 2014, Dianatkhah M. et al. 2017). Melatonin is secreted from the pineal gland and has effects

on circadian entrainment inducing night-state physiological metabolism which improves the quality of sleep. In addition, melatonin is an antioxidant and anti-inflammatory agent, working through inhibition of mitochondrial metabolism which reduces glycolytic metabolism and creates a quiescent/inactivated state in immune cells. This has been investigated in the suppression of the hyperinflammatory syndrome in different viral infections including influenza and COVID-19 (Anderson G. and Reiter R. J. 2020, Cross K. M. et al. 2021). This drug has a good safety profile for clinical use in both adults and children (Gitto E. et al. 2011). Using melatonin for patients with dengue infection has been explored, (Dianatkhah M. et al. 2017, Roodaki Navid P 2021) but as yet there has been no research on the use of melatonin for severe dengue patients in ICU. Future trials could consider these host-directed therapies in addition to fluid optimisation strategies. Patients who experience multiorgan impairment have the highest risk of death and novel therapeutics should be prioritized in this group.

7.2.3 Health economics of future interventions

Health economics including cost-effectiveness studies is an important factor to consider while comparing the pros and cons of different interventions. It is particularly important for policymakers in LMICs, as dengue causes a large economic burden (as described in chapter 1 pages 4-6). No cost information was included in any of the RCTs on different fluid interventions, therefore future fluid studies should take health economics into account. Recent reports on the economic burden of dengue in Vietnam addressed some important gaps and included direct and indirect costs associated with dengue, like lost income even after disease resolution due to prolonged fatigue and depression (Hung T. M. et al. 2018). Further studies on the cost of severe dengue in ICU need to be assessed, especially in children where there is a lack of data.

7.2.4 Point-of-care ultrasound (POCUS) training

The results of my study using POCUS to assess fluid status have shown the importance of using this technique to provide essential information on haemodynamic status to guide fluid

therapy. POCUS is a non-invasive and helpful tool to identify shock types and guide resuscitation for patients in emergency and intensive care settings. However this essential skill needs staff training and supervision to achieve competence. Research on POCUS training has shown that even a short 2-day ultrasound course was helpful for clinicians to be able to apply it for daily practice. However important barriers to applying POCUS in practice were identified, including lack of supervision of experts, practice time and access to an ultrasound machine (Rajamani A. et al. 2019, Olgers T. J. et al. 2020). These barriers would be even more important in LMICs where dengue is prevalent, because of a lack of health care staff, overloaded hospital settings, and limited monitors and echo machines. All these factors may deter the implication of using POCUS in routine practice. While there is currently a lack of evidence regarding the use of POCUS for improving patient outcomes, we cannot refute the important role of POCUS in assessing severe patients and guiding fluid management (Soliman-Aboumarie H. et al. 2021). Therefore, more training courses and continuous supervision programs should be organised in dengue-endemic settings to provide this essential skill for clinicians, particularly intensivists. POCUS training would also facilitate research collaboration which is one of the most important steps in conducting a multi-centre study.

7.2.5 Public engagement, public communication and qualitative research on patient-centred care

The number of clinical trials in children is far fewer compared to research in adults, resulting in a less evidence base for paediatric international guidelines and also fewer novel therapies for children and young patients (Joseph P. D. et al. 2015). There are numerous barriers to conducting clinical research with children, including ethical concerns, local laws and regulations, complexities of study protocols due to the high heterogeneity of the paediatric population, early patient drop-out, psychology of children, consent process, misinformation and concerns of parents as well as hesitancy of clinicians. Children are often not the persons who decide to participate in a study rather it is their parents or guardians, therefore, the informed consent process is more complex. One solution for this problem is to incorporate paediatric

patients, their parents and clinicians into the protocol designing stage. Activities of gathering patients' and parents' thoughts, sharing their experience stories through different social communication tools, and raising their unmet demands are all important works that should be taken into account in the early phase of clinical research and throughout all stages in parallel with the main research protocol. We have been running multiple innovative studies but there is still a lack of routine communication between researchers –clinicians- parents - patients and the study hospital. Publishing the results of a clinical study is not sufficient to impact or change management guidelines. Further engagement is required with all stakeholders to discuss the potential applications. The approach for running clinical research on dengue or other infections in LMICs should be "protected through research but not from research" (Nuffield Council on Bioethics 2015). I do think there should be further public engagement, communication activities, and qualitative research in this area to explore more patients' experiences, address patients' and parents' needs and record clinician experiences and concerns in managing severe dengue cases through different research stages. This would help to find good ways to conduct good clinical research. Patient-centred care is now the preferred approach to designing trials and facilitating research with children. I believe these approaches would be helpful for both patients and researchers as the results of any clinical studies are finally for patients' benefit.

After the COVID-19 pandemic, in 2022, the number of dengue cases has surged in Vietnam including the number of severe cases admitted to ICU. The clinical phenotype also appears to be different, with patients developing shock earlier (on day 3, day 4 of illness); plasma leakage is more severe resulting in multiple episodes of shock (almost 50% develops reshock compared to 25% in previous years); multi-organ impairment and prolonged fever. The underlying cause of these changes needs further investigation. One hypothesis is that the endothelial injury and vascular complications following SARS-CoV-2 infection may predispose to more severe vascular manifestations in dengue infections. Work at OUCRU is underway to investigate this and other potential causes of these severe manifestations.

In the future, I anticipate that there will be an efficacious dengue vaccine, and some effective antivirals as well as host-directed drugs. However at present, without a vaccine or specific therapy, we need multiple efforts to find better treatment for severe dengue. I hope my research and suggestions for future trials may eventually impact the management for severe dengue and improve patient outcomes.

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