



## Risk Factors for Mortality in Dengue Shock Syndrome (DSS)

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### ABSTRACT

**Background:** Dengue shock syndrome (DSS) is the most severe form of dengue hemorrhagic fever (DHF) and has a high mortality. There are two major pathological changes in DHF determining the severity of disease, plasma leakage and bleeding. Cytokines released during the immune response to dengue virus have been thought to be mediators of the process.

**Methods:** The study involved 50 children with DSS, of whom 13 (26%) died. We investigated which clinical signs and laboratory findings are related to mortality.

**Results:** We found that gastrointestinal bleeding and bilateral pleural effusion were significantly more frequent in non-survivors than in survivors ( $p < 0.02$  and  $p = 0.0006$ , respectively). Also, mean admission levels of thrombin-antithrombin complexes (TATc) and plasminogen activator inhibitor type 1 (PAI-1), activation markers of coagulation and fibrinolysis, respectively, were significantly higher in non-survivors ( $p = 0.004$  and  $p = 0.0006$ , respectively). In regression analysis, bilateral pleural effusion and admission levels of TATc were significantly associated with mortality ( $p = 0.007$  and  $p = 0.048$ , respectively).

**Conclusions:** Our data provide evidence for a relationship of mortality with pleural effusion, a marker of plasma leakage, and coagulation activation, both characteristic pathological changes in dengue shock syndrome.

**Keywords:** Dengue shock syndrome, mortality, risk factor.

### ABSTRAK

Faktor risiko kematian pada demam berdarah dengue dengan sindroma syok (DSS)

**Latar belakang:** DSS merupakan bentuk klinik yang paling berat dari demam berdarah dengue (DBD) dan mempunyai angka kematian yang tinggi. Terdapat dua kelainan patologik utama pada DBD yang menentukan beratnya penyakit, yakni kebocoran plasma dan perdarahan. Sitokine yang dilepas sewaktu terjadi respon imun virus dengue diduga merupakan mediator proses ini.

**Metode:** Studi dilaksanakan pada 50 penderita DSS anak, dimana 13 (26%) diantaranya meninggal. Investigasi dilakukan untuk mencari temuan klinik dan laboratorik yang berhubungan dengan kematian.

**Hasil:** Perdarahan gastrointestinal dan efusi pleura bilateral secara bermakna lebih banyak ditemukan pada penderita yang meninggal dibandingkan dengan penderita yang hidup (berturut-turut  $p < 0,02$  dan  $p = 0,0006$ ). Rerata kadar thrombin-antithrombin complexes (TATc) sewaktu masuk rumah sakit dan kadar plasminogen activator inhibitor type 1 (PAI-1) juga merupakan petanda aktivasi koagulasi dan fibrinolisis, secara bermakna (berturut-turut  $p = 0,004$  dan  $p = 0,0006$ ) lebih tinggi pada penderita yang meninggal. Pada analisis regresi didapatkan bahwa efusi pleura bilateral dan kadar TATc sewaktu masuk rumah sakit berhubungan bermakna dengan kematian (berturut-turut  $p = 0,007$  dan  $p = 0,048$ ).

**Simpulan:** Data dari studi ini membuktikan adanya hubungan antara kematian dan efusi pleura bilateral (suatu petanda kebocoran plasma) dan aktivasi koagulasi dimana keduanya merupakan kelainan patologik khas untuk DSS.

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## BACKGROUND

Dengue virus infection is an arthropod-borne disease, caused by any of four closely related serotypes of dengue virus, belonging to the genus *Flavivirus*, family *Flaviviridae*: DEN-1, DEN-2, DEN-3 and DEN-4. Infection confers life-long homotypic, but not heterotypic, immunity. Clinical manifestation can be asymptomatic infection, dengue fever (DF) and dengue hemorrhagic fever (DHF). The severity of DHF can vary from DHF I to DHF IV. The severe forms (DHF III and IV) are also referred to as dengue shock syndrome (DSS), characterized by shock due to leakage of plasma from blood vessels.<sup>1</sup>

The pathogenesis of DSS is controversial. Epidemiological studies indicate that pre-existing dengue virus antibodies may predispose an individual to DHF on subsequent infection with another dengue virus serotype.<sup>2-4</sup> Other studies have demonstrated that DEN-2 and DEN-3 are more virulent than DEN-1 and DEN-4, but all four serotypes can cause severe or fatal disease.<sup>5-9</sup> Epidemiological observations have provided evidence that age and race may be risk factors for DHF. DSS has been almost confined to children<sup>10</sup> and the age group most severely affected was 5-9 years.<sup>11</sup> Blacks are less susceptible to dengue shock syndrome than whites and Asians.<sup>3</sup>

There are two major pathological changes in DHF determining the severity of disease, plasma leakage and bleeding. The mechanism responsible for the increased permeability of vascular endothelial cells and bleeding in DHF has not been elucidated, although cytokines released during the immune response to dengue virus have been thought to be mediators of the process.<sup>12-14</sup> The mortality rate of DHF is high, ranging from 2 to 10.9%,<sup>15-19</sup> reaching 47% in patients with profound shock.<sup>20</sup>

We conducted a prospective clinical study, to investigate which clinical signs and laboratory parameters are associated with mortality in DSS.

## METHODS

This prospective study was performed in Dr. Kariadi Hospital, the university hospital of Diponegoro University, Semarang, Indonesia. The research protocol was approved by the Review Board of the Dr. Kariadi Hospital. Written informed consent was obtained from children's parents or legal guardians.

Between June and November 1996, during an outbreak of dengue in Indonesia, 50 consecutive children with the clinical diagnosis of DSS, who were admitted to the Paediatric Intensive Care Unit were enrolled in the study. Only children with age  $\geq 3$  years were included.

The clinical diagnosis of DSS (DHF grade III and grade IV) was finally based on the 1997 WHO criteria.<sup>(1)</sup> Patients presenting all criteria for DHF: (i) fever or history of acute fever 2-7 days; (ii) bleeding (mild to severe); (iii) thrombocytopenia  $100.000/\text{mm}^3$  or less (iv) evidence of plasma leakage plus evidence of circulatory failure manifested by (a) rapid and weak pulse; (b) narrow pulse pressure (20 mmHg); (c) hypotension and (d) cold, clammy skin and restlessness, were classified DHF III. If profound shock with undetectable blood pressure and pulse was present, patients were classified as DHF IV. Decreased consciousness was based on the Glasgow Coma Scale.<sup>21</sup>

Through clinical assessment was performed daily during hospitalization, using a medical record form. Laboratory tests to support clinical management included blood cell counts, tests for hemostasis (prothrombin time and activated partial thromboplastin time) as well as biochemical tests for kidney and liver functions and electrolyte status. Thrombin-antithrombin complexes (TATc) and plasminogen activator inhibitor type 1 (PAI-1), activation markers for coagulation and fibrinolysis, respectively, were also assayed. Chest x-ray and ECG were done routinely.

Blood specimens were collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ 07417). Two ml of blood for serological assays was collected on day of admission and at discharge. Blood was centrifuged 1000-1500 rpm for 10 minutes, after which serum was transferred to screw cap Eppendorf tubes and stored at  $-80^{\circ}\text{C}$ , until assayed.

Full blood counts were measured with QBC, Becton Dickinson, NJ 07417. For measurement of aPTT, PT and TATc, venous blood (9 vol.) was drawn into vacutainer tubes containing 0.105 M sodium citrate (1 vol.). aPTT and PT were measured by a coagulometer, KG Behnk Elektronik, and TATc levels were determined by means of a commercially available ELISA kit, according to the manufacturer's instructions (Enzygnost TAT micro, Dade Behring, Marburg Germany); normal value  $<4.1\text{mg/l}$ . For measurement of PAI-1, blood was collected in siliconized vacutainer tubes containing Polybrene (Janssen Chimica, Belgium) and EDTA (0.05%, w/v, and 10 mM, respectively, final concentrations), to prevent *in vitro* complex formation. PAI-1 was measured with sandwich ELISA kits using specific monoclonal antibodies as described.<sup>22</sup> All blood samples for analysis of coagulation and fibrinolysis were immediately immersed in melting ice and subsequently centrifuged at  $4^{\circ}\text{C}$  for 20 min, at  $1600 \times g$ . Plasma samples were stored at  $-80^{\circ}\text{C}$ , until assayed. Normal value for PAI-1 is 30-60 ng/ml. Biochemical tests, including liver as well as renal function tests and

electrolytes, were measured with Auto Analyzer, Hitachi, 7050. Normal value for serum protein were 5.9-8.6 g/dl, and for serum albumin were 3.5-5.6 g/dl. TATc and PAI-1 measurements were done at the Slotervaart Hospital, Amsterdam, The Netherlands. For the transport from Indonesia to The Netherlands (which last longer than 15 hours) the samples for coagulation, fibrinolysis, and serological assays were kept on dry ice.

The diagnosis of dengue virus infection was confirmed by serological assays. A capture and indirect enzyme-linked immunosorbent assay (ELISA) detected dengue specific IgM and IgG antibodies in serum samples, according to a previously described procedure.<sup>23</sup>

Continuous data were described in mean (SD) and nominal data in number (%). Nominal variables between survivors and non-survivors were compared using  $\chi^2$  Test. Fisher's Exact test was performed if the number of cells with expected frequency less than 5 were more than 20%. Independent t-tests were done to compare numeric variables between survivors and non-survivors. The assumption of normality of the data was checked before the t-test. Mann-Whitney U tests were done when the data were not normally distributed. The cut-off-point of significance was  $p=0.05$  with 95% confidence interval. Regression analysis was done to determine factors associated with mortality. Survival time was calculated with Kaplan-Meier curves. Log-rank test was used for curve comparison. All statistical analyses were performed with SPSS for Windows version 9.0.

## RESULTS

Between June and November 1996, 50 children with a clinical diagnosis of DSS, were enrolled in the study. Thirteen patients (26%) died during follow up in the Intensive Care Unit. The clinical diagnosis of DSS was confirmed by serological assays in all patients, either by an IgM response or a fourfold rise in IgG titers. All antibody profiles were typical for secondary dengue infection.

As shown in table 1, most patients were young children who presented after about 4 days of illness. Relatively more female patients died than males. Of the non-survivors 4 of 13 (30.7%) were classified as DHF IV, as compared with 3 of 37 (8.1%) survivors. The proportion of patients with gastrointestinal and gum bleeding was significantly higher in non-survivors than in survivors. A clammy skin, delirium, decrease of consciousness and bilateral pleural effusion were significantly more frequent in non-survivors than in survivors.

Table 2 shows that the prothrombin time (a measure for both the extrinsic and the common coagulant pathway) was significantly longer in non-survivors than in

survivors. Also the levels of thrombin-antithrombin complexes (TATc) and plasminogen activator inhibitor type 1 (PAI-1), activation markers of coagulation and fibrinolysis, respectively, were significantly higher in non-survivors than in survivors. The levels of serum protein were significantly lower in non-survivors than in survivors.

In the logistic regression analysis for mortality of day of admission levels of TATc and PAI-1, activation markers of coagulation and fibrinolysis, respectively, we found a significant association of TATc with mortality ( $p=0.048$ ; regression coefficient: 0.023). Such association was not found for PAI-1 (Table 3).

In the logistic regression analysis of albumin/protein ratio on day of admission and bilateral pleural effusion for mortality, a significant association was found for bilateral pleural effusion but not for albumin/protein ratio. The odds ratio for bilateral pleural effusion was 12.2 and p-value 0.007, implying that patients with bilateral pleural effusion had a 12.2 times increased risk of death (Table 3). This effect is also shown in the cumulative survival curve of patients with and without bilateral pleural effusion (Figure).

## DISCUSSION

In this prospective study of 50 children with DSS, we found that bilateral pleural effusion and elevated TATc concentrations were significantly associated with mortality in logistic regression analysis. Bilateral pleural effusion indicates severe vascular leakage whereas high TATc concentrations reflect markedly activated coagulation. Our finding that bilateral pleural effusion is associated with poor prognosis corroborates the data of a study from Jakarta of 30 fatal cases of virologically confirmed dengue virus infection. In that study plasma leakage appeared to be the main cause of death, because 19 of the 30 cases (63.3%) had profound shock with hemoconcentration.<sup>24</sup> In an autopsy study from Thailand of 100 children with fatal DSS plasma leakage was also likely the main cause of death, as evidenced by  $>4$ g/dl protein content of serous effusions in the pleural, abdominal and pericardial cavities. Also, the mucosa, submucosa and serosa of the gastrointestinal tract of these children showed oedema and hemorrhage, especially in children with prolonged shock.<sup>25,26</sup>

The admission level of TATc appeared to be a strong predictor of mortality, indicating the pathophysiological implication of coagulation activation. This finding is in line with the result from an earlier study, in which we found that high PAI-1 levels and hence activation of coagulation were most pronounced in non-survivors, indicating an ongoing procoagulant state.<sup>27</sup> Studies on

Table 1. Clinical characteristics of survivors and non-survivors in 50 patients with dengue shock syndrome

	Survivors (n=37)	Non-Survivors (n=13)	p value
<i>Clinical characteristics</i>			
Age (years)	6.8 ± 2.8	6.0 ± 2.8	
Sex ratio (M/F)	20 / 17	4 / 9	
DHF III/DHF IV	34 / 3	9 / 4	
Days since onset of disease	4.2 ± 0.9	4.0 ± 1.3	
<i>Bleeding manifestations</i>			
Positive Tourniquet test	16	7	0.51
Skin bleeding	14	7	0.31
Epistaxis	7	3	0.74
Gum bleeding	0	3	<0.003
Gastrointestinal bleeding	17	11	<0.02
Hematuria	1	1	0.43
<i>Signs of disease severity</i>			
Cold extremities	35	13	0.39
Clammy skin	3	6	0.002
Delirium	13	11	0.002
Decreased consciousness	16	11	0.01
Convulsions	2	3	0.07
Bilateral pleural effusion	2	6	0.0006
Lowest systolic blood pressure	92 ± 11	83 ± 11	
Lowest diastolic blood pressure	59 ± 24	45 ± 31	

Values indicated are mean±SD and number

Table 2. Laboratory tests for hemostasis and markers of plasma leakage on day of admission in survivors and non-survivors in 50 patients with dengue shock syndrome

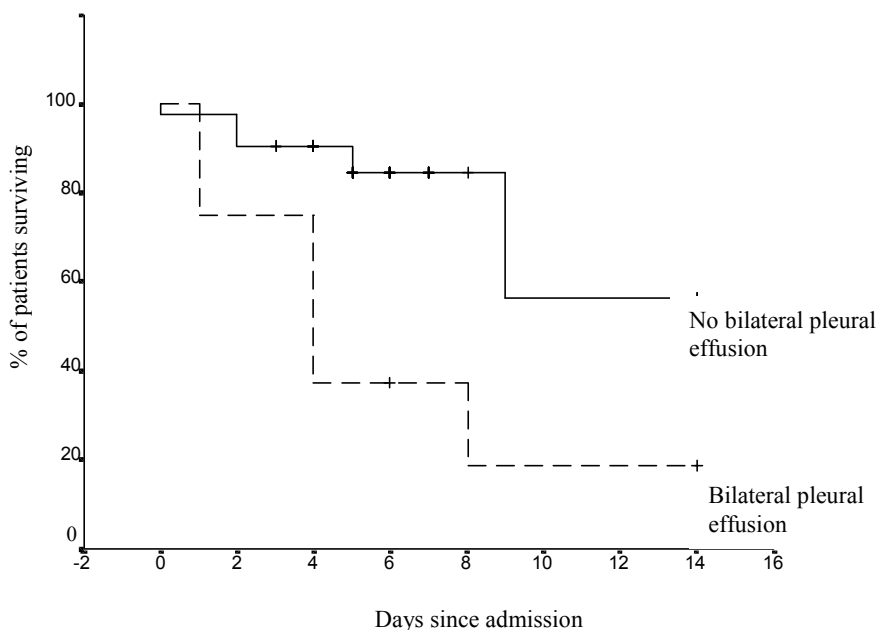
	Survivors (n=37)	Non-Survivors (n=13)	p value *)
<i>Tests for hemostasis</i>			
APTT (sec)	56.6 ± 24.1	107.0 ± 102.2	0.06
PT (sec)	15.3 ± 4.7	24.2 ± 12.0	0.001
PAI-1 (ng/ml)	285.1 ± 491.8	1126.4 ± 1314.6	0.0006
TATc (ng/ml)	32.2 ± 27.3	67.5 ± 43.3	0.004
Platelets (x10 <sup>9</sup> /l)	63.8 ± 35.7	48.6 ± 12.9	0.15
<i>Markers of plasma leakage</i>			
			p value (95% CI)
Serum protein (g/l)	5.2 ± 1.0	4.5 ± 0.9	0.035 (0.052 - 1.335)
Serum albumin (g/l)	3.5 ± 0.7	3.2 ± 0.6	0.22 (-0.164 - 0.682)
Albumin/protein ratio	0.67 ± 0.08	0.71 ± 0.07	0.10 (-0.103 - 0.010)
Plasma sodium (mmol/l)	134.9 ± 6.2	133.8 ± 8.0	0.63 (-3.639 - 5.872)
Hematocrit (%)	38.3 ± 6.4	36.4 ± 7.4	0.39 (-2.519 - 6.227)

\* Mann-Whitney U test for differences of APTT, PT, PAI-1, TATc, and platelets; Independent t-test for serum protein, serum albumin, albumin/protein ratio, plasma sodium and hematocrit; APTT: activated partial thromboplastin time (control value: 31.9±1.7 sec); PT: prothrombin time (control value: 12±0.6 sec); PAI-1: plasminogen activator inhibitor-type 1 (normal value 30-60 ng/ml); TATc: thrombin-antithrombin complex (normal value <4.1 ng/ml).

Table 3. Association of markers of coagulation and fibrinolysis and of plasma leakage with mortality in 50 patients with dengue shock syndrome

	$\beta$	<i>p</i> value	Exp ( $\beta$ )/OR
<i>Activation markers of coagulation and fibrinolysis*</i>			
TATc (ng/ml)	0.02	0.048	1.02
PAI-1 (100 ng/ml)	0.08	0.12	1.08
Constant	-2.79	0.0002	
<i>Markers of plasma leakage</i>			
Bilateral pleural effusion	2.5	0.007	12.2
Albumin protein ratio*)	3.7	0.39	40.7
Constant	-4.109	0.172	

$\beta$ : regression coefficient; Exp.  $\beta$ : exponent  $\beta$ ; OR: odds ratio; TATc: thrombin-antithrombin complex; PAI-1: plasminogen activator inhibitor type-1; \*) day of admission levels



Legend to figure.

Figure. Kaplan Meier survival curves of patients with bilateral pleural effusion compared with those without bilateral pleural effusion ( $p=0.002$ ). The median survival time of patients with bilateral pleural effusion was 4 days (95% CI, 1.32 - 6.68).

fatal cases demonstrated evidence of severe bleeding followed by shock<sup>24</sup>, foci of hemorrhage in the brain<sup>28</sup>, histological evidence of intravascular coagulation,<sup>26,29</sup> sero-hemorrhagic effusions<sup>29</sup> and renal impairment.<sup>30</sup>

We also found that prothrombin time was significantly longer in non-survivors than in survivors. This suggests that the extrinsic (tissue factor/factor VIIa) pathway may be the main route in the activation of coagulation in DSS.

A number of clinical signs were more frequently seen in non-survivors than in survivors although these signs did not qualify in the logistic regression analysis, because these are not specific for DSS. Clammy skin was significantly more frequent ( $p=0.0002$ ) and the systolic and diastolic blood pressures were lower in non-survivors (Table 1). In addition, we found that delirium and decreased consciousness were significantly more frequent in non-survivors than in survivors ( $p=0.002$ ).

and  $p=0.010$ , respectively). Dengue virus subtypes 2 and 3 have been isolated from cerebrospinal fluid and have known to be neurovirulent.<sup>31</sup> Histological studies from fatal DHF cases with signs of encephalopathy have provided evidence that the brain cell is also a target cell for dengue virus.<sup>32-36</sup> In addition, loss of integrity of the cerebral vascular endothelium leading to cerebral oedema and cerebral bleeding has been observed.<sup>28,37</sup>

Conclusion our data provide evidence for a relationship of mortality with both, plasma leakage and coagulation activation in dengue shock syndrome. In the absence of interventions to stop vascular leakage, close monitoring and the use of plasma expanders to increase intravascular oncotic pressure is mandatory, especially in children with pleural effusion. In addition strategies to control coagulation activation would be an important area of development.

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