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# Increase of Plasminogen Activator Inhibitor-1 and Decrease of Transforming Growth Factor-B1 in Children with Dengue Haemorrhagic Fever in Indonesia

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## Summary

**Mortality in children with severe dengue haemorrhagic fever (DHF) in Indonesia is high. The origin of the elevated plasminogen activator inhibitor-1 (PAI-1) levels in these children is unclear. We measured PAI-1, transforming growth factor-β1 (TGF-β1), platelet counts, plasma leakage and liver function in 71 children with DHF (3–15 years old) and in 30 healthy children. We found that PAI-1 concentrations in children with DHF were significantly higher on admission than on Day 2. Circulating TGF-β1 concentrations on admission were significantly lower in DHF than in controls, but on Day 2 increased towards levels in controls. TGF-β1 and PAI-1 concentrations were not correlated on either day. PAI-1 was correlated with platelet count and serum albumin on admission, and with degree of pleural effusion. Liver function tests were mildly elevated but not correlated with PAI-1. In conclusion, elevated PAI-1 concentrations in DHF were associated with platelet counts and plasma leakage.**

**Key words: dengue, PAI-1, TGF-β1, plasma leakage.**

## Introduction

The clinical manifestations of dengue virus infection range from mild disease to the more severe dengue haemorrhagic fever (DHF), of which the most severe form is called dengue shock syndrome (DSS). Globally, an estimated 24 000–50 000 patients die out of 250 000–500 000 DHF/DSS cases that occur annually, depending on the epidemic activity [1, 2]. Death in severe DHF and DSS is caused by prolonged shock and massive haemorrhage [3]. DSS children who died in Kariadi hospital had persistently high PAI-1, contributing to an ongoing pro-coagulant state by a relative inhibition of fibrinolysis [4]. Increased PAI-1 concentrations in patients with severe dengue virus infections were also found in a study from Vietnam, whereby PAI-1 levels correlated with bleeding severity [5]. During *Neisseria meningitidis* infection, 4G/5G polymorphism in the promotor gene of PAI-1 was associated with increased plasma PAI-1 concentration and with the development of shock and death [6, 7]. However, this polymorphism had no influence on PAI-1 concentrations and the risk of death in children with dengue [8].

Other factors may also influence the plasma PAI-1 concentration in severe dengue virus infection. PAI-1 concentration is modulated by cytokines such as TGF-β1 [9]. Studies in dengue virus-infected patients suggested that TGF-β1 may play a role in severe clinical manifestations [10, 11]. However, the relation between PAI-1 and TGF-β1 concentrations in dengue has never been explored. A rich source of TGF-β1 [12] and PAI-1 [13] are platelets. In addition to the platelets, leukocytes are also a rich source of TGF-β1. *In vitro* studies show that leukocytes release

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a large amount of TGF-β into the medium [14–16]. Therefore, in our study we also assessed *ex vivo* lipopolysaccharide (LPS)-induced TGF-β production in whole blood of dengue virus-infected children.

Plasma PAI-1 is produced by various cells including endothelial cells [17] and is a marker of endothelial function [18]. Dysfunction of the endothelial cells can affect vascular permeability and cause plasma leakage which is an important clinical manifestation in severe dengue. However, the relation between plasma PAI-1 concentration and plasma leakage in children with severe dengue has not been studied. Liver disease results in reduced plasma PAI-1 clearance [19–22], and liver dysfunction usually occurs in severe dengue [23–25]. Whether plasma PAI-1 concentration in patients with severe DHF is associated with liver injury remains unclear.

## Methods

The study was done in 2005–06 at Dr Kariadi Hospital, Semarang, Indonesia. We enrolled children below 15 years of age who were admitted to the paediatric intensive care unit or the paediatric ward and were suspected clinically of DHF. The WHO classification was used in determining whether children had DHF Stages I and II or DHF Stages III and IV (DSS; WHO, 1997). Cases were included only if acute dengue virus infection was confirmed serologically by capture and indirect ELISA (Focus Technologies, Cypress, CA, USA) showing positive dengue-specific IgM and IgG antibodies (optical density of the sample above the cut-off value of the serum provided by the manufacturer). Thirty children, age 6–14 years and found healthy after clinical examination by the medical doctor, were included as controls.

Blood was collected on day of admission and on Day 2 after admission. All blood samples were centrifuged at 15°C for 20 min at 1600g. Plasma samples were collected and stored at −80°C. Plasma PAI-1 concentrations (normal value: 7–43 ng ml$^{-1}$) were measured using a commercially available Technozym PAI-1 antigen ELISA kit (Technoclone GmbH, Vienna, Austria). Serum albumin and protein concentrations (normal values for 7-year-old children: 3.26 and 5.97 g dl$^{-1}$ or above, respectively) were measured using Bromocresol–Green and Biuret method, respectively. Concentrations of albumin and protein were used as plasma leakage markers. Pleural effussion index (PEI), used as a marker for plasma leakage and calculated as 100 times the maximum width of the (right or left) pleural effusion, divided by the maximum width of the hemi thorax on that side, was measured on day of admission and Day 2 after admission. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (normal values for 7-year-old children: up to 35 and 40 U l$^{-1}$, respectively) were measured using colorimetric method (HITACHI 7050; Boehringer Ingelheim, Germany).

TGF-β1 and *ex vivo* TGF-β1 production were measured as follows. Ethylenediaminetetra-acetic acid (EDTA) whole-blood samples were centrifuged, plasma was obtained and stored at −80°C until analysis for circulating TGF-β1. One hundred microlitres of whole blood was added to 900 µl complete RPMI (Sigma Chemical Co., St Louis, MO, USA) with 10% fetal bovine serum (FBS) in a 24-well sterile culture plate. *Escherichia coli* LPS (10 µg ml$^{-1}$; Sigma) was added, then the samples were incubated at 37°C in 5% $CO_2$ atmosphere for 24 h. Supernatants were obtained after centrifugation and stored at −80°C until immunoassay. TGF-β1 was measured using a commercially available TGF-β1 $E_{max}$® ImmunoAssay (Promega, Madison, WI, USA). For measuring circulating TGF-β1 plasma concentrations, plasma was acid treated and the samples neutralized as described in the ELISA protocol. This procedure was also performed for measuring *ex vivo* LPS stimulated total TGF-β1 concentrations in the supernatant. For measuring *ex vivo* LPS stimulated naturally activated TGF-β1 concentrations, culture supernatant was processed directly using the ELISA protocol without acid treatment.

The study was approved by the Research Ethics Committee of Dr Kariadi Hospital. Informed consent was obtained from parents or legal guardians of the patients and of the healthy controls.

Analysis was performed using SPSS 11.5 (Jakarta, Indonesia). Mean (SD) of normally distributed data were compared using Student's *t*-test. If data distribution was not normal, Mann–Whitney U-test was used to compare medians with inter-quartile range (IQR). Wilcoxon rank test was used to compare medians with IQR between observation days. To quantify correlations between continuous data, Spearman's rank correlation coefficient was measured. A $p < 0.05$ was used to indicate statistical significance.

## Results

### *Characteristics of the study population*

A total of 71 children with DHF were included in the study, 43 with DHF Stages I and II, and 28 with DHF Stages III and IV (DSS; Table 1). The children were 3–14 years old, 24 males and 47 females. Median duration of fever on admission was 4 days. Blood pressure of the DHF Stage IV patients was unmeasurable on admission, the value mentioned in Table 1 was that after initial fluid replenishment. In-hospital mortality occurred in 6 out of 71 patients, all of them DSS cases.

### *PAI-1 concentration and correlation with circulating plasma TGF-β1*

Plasma PAI-1 concentrations were significantly elevated on both observation days, and higher on

TABLE 1
*Characteristics of the study population* (n = 71)

| Characteristic | DHF I and II ($n=43$) | DHF III and IV (DSS) ($n=28$) | $p$-value |
|---|---|---|---|
| Male sex, $n$ (%) | 15 (34.9) | 9 (32.1) | 0.057 |
| Age (years) | 8.00 (6.00–9.00) | 7.00 (6.00–9.75) | 0.465 |
| Duration of fever until admission (days) | 4.00 (3.00–5.00) | 4.00 (4.00–5.00) | 0.732 |
| Body weight (kg) | 20.00(18.00–28.00) | 22.00 (17.13–29.75) | 0.650 |
| Body height (cm) mean (SD) | 122.97(15.14) | 123.25 (17.81) | 0.948[a] |
| Body temperature (°C) | 37.60 (37.00–38.40) | 38.00 (37.13–38.50) | 0.426 |
| Pulse per minute, mean (SD) | 101.43 (15.91) | 108.04 (15.78) | 0.092[a] |
| Systolic blood pressure (mmHg) | 100.00 (100.00–110.00) | 100.00 (90.00–100.00)[b] | 0.169 |
| Diastolic blood pressure (mmHg) | 70.00 (60.00–70.00) | 70.00 (60.00–70.00)[b] | 0.474 |
| Mean arterial pressure (mmHg) | 80.00 (73.30–85.00) | 80.00 (70.00–85.00)[b] | 0.299 |
| Respiratory rate per minute | 28.00 (24.00–28.50) | 28.00 (24.00–30.00) | 0.505 |
| Tourniquet test positive, $n$ (%) | 29 (67.4) | 14 (50.0) | 0.100 |
| Tourniquet test: petechiae per 2.5 $cm^2$ | 20.00 (10.00–25.00) | 20.00 (0.00–23.75) | 0.217 |
| Petechiae, $n$ (%) | 7 (16.3) | 7 (25.0) | 0.367 |
| Epistaxis, $n$ (%) | 5 (11.6) | 0 (0.0) | 0.071[c] |
| Gum bleeding, $n$ (%) | 1 (2.3) | 0 (0.0) | 1.000 |
| Haematemesis, $n$ (%) | 2 (4.7) | 1 (3.6) | 1.000[c] |
| Melena, $n$ (%) | 0.0 (0.0) | 1 (3.6) | 0.394 |
| Pleural effusion (left, right, both) on Day 0, $n$ (%) | 31 (72.1) | 25 (89.3) | 0.092 |
| Haemoglobin (g $dl^{-1}$) | 13.10 (12.40–14.00) | 13.30 (11.93–14.20) | 0.902 |
| Hematocrit (%) | 38.20 (36.40–41.70) | 39.80 (35.60–43.25) | 0.878 |
| Thrombocyte count ($\times$1000 cell $ml^{-1}$) | 63.00 (40.00–85.00) | 38.00 (25.70–66.50) | 0.074 |
| White blood cell count ($\times 10^3$ cell $ml^{-1}$) | 4.40 (3.10–6.30) | 5.00 (3.00–9.50) | 0.221 |

Data are median (IQR) and were analysed using Mann–Whitney U-test unless otherwise specified; data presented as $n$ (%) were analysed using $\chi^2$-test unless otherwise specified.
*Significant difference at $p < 0.05$.
[a]Independent $t$-test.
[b]Blood pressure after initial fluid replenishment.
[c]Fisher exact test.

admission than on Day 2 ($p=0.033$; Table 2). PAI-1 levels on admission of DSS [mean ± SD (75.77 ± 28.48 ng $ml^{-1}$)] and DHF without shock (68.30 ± 29.32) groups were not significantly different ($p=0.336$). PAI-1 levels on Day 2 of DSS (73.78 ± 37.32) were significantly higher ($p=0.014$) than those of DHF without shock (52.33 ± 29.23).

Circulating plasma TGF-β1 concentrations on admission were significantly lower than those of healthy children (Table 3). A significant increase of circulating plasma TGF-β1 concentrations was found from admission to Day 2 ($p=0.017$), despite a decrease in haematocrit. On Day 2, after admission circulating plasma TGF-β1 concentrations of children with DHF approached values found in the controls (Table 3). Circulating plasma TGF-β1 concentrations did not significantly correlate with PAI-1 concentrations on both observation days (Fig. 1C).

*Correlation of LPS-stimulated TGF-β1 with PAI-1*
LPS-stimulated naturally activated and total TGF-β1 concentrations on both observation days were significantly lower than those of control children (Table 3). A significant increase from admission to Day 2 was observed of LPS stimulated total TGF-β1 concentrations. There were no significant correlations between PAI-1 concentrations and LPS-stimulated TGF-β1 concentrations, neither on admission, nor on Day 2 (data not shown).

*Platelet counts and correlation with PAI-1 and TGF-β1*
Platelet count on admission was not significantly different from that on Day 2 (Table 2). A significant but weak inverse correlation was found between platelet count and PAI-1 concentration on admission ($r=-0.293$; $p=0.021$; Fig. 1A). On Day 2, there were also significant but weak correlations between platelet count and circulating TGF-β1 plasma concentration ($r=0.320$; $p=0.013$; Fig. 1B) as well as LPS-stimulated total TGF-β1 concentration ($r=0.310$; $p=0.021$).

*Plasma leakage and correlation with PAI-1*
Serum albumin and total protein concentration on admission were decreased, and significantly lower than on Day 2 ($p=0.006$ and $p=0.001$, respectively), when concentrations had normalized. From

TABLE 2
*Laboratory parameters, PAI-1 and PEI on admission and on Day 2 in children with DHF (n = 71)*

| Marker | Day 0 Median (IQR) | Day 2 Median (IQR) | *n* | *p*-value |
|---|---|---|---|---|
| Platelet count ($\times 1000\,cell\,ml^{-1}$) | 58.00 (32.75–83.50) | 54.00 (40.25–89.75) | 64 | 0.937[a] |
| Leukocytes counts ($\times 1000\,cell\,ml^{-1}$) | 4.45 (3.03–6.88) | 6.74 (4.73–9.90) | 64 | <0.0001[a] |
| Total protein ($g\,dl^{-1}$) mean (SD) | 5.39 (1.07) | 5.99 (1.25) | 62 | 0.001[b] |
| Albumin ($g\,dl^{-1}$) mean (SD) | 3.20 (0.61) | 3.42 (0.57) | 62 | 0.006[b] |
| Haemoglobin ($g\,dl^{-1}$) | 13.10 (12.23–14.00) | 12.20 (11.43–13.10) | 64 | 0.008[a] |
| Hematocrit | 38.45 (36.00–42.00) | 36.25 (33.58–39.23) | 64 | 0.007[a] |
| SGPT ($U\,l^{-1}$) | 47.50 (29.75–77.50) | 51.00 (33.00–75.00) | 62 | 0.971[a] |
| SGOT ($U\,l^{-1}$) | 117.50 (83.00–188.25) | 103.50 (72.00–148.00) | 62 | <0.0001[a] |
| PAI-1 ($ng\,ml^{-1}$) mean (SD) | 70.52 (29.30) | 59.96 (33.16) | 55 | 0.033[b] |
| PEI (%) | 16.12 (3.89–24.63) | 25.50 (18.00–36.56) | 62 | <0.0001[a] |

Data are median (IQR) unless otherwise specified. PEI—in 62 out of 71 children, chest X-ray was available on both the days.
[a]Wilcoxon sign rank test.
[b]Paired *t*-test.

TABLE 3
*TGF-β1 in children with DHF and healthy controls*

| Cytokines ($pg\,ml^{-1}$) | DHF Children ($n = 71$) | | | Healthy Children ($n = 30$) | *p*-value |
|---|---|---|---|---|---|
| | *n* | Day 0 | Day 2 | | |
| Circulating plasma TGF-β1 | 59 | 33 990.00 (8106.00–51 450.00) | 39 530.00 (24 850.00–52 590.00) | 50 197.00 (30 051.00–84 548.00) | <0.0001[a]<br>0.150[b]<br>0.017[c] |
| LPS stimulated naturally activated TGF-β1 | 56 | 12.71 (0.00–62.21) | 28.21 (7.27–54.09) | 2566.35 (2347.33–3363.20) | <0.0001[a]<br><0.0001[b]<br>0.310[c] |
| LPS stimulated total TGF-β1 | 52 | 4030.50 (2142.45–6936.50) | 7629.00 (3850.75–15 050.50) | 102 209.00 (82 265.00–123 020.00) | <0.0001[a]<br><0.0001[b]<br><0.0001[c] |

Data are median (IQR).
*Significant difference at $p < 0.05$.
[a]Mann–Whitney U-test between day on admission and healthy children.
[b]Mann–Whitney U-test between Day 2 after admission and healthy children.
[c]Wilcoxon sign rank test between admission day and Day 2 after admission of DHF children.

admission to Day 2, the PEI of patients increased significantly ($p < 0.0001$; Table 2), while haematocrit values decreased significantly ($p = 0.007$). On admission, a significant but moderate negative correlation was observed between PEI and both albumin ($r = -0.483$; $p < 0.0001$) and total protein ($r = -0.431$; $p < 0.0001$) concentrations. This correlation remained also on Day 2 (data not shown). On admission, a significant weak negative correlation was observed between PAI-1 and serum albumin concentration ($r = -0.306$; $p = 0.017$ Fig. 2A). Also, we found a significant positive correlation between PAI-1 and PEI ($r = 0.283$; $p = 0.026$) and ($r = 0.343$; $p = 0.010$) on admission and Day 2, respectively (Fig. 2B). Haematocrit correlated with serum albumin ($r = 0.370$; $p = 0.004$) on Day 2, but did not correlate with PAI-1 in either observation days.

*Liver function and correlation with PAI-1*

The mean serum SGOT and SGPT concentrations were significantly but mildly elevated on both observation days. Values for SGPT on admission and on Day 2 were similar; however, SGOT concentrations on admission were significantly higher than on Day 2 ($p < 0.0001$; Table 2). A correlation was found between PAI-1 and both, SGPT and SGOT concentration, ($r = 0.279$; $p = 0.028$) and ($r = 0.454$; $p < 0.0001$) on Day 2.

## Discussion

Our study showed that PAI-1 levels were elevated, both on admission and on Day 2 after admission, and that admission levels in DSS were not different from those in DHF without shock. This is in line with

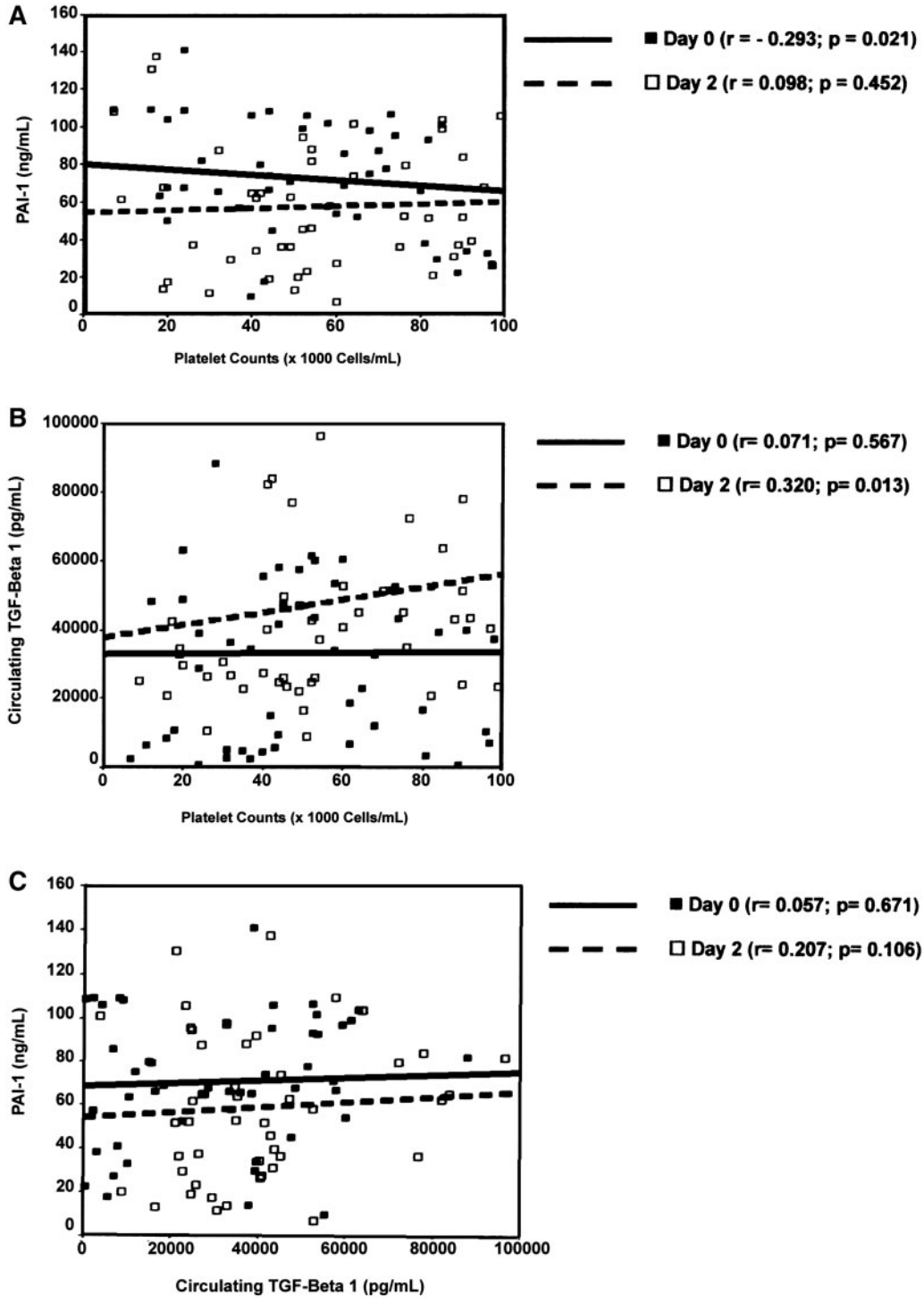


FIG. 1. Correlation of platelet count with plasma PAI-1 on admission ($n = 62$) and on Day 2 ($n = 61$) (**A**), and circulating plasma TGF-β1 on admission ($n = 67$) and on Day 2 ($n = 60$) (**B**), correlation of plasma PAI-1 with TGF-β1 on admission ($n = 59$) and on Day 2 ($n = 62$) (C) in children with DHF.

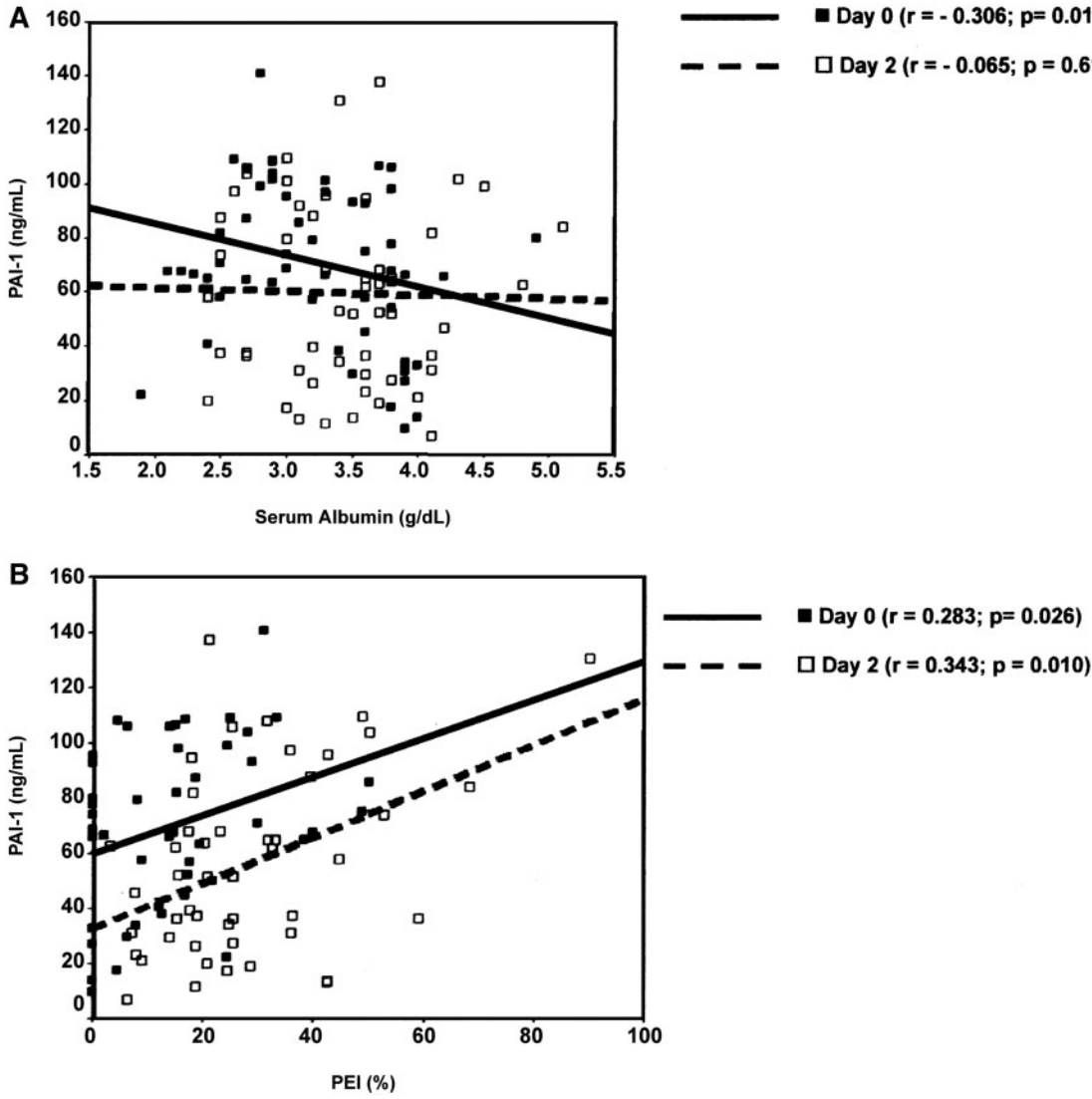


FIG. 2. Correlation of plasma PAI-1 with serum albumin on admission ($n = 61$) and on Day 2 ($n = 62$) (**A**), and PEI on admission ($n = 62$) and on Day 2 ($n = 55$) (**B**) in children with DHF.

a previous study done in the pediatric department in the same hospital [8]. In that study, PAI-1 levels on admission in DHF and DSS patients were 60 (IQR 42.3–82.5) and 87.0 (IQR 35.0–180.8) ng ml$^{-1}$, respectively, without significant difference between those groups. We found, however, that on Day 2 PAI-1 levels in children with DSS were significantly higher than in children with DHF without shock ($p = 0.014$), suggesting that PAI-1 levels are associated with the presence of shock.

In the present study, significant lower TGF-β1 concentrations were found in children with DHF compared with healthy control children. This finding is in contrast with earlier studies that reported higher TGF-β1 concentrations in children with DHF compared with children with dengue fever as well as control children [10, 11]. Several factors may explain this difference. First, the selection of the controls. In our study, healthy children without any diseases were included as controls while Laur *et al.* [11] included children who underwent pre-operative investigation. Interestingly, also Laur *et al.* [11] found lower TGF-β1 concentrations in the first few days after onset of fever, than later in the disease. Secondly, the difference may also be explained by the type of sample that was used for the measurement of

TGF-β1. We used EDTA plasma to avoid platelet degranulation as suggested in the guidelines for the TGF-β1 assay (Promega Co protocol, 2006). The other studies used platelet free citrated plasma [11] and serum [10], respectively. Citrated-plasma samples will interfere with the assay as mentioned by the manufacturer (Promega Co protocol, 2006) although the assay used by Laur *et al*. does not specify this. The use of serum, whereby all platelets are allowed to degranulate, will assess mainly the TGF-β1 content of platelets [26]. Thirdly, collection of samples is important. Platelet degranulation during plasma collection and preparation may contribute to an over-estimation of plasma TGF-β1 levels [26]. This can be avoided by using the procedure developed by Wakefield *et al*. [27] who used a wide-gauge butterfly needle without using tourniquet during blood drawing. We were not able to use large needles due to the small veins in the children, and shock in children with DSS. No correlation between plasma TGF-β1 concentrations and platelet counts on admission was found, while a weak correlation ($r = 0.320$; $p = 0.013$) (Fig. 1B) was found on Day 2. Also on Day 2, a weak correlation was observed between platelet counts and LPS-stimulated total TGF-β1 production ($r = 0.310$; $p = 0.021$). Previous study in healthy individuals showed no significant correlation between those variables in plasma ($n = 15$), while in serum a strong correlation was found ($r = 0.86$; $p < 0.001$; $n = 16$) [28]. Platelet-large latent TGF-β1 released during clotting was not activated by acid treatment in the absence of urea [29]. This procedure was performed in our study and we therefore avoided over-estimation of TGF-β1 levels caused by platelet degranulation during plasma collection and preparation. This is also supported by a previous study using plasma TGF-β1 chromatography analysis whereby almost complete absence of platelet-large latent TGF-β1 complex in plasma was found [26]. TGF-β1 produced by activated platelets is able to induce the PAI-1 secretion by human umbilical cord endothelial cells [9]. LPS receptor-signalling complex including TLR4 and MyD88 are expressed by platelets, and LPS-induced platelet secretion uses TLR4/MyD88-dependent pathway [30]. Our study, therefore, suggests that activated platelets might be the source of TGF-β1. In addition, thrombocytopenia might be underlying the reduced LPS-stimulated TGF-β1 production in complicated dengue.

We found that plasma TGF-β1 concentrations on admission were similar in children with DSS and children with DHF without shock. This finding is not in line with the study by Argawal *et al*. [10], who reported higher serum TGF-β1 concentrations in patients with DHF grade IV. On Day 2, plasma TGF-β1 concentrations increased significantly to levels of healthy controls (not significantly different anymore). The difference with our findings may be due to the sample used (serum, not plasma). Alternatively, TGF-β1 might be lost from blood circulation during plasma leakage or bound to the receptor.

The genetic background of patients may also influence TGF-β1 levels. TGF-β1-509 CC genotype showed low TGF-β1 in plasma [31]. This TGF-β1 polymorphism in combination with CTLA4 gene polymorphism was associated with DHF susceptibility [32]. Most of DHF patients demonstrated lower plasma and *ex vivo* LPS stimulation TGF-β1 levels. The TGF-β1 gen polymorphisms might therefore influence plasma level and production capacity of TGF-β1 in DHF patients. Our study showed normalized plasma TGF-β1 levels accompanied by a significant increase of leukocyte counts. This in line with a study showing that leukocytes, including mononuclear cells and granulocytes, contained large amount of TGF-β1 [26].

TGF-β1 is an immunosuppressive cytokine and the low plasma concentrations, as we found in our study in severe dengue patients, may therefore contribute to the pathogenesis of DHF. The fact that we found low plasma TGF-β1 concentrations and high PAI-1 levels suggest that plasma TGF-β1 is not the major signal for PAI-1 expression in severe dengue. Furthermore, plasma TGF-β1 concentrations did not correlate with PAI-1 concentrations on both observation days (Fig. 1C), while PAI-1 concentrations also did not correlate with *ex vivo* LPS-stimulated TGF-β1 production in whole blood.

There is a continuous production of large amounts of active PAI-1 in platelets [13]. We found high PAI-1 levels on the day of admission, while the concentration of TGFβ was low. To explain the difference between these two mediators, we should consider that PAI-1 is almost exclusively released from platelets, while TGF-β is not only released from platelets, but also from the immune cells. Critically ill patients with severe infections are long known to have a diminished production of cytokines [33], which most likely explains the low TGF-β1 in children with DHF. In contrast, the PAI-1 concentrations are not affected by this immunological-driven effect, and depend exclusively on the release from the alpha granules of the platelets.

An *in vitro* study demonstrated that dengue 2 virus was able to directly interact with and activate platelets, an event that might be important in the pathogenesis of dengue-associated thrombocytopenia [34]. We observed elevated plasma PAI-1 concentrations and thrombocytopenia in children with DHF on both observation days. Our study also showed that platelet counts had a negative correlation with plasma PAI-1 concentrations on admission (Fig. 1A). This finding is in line with the notion that platelets are the main source of plasma PAI-1.

Endothelial dysfunction results in plasma leakage, pleural effusion (measured by PEI) and decrease of

serum albumin concentration. Serum albumin and PEI on admission were significantly lower than on Day 2 ($p = 0.006$ and $p < 0.0001$, respectively; Table 2). Serum albumin had no significant correlation with liver injury (measured by SGPT) or renal function. However, serum albumin concentrations were correlated with PEI on admission, suggesting that albumin concentration was related to plasma leakage, but not to liver and renal function. The correlation we observed between PAI-1 concentrations and serum albumin on admission (Fig. 2A), and PEI on either observation days (Fig. 2B), suggests that plasma leakage, which may result in intravascular volume depletion, influences the elevation of plasma PAI-1 concentration. Our study is in line with a previous study which found that plasma leakage contributed significantly to several plasma protein concentrations in DSS children [35]. The results of serum albumin and PEI measurement of our study in fact contradicted each other. Because serum albumin is influenced by several factors, PEI may be a more reliable measure for plasma leakage in DHF patients.

PAI-1 levels are also influenced by PAI-1 clearance in the liver [19–22]. Previous studies found disturbance of liver function in severe dengue [23–25]. However, in the present study, only mild increase of SGOT and SGPT was found which may have little contributed to PAI-1 clearance.

In conclusion, the elevated PAI-1 concentrations observed in children with DHF may be the result of plasma leakage, and are associated with platelet counts. In contrast to previous studies, low plasma TGF-β1 concentrations were found in children with severe dengue compared with healthy controls. We did not find evidence that liver injury or TGF-β1 can explain the elevated PAI-1 levels.

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