Effect of oseltamivir phosphate versus placebo on platelet recovery and plasma leakage in adults with dengue and thrombocytopenia; a phase 2, multicenter, double-blind, randomized trial

Rahajeng N. Tunjungputri1,2, Silvita Fitri Riswari1,3,4, Setyo G. Pramudo2,5, Lydia Kuntjoro6, Bachti Alisjahbana3,7, Harry Galuh Nugraha8, Andre van der Ven1, Muhammad Hussein Gasem2,5, Quirijn de Mast1*

1 Department of Internal Medicine and the Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands, 2 Center for Tropical and Infectious Disease (CENTRID), Faculty of Medicine Diponegoro University, Dr. Kariadi Hospital, Semarang, Indonesia, 3 Research Center for Care and Control of Infectious Disease (RC3ID), Universitas Padjadjaran, Bandung, Indonesia, 4 Parasitology Division, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia, Indonesia, 5 Department of Internal Medicine, Diponegoro National University Hospital, Faculty of Medicine Diponegoro University, Semarang, Central Java, Indonesia, 6 Department of Radiology, Diponegoro National University Hospital, Faculty of Medicine Diponegoro University, Semarang, Central Java, Indonesia, 7 Department of Internal Medicine, Hasan Sadikin General Hospital, Faculty of Medicine Universitas Padjadjaran, Bandung, West Java, Indonesia, Indonesia, 8 Department of Radiology, Hasan Sadikin General Hospital, Faculty of Medicine Universitas Padjadjaran, Bandung, West Java, Indonesia, Indonesia

* Quirijn.deMast@radboudumc.nl

Abstract

Background
Thrombocytopenia, bleeding and plasma leakage are major complications of dengue. Activation of endogenous sialidases with desialylation of platelets and endothelial cells may underlie these complications. We aimed to assess the effects of the neuraminidase inhibitor oseltamivir on platelet recovery and plasma leakage in dengue.

Methods
We performed a phase 2, double-blind, multicenter, randomized trial in adult dengue patients with thrombocytopenia (<70,000/μl) and a duration of illness ≤ 6 days. Oseltamivir phosphate 75mg BID or placebo were given for a maximum of five days. Primary outcomes were the time to platelet recovery (≥ 100,000/μl) or discharge from hospital and the course of measures of plasma leakage.

Results
A total of 70 patients were enrolled; the primary outcome could be assessed in 64 patients (31 oseltamivir; 33 placebo). Time to platelet count ≥100,000/μl (n = 55) or discharge (n = 9)
were similar in the oseltamivir and placebo group (3.0 days [95% confidence interval, 2.7 to 3.3] vs. 2.9 days [2.5 to 3.3], \( P = 0.055 \)). The kinetics of platelet count and parameters of plasma leakage (gall bladder thickness, hematocrit, plasma albumin, syndecan-1) were also similar between the groups.

**Discussion**

In this trial, adjunctive therapy with oseltamivir phosphate had no effect on platelet recovery or plasma leakage parameters.

**Trial registration**

ISRCTN35227717.

**Author summary**

Moderate to severe thrombocytopenia is common in the febrile and/or critical phase of dengue virus infection. Platelets are important for preservation of vascular integrity, especially during inflammation, and low platelet counts may contribute to plasma leakage. Currently, no therapeutic intervention that targets the pathogenic pathway is available for DENV infection, including therapies to prevent or reduce thrombocytopenia or plasma leakage. Oseltamivir phosphate is widely used for prevention and treatment of influenza by inhibiting viral neuraminidase. However, oseltamivir may also inhibit human endogenous neuraminidase involved in sialic acid metabolism, and as such extend the lifespan of platelets. In the phase 2 TOTO trial (Treatment Of Thrombocytopenia with Oseltamivir in acute dengue virus infection: a randomized, placebo controlled, multicenter trial) we investigated the potential of oseltamivir phosphate to shorten the time to platelet recovery and reduce plasma leakage in patients with DENV infection. In this trial involving 70 adult thrombocytopenic patients, hospitalized with acute DENV infection, adjunctive therapy with oseltamivir phosphate did not shorten platelet recovery time compared with placebo. The trial also did not show an effect of adjunctive oseltamivir on plasma leakage parameters. The reasons that oseltamivir had no apparent effect on platelet counts, markers of plasma leakage and glycocalyx distortion in this study remain speculative, but may involve one or more of the following; first, dengue-associated thrombocytopenia and plasma leakage are both multifactorial in origin and targeting neuraminidase alone may be insufficient to impact these processes. Second, oseltamivir phosphate was designed to inhibit viral neuraminidase, and data of its inhibitory actions on human neuraminidases are inconclusive. The finding in this study also suggest that while laboratory works may lead to hypotheses for novel treatment, proof of concept studies are essential to test them in a clinical setting.

**Background**

Dengue is the most common arboviral infection globally and is associated with a substantial global economic burden [1]. Dengue virus (DENV) infection may be asymptomatic or result in clinical manifestations ranging from a mild febrile illness to a life-threatening shock syndrome. The latter usually occurs around or shortly after the time of defervescence during the
so-called critical phase. Endothelial dysfunction leading to a transient vascular leak syndrome and bleeding are hallmarks of severe dengue. Moderate to severe thrombocytopenia is common in the febrile and/or critical phase of dengue and severe manifestations of dengue are usually preceded by a rapid drop in platelet count [2]. Different guidelines advise to measure platelet count daily and it is a commonly used parameter to guide timing of hospital admission and discharge [3].

Thrombocytopenia and platelet dysfunction may contribute to bleeding manifestations in dengue [4–7], which usually manifest as skin or mucosal bleeding and can be life-threatening. In addition, an increasing body of evidence highlights a role for platelets in maintaining vascular homeostasis, both in inflammatory conditions, as well as in absence of injury or inflammation [8–10]. Nonetheless, it should be acknowledged that thrombocytopenia is also common in patients with non-severe dengue and that platelets may release pathogenic molecules. This highlights the complex and still incompletely understood role of platelets in dengue pathophysiology.

Currently, no therapeutic intervention that targets the virus or pathogenic pathways is available for DENV infection, including therapies to prevent or reduce thrombocytopenia or plasma leakage. Intravenous immunoglobulins or corticosteroids were ineffective in treating thrombocytopenia [11,12], whereas a large multicenter trial failed to demonstrate a beneficial effect of prophylactic platelet transfusion [13].

The cause of thrombocytopenia in dengue is multifactorial, including increased clearance and reduced production [14]. Both platelets and endothelial cells have an abundance of sialic acids on their surface. Loss of sialic acid residues from platelet glycoproteins by endogenous sialidases, such as neuraminidase-1 and neuraminidase-3, results in rapid removal of platelets from the circulation by the hepatic Aswell Morell receptor [15]. This is a physiological clearance mechanism of senescent platelets, but may also lead to accelerated platelet clearance in pathological conditions. We recently showed that platelet desialylation also occurs in dengue [16]. In addition, DENV non-structural protein-1 (NS1) activates sialidases in endothelial cells leading to desialylation and disruption of the endothelial glycocalyx and vascular leak [17,18]. Oseltamivir phosphate is widely used for prevention and treatment of influenza by inhibiting viral neuraminidase. However, oseltamivir may also inhibit human endogenous neuraminidase involved in sialic acid metabolism [19], and as such extend the lifespan of platelets. Studies indeed showed a significant increase in platelet number in individuals prescribed oseltamivir for (suspected) influenza [20,21], and the successful use of oseltamivir to increase platelet numbers in immune thrombocytopenia (ITP) [22–25] and sepsis [26].

In the phase 2 TOTO trial (Treatment Of Thrombocytopenia with Oseltamivir in acute dengue virus infection: a randomized, placebo controlled, multicenter trial) we investigated the potential of oseltamivir phosphate to shorten the time to platelet recovery and reduce plasma leakage in patients with DENV infection.

**Methods**

**Ethics statement**

The study protocol was approved by the ethical review boards of Faculty of Medicine Diponegoro University and Universitas Padjajaran as well as the Indonesian National Agency of Drug and Food Control. All participants provided written informed consent.

**Study population and sample size calculation**

Patients were recruited among patients hospitalized for suspected DENV infection in six hospitals in Central and West Java Indonesia: RS Nasional Diponegoro, RSUD K.R.M.T. Wongso-negoro, William Booth Hospital, RSUD Kartini, Hasan Sadikin General Hospital and RSAU
dr. M. Salamun; recruitment in the two latter hospitals was started halfway the trial to increase patient recruitment. Patients were eligible for inclusion if they were aged at least 16 years; had a platelet count below 70,000/$\mu l$; had fever for six days or less; were positive for DENV NS-1 or positive for acute dengue serology with probable dengue criteria defined in WHO 2009. Restriction of enrolment to participants aged 16 years and above was done for safety and ethical reasons and because we considered it unlikely that fundamental differences exist in the pathophysiology of dengue-associated thrombocytopenia and plasma leakage between adults and children.

Patients were excluded when they used platelet function inhibitors or anticoagulants; had an estimated creatinine clearance <70 ml/min and/or an alanine aminotransferase (ALT) value $>3x$ ULN; were pregnant or breastfeeding; had a platelet transfusion during the current hospitalization; had an already recovering platelet number; or had persistent or recurrent clinically significant bleeding. All patients provided written informed consent.

**Study design and procedures**

This was a phase 2, double-blind, multicenter, randomized, placebo-controlled trial. Eligible patients were randomized using block randomization in a 1:1 ratio to receive oral oseltamivir phosphate 75mg twice daily or placebo. Trial drugs were administered orally under supervision until the primary endpoint was reached or until a maximum of five days of treatment. In patients with an estimated creatinine clearance between 30 and 60 ml/min, a dose reduction of 75mg OD was used. Generic oseltamivir phosphate was manufactured by PT Indofarma (Bekasi, Indonesia) in compliance with international manufacturing practice standards; trial drugs were prepared by Kimia Farma (Jakarta, Indonesia).

Patients were followed daily by members of the study team for up to five days of treatment or until hospital discharge. Daily assessments included assessment of possible side effects and bleeding manifestations (WHO bleeding score), ultrasonography (Philips Lumify portable ultrasound) to assess the thickness of the gall bladder wall and the presence of ascites and pleural fluid, and laboratory examinations. The latter consisted of a complete blood count (Hb, hematocrit, leukocytes, platelet count) twice daily using a standard hematology analyzer and daily plasma creatinine and ALT concentration. Plasma concentrations of albumin (ALB Flex Dimension, Siemens Healthcare Diagnostics, Ltd) and syndecan-1 (human syndecan-1 ELISA kit, Abcam, ab46506) were determined in stored plasma. Patients who were discharged before their platelet numbers had reached 100,000/$\mu l$ were visited at home whenever possible. A post-discharge visit was scheduled approximately three weeks after enrolment to assess for possible late complications and to obtain reconvalescence laboratory measurements.

An independent data monitor and an independent safety monitor reviewed study data at predefined intervals. On-site monitoring visits were performed by the data monitor and the Indonesian Food and Drug Authority (BPOM, Badan Pengawas Obat dan Makanan).

**Outcomes**

Primary outcomes measures were: (a) the time to platelet recovery, defined as the time between study enrolment and the platelet count reaching a value of $\geq 100,000/$\mu l, or discharge from hospital when the platelet number was still $<100,000/$\mu l and without follow-up samples at home, and (b) measures of plasma leakage, including hematocrit, concentrations of plasma albumin and the glycoscalyx marker syndecan-1 and results of daily ultrasonography (gall bladder wall thickness; presence of ascites or pleural fluid). Secondary outcome parameters included safety, the rate of change of platelet count at 24 and 48 hours and 5 days, occurrence of severe thrombocytopenia and the occurrence of clinical bleeding.
Statistical analyses

The sample size calculation was based on data from a previous study in dengue patients in the same area [16]. We assumed that a mean time for platelet number to reach 100,000/µl in those with enrolment platelet count of less than 70,000/µl was 4 days (SD 1.4). Considering a shortening of platelet recovery to 3 days in the oseltamivir group as clinically relevant, 31 participants per group had to be enrolled using a two-sided approach (alpha 0.05) with power 80%. Accounting loss to follow up, enrolment of 35 participants per group was planned.

All efficacy analyses were conducted in the intention-to-treat population (which included all patients who underwent randomization). The time to platelet count recovery or discharge was compared with the use of a two-sided Student T-test. Differences between the groups in kinetics of daily platelet count, hematocrit, plasma albumin and syndecan-1 concentrations and gallbladder wall thickness were compared using mixed model methods. Differences in the percentage of participants with pleural fluid or ascites were analyzed using Chi square test. All analyses were performed using Graphpad Prism 9 (Graphpad).

Results

A total of 70 patients were randomly assigned to receive oseltamivir (35 patients) or placebo (35 patients) during the period from January 2018 to February 2019. Demographic and baseline disease characteristics were generally similar in the two groups, except for ascites being more common in the placebo group at baseline (Table 1).

The mean duration of illness at enrolment was 4.4 days (1.1 days) in the oseltamivir group and 4.7 days (0.9 days) in the placebo group (P = 0.19). Randomization and receipt of intervention was done immediately following enrolment. The platelet count at enrolment was similar in both groups with a mean (SD) value of 44,000/µl (23,000/µl) in the oseltamivir group and 43,000/µl (16,000/µl) in the placebo group. Dengue was diagnosed by a positive NS1 rapid test in 46 (66%) patients and by serology in the remainder; serotyping by PCR was not performed. Twenty nine of all 70 patients had positive IgG, suggestive of a secondary infection in these patients. According to the 2009 WHO Guideline, none of the patients classified as severe dengue.

Fig 1 shows the trial profile. All the participants received at least one dose of the assigned oseltamivir or placebo. The trial regimen was prematurely discontinued in five different patients, because of withdrawal of consent (one in oseltamivir group and two in placebo group), administration of a platelet transfusion (one in oseltamivir group) or fresh frozen plasma (FFP) transfusion (one in oseltamivir group). One patient assigned to the oseltamivir group died. Seven patients in the oseltamivir group and three in the placebo group received less than 4 doses of the study drug; an overview of the number of study medication doses taken is given in S1 Fig.

Platelet recovery

The primary combined outcome measure, time to platelet count >100,000/µl or discharge, could be assessed in 64 patients using the intention to treat analysis. Fifty-five patients (27 in the oseltamivir group and 28 in the placebo group) reached a platelet count of 100,000/µl or above. Nine patients (four in the oseltamivir group and five in the placebo group) were discharged with a platelet count below 100,000/µl, of whom three had a platelet count between 95,000 and 99,000/µl. The time to the composite primary endpoint was similar across the groups: 3.0 ± 0.8 days in the oseltamivir group and 2.9 ± 1.1 days in the placebo group (P = 0.055). In the 55 patients who reached a platelet count >100,000/µl, there was also no difference in time to reach this time point (oseltamivir group 2.9 ± 0.8 days vs. placebo 2.6 ± 1.0 days; P = 0.17). The kinetics of the increase in platelet count between the groups was similar as
The median (IQR) change from baseline platelet count in the placebo group was -3,000/μl (-13,000 to 9,000/μl) at 24 hrs. after start of study medication and 17,000/μl (-7,500 to 44,750/μl) at 48 hrs. In the oseltamivir group, these changes were 0/μl (-9,000 to 3,000/μl; P = 0.6 vs. placebo group) at 24 hrs. and -3,000/μl (-16,000 to 15,500/μl; P = 0.06) at 48 hrs. The change in platelet count at day five was not calculated because only two and four patients in the placebo and oseltamivir groups were still hospitalized at that day. In participants with a baseline platelet count ≥ 20,000/μl (n = 61), marked thrombocytopenia below 20,000/μl developed in two patients in the placebo group and in six patients in the oseltamivir group. There were no differences in bleeding manifestation (skin petechiae, epistaxis and melena) across both groups during hospitalization (S1 Table). Finally, we evaluated whether the time between symptom onset and onset of the intervention might have impacted the results. Adding ‘time since symptom onset’ as a covariate in a linear multivariate model did not result in a significant difference in the primary outcome between the groups (P = 0.25).

### Plasma leakage parameters

Plasma leakage was assessed by a combination of laboratory parameters and daily bedside ultrasonography. The hematocrit and plasma concentrations of albumin and of the glyocalyx marker syndecan-1 were similar between the oseltamivir and placebo groups at the different time points (Fig 3). In addition, thickness of the gall bladder wall was similar between the groups (Fig 4). At enrollment, pleural fluid was more common in the oseltamivir group,
Fig 1. Study flow chart.

https://doi.org/10.1371/journal.pntd.0010051.g001

Fig 2. Twice daily platelet count. Data depicted are individual values (transparent lines) and geometric mean (solid line) with 95% confidence interval. P value calculated using mixed model method.

https://doi.org/10.1371/journal.pntd.0010051.g002
whereas ascites was more common in the placebo. These differences persisted until day 3 of follow up with ascites remaining significantly more common in the placebo group (Fig 4).

Safety

Treatment with oseltamivir was generally well-tolerated. The incidence of reported side effects in the oseltamivir was similar to the control group (Table 2). Mild elevations in serum transaminases were common in both groups. In one event, oseltamivir was interrupted at day 3 due to an ALT concentration more than 10 times the upper limit of normal (614 U/L), which normalized thereafter. One death occurred in the oseltamivir group; this participant was enrolled on the basis of a positive result of dengue IgM with negative NS1 RDT. The patient received two dosages of oseltamivir before this was stopped because of suspected septic shock with progressive multi-organ failure. The participant died on day 3; death was considered unrelated to the study drug.

Discussion

In this trial involving adult thrombocytopenic patients, hospitalized with acute DENV infection, adjunctive therapy with oseltamivir phosphate did not shorten platelet recovery time

Fig 3. Daily hematocrite, serum albumin and syndecan-1 concentrations. Data depicted are individual values (transparent lines) and geometric mean (solid line) with 95% confidence interval. P value calculated using mixed model method.

https://doi.org/10.1371/journal.pntd.0010051.g003

Fig 4. Daily gallbladder wall thickness and presence of pleural fluid or ascites. Gallbladder wall thickness and presence of pleural fluid or ascites was assessed daily by ultrasonography. Gallbladder thickness data are depicted as individual values (transparent lines) and geometric mean (solid line) with 95% confidence interval. P value for gallbladder thickness calculated using mixed model method. Differences in presence pleural fluid or ascites calculated using chi-square test (* P<0.05).

https://doi.org/10.1371/journal.pntd.0010051.g004
compared with placebo. The trial also did not show an effect of adjunctive oseltamivir on plasma leakage parameters.

Transient thrombocytopenia with hemorrhagic manifestations and plasma leakage are hallmarks of DENV infection. The hypothesis that the sialidase inhibitor oseltamivir may serve as a possible therapeutic adjunct to shorten platelet recovery time and decrease plasma leakage in dengue was based on earlier findings suggesting a role for human sialidases in dengue-associated platelet clearance and endothelial hyperpermeability [16,17].

We previously showed a reduced binding of *Sambucus nigra* lectin (SNA) and *Maackia amurensis* lectin II (MAL-II) to sialic acid residues on the platelet surface of dengue patients relative to patients with non-dengue febrile illness or healthy controls [16]. This loss of sialic acid was caused by translocation of platelet neuraminidase-1 (Neu-1) to the platelet membrane following binding of plasma von Willebrand factor to platelet glycoprotein (GP)-1b. *In vitro*, oseltamivir was shown to lower sialidase activity and β-galactose exposure (indicative of reduces desialylation) at the platelet surface [16,27]. This was further confirmed in a patient with anti-GP1b antibody-mediated ITP, in whom oseltamivir phosphate reduced desialylation of platelet glycoproteins [23]. Different retrospective studies in conditions with accelerated platelet clearance, such as anti-GPIb antibody-mediated ITP [22,23] or suspected influenza [24], and an open-label randomized trial in sepsis [26], have supported the notion that Neu-1 inhibition by oseltamivir increases platelet counts. This was further supported by the results of a recent multicentre, randomized, open-label phase 2 trial, which showed that oseltamivir in combination with dexamethasone resulted in a better platelet response compared with dexamethasone alone in patients with ITP [25]. Besides platelet neuraminidase, DENV NS1 has been shown to directly activate endothelial neuraminidases leading to disruption of endothelial glycocalyx components [17]. The neuraminidase inhibitor zanamivir partially prevented DENV NS1-induced endothelial hyperpermeability in endothelial cell monolayers [28]. Syndecan-1 is a component of the endothelial glycocalyx and the strong increase in plasma syndecan-1 concentrations in the participants in our study reinforces the importance of glycocalyx disruption in DENV infection.

The reasons that oseltamivir had no apparent effect on platelet counts, markers of plasma leakage and glycocalyx distortion in this study remain speculative, but may involve one or more of the following: first, dengue-associated thrombocytopenia and plasma leakage are both multifactorial in origin and targeting neuraminidase alone may be insufficient to impact these processes. Second, oseltamivir phosphate was designed to inhibit viral neuraminidase, and

---

**Table 2. Reported side effects.**

| Side effects, n (%) | Oseltamivir | Placebo | P value |
|--------------------|------------|---------|---------|
| Nausea             | 9 (26)     | 11 (31) | 0.8     |
| Vomiting           | 8 (23)     | 6 (17)  | 0.8     |
| Headache           | 3 (9)      | 1 (3)   | 0.6     |
| Abdominal pain     | 5 (14)     | 3 (9)   | 0.7     |
| Coughing           | 1 (3)      | 0 (0)   | 0.99    |
| Diarrhea           | 1 (3)      | 2 (6)   | 0.99    |
| Dizziness          | 1 (3)      | 0 (0)   | 0.99    |
| Itching            | 1 (3)      | 1 (3)   | 0.99    |
| ALT >10x ULN       | 1 (3)      | 0 (0)   | 0.99    |
| ALT >5x ULN        | 2 (6)      | 3 (9)   | 0.8     |
| Creatinine >0.5 mg/dl | 2 (6) | 2 (6) | 0.8 |

ALT, alanine-aminotransferase; ULN, upper limit of normal

[https://doi.org/10.1371/journal.pntd.0010051.t002](https://doi.org/10.1371/journal.pntd.0010051.t002)
data of its inhibitory actions on human neuraminidases are inconclusive. In fact, whereas different studies have provided evidence of oseltamivir inhibiting mammalian neuraminidases [23,29], Hata et. al. did not observe a substantial inhibiting effect of oseltamivir on recombinant soluble human neuraminidases [30]. Whether other neuraminidase inhibitors, such as zanamivir, would have yielded different results remains speculative. Third, our study did not include the pharmacokinetics of oseltamivir phosphate. Data from preclinical studies regarding the required dose of oseltamivir to exert the desired platelet effects is limited. However, the fact that a standard dose of 75mg BID was sufficient in different observational and interventional studies to exert an effect on platelet number, as well as to reduce platelet desialylation (assessed by flow cytometry) in a chronic ITP patient, suggests that the standard oseltamivir phosphate dose is sufficiently high [20–27]. Fourth, we cannot exclude that oseltamivir prevents or limits the development of thrombocytopenia when given earlier in the course of DENV infection, although we consider this scenario unlikely given the complete absence of difference in platelet counts between the two groups in this study.

Limitations of our study include the fact that assessment of changes in sialic acid residues on the platelet membrane was not feasible in the trial participants. This assessment requires a rather complicated flow cytometry assay that needs to be performed on fresh blood samples within hours after blood sampling. With six participating centers, this was logistically not feasible, but it should be considered in future studies as it may yield important mechanistic information. In addition, molecular confirmation of DENV infection with serotype identification was not performed in our study.

In conclusion, in adult patients with DENV infection and thrombocytopenia (<70,000/μl), adjunctive therapy with oseltamivir phosphate did neither improve platelet recovery, nor affect plasma leakage parameters.

Supporting information

S1 Fig. Number of study medication doses taken by every participant. (DOCX)

S1 Table. Bleeding complications during admission. (DOCX)

Acknowledgments

We acknowledge the contributions of Josephine Rahma, Felicia A. Tjahjadi, and Fadel M. Gharishah. We thank Prof. Sultana M. H. Faradz from the Center for Biomedical Research (CEBIOR) for her generous support of our work and the laboratory facilities in Diponegoro University, Semarang, Indonesia. We acknowledge the Integrated Laboratory Unit (UPT) of Diponegoro University and Evi Nurwulan for providing technical assistance. We thank dr. Haneng Marissangan., SpPD and dr. Any Yuliani., SpPK., M.Kes from dr. M. Salamun Hospital in Bandung and its staff for their support. We also thank the Immunology Laboratory, Faculty of Medicine, Universitas Padjadjaran, and Siti Rasnawati Mony, Dera Darmayanti, Tri Kusniati, and Qireneu Dwiputri for technical assistance. We acknowledge the contributions of Josephine Rahma, Raras Rahmandiar, Mutiara Chairsabela and Ikha H. Savitri in Semarang, as well as Agni Laili Perdani, Dwi Putri Sekarini, Ghina Rahmadiani and Gabriela Grace in Bandung.

Author Contributions

Conceptualization: Rahajeng N. Tunjungputri, Andre van der Ven, Muhammad Hussein Gasem, Quirijn de Mast.
Data curation: Rahajeng N. Tunjungputri, Quirijn de Mast.

Formal analysis: Rahajeng N. Tunjungputri, Quirijn de Mast.

Funding acquisition: Quirijn de Mast.

Investigation: Rahajeng N. Tunjungputri, Silvita Fitri Riswari, Setyo G. Pramudo, Lydia Kuntjoro, Harry Galuh Nugraha, Quirijn de Mast.

Methodology: Rahajeng N. Tunjungputri, Quirijn de Mast.

Project administration: Rahajeng N. Tunjungputri, Silvita Fitri Riswari, Setyo G. Pramudo, Lydia Kuntjoro, Harry Galuh Nugraha, Quirijn de Mast.

Resources: Rahajeng N. Tunjungputri, Silvita Fitri Riswari, Setyo G. Pramudo, Bachti Alisjahbana, Muhammad Hussein Gasem, Quirijn de Mast.

Supervision: Bachti Alisjahbana, Andre van der Ven, Muhammad Hussein Gasem, Quirijn de Mast.

Validation: Quirijn de Mast.

Visualization: Rahajeng N. Tunjungputri, Quirijn de Mast.

Writing – original draft: Rahajeng N. Tunjungputri, Quirijn de Mast.

Writing – review & editing: Rahajeng N. Tunjungputri, Silvita Fitri Riswari, Setyo G. Pramudo, Lydia Kuntjoro, Bachti Alisjahbana, Harry Galuh Nugraha, Andre van der Ven, Muhammad Hussein Gasem.

References
1. Shepard DS, Undurraga EA, Halasa YA, Stanaway JD. The global economic burden of dengue: a systematic analysis. The Lancet infectious diseases 2016; 16:935–41. https://doi.org/10.1016/S1473-3099(16)00146-8 PMID: 27091092
2. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. 2009.
3. World Health Organization. Dengue: guideline for diagnosis, treatment, prevention and control—New edition., 2009.
4. Mourao MP, Lacerda MV, Macedo VO, Santos JB. Thrombocytopenia in patients with dengue virus infection in the Brazilian Amazon. Platelets 2007; 18:605–12. https://doi.org/10.1080/09537100701426604 PMID: 18041652
5. Tomashek KM, Lorenzi OD, Andujar-Perez DA, Torres-Velasquez BC, Hunasperger EA, Munoz-Jordan JL, et al. Clinical and epidemiologic characteristics of dengue and other etiologic agents among patients with acute febrile illness, Puerto Rico, 2012–2015. PLoS Negl Trop Dis 2017; 11:e0005859. https://doi.org/10.1371/journal.pntd.0005859 PMID: 28902845
6. Potts JA, Rothman AL. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Trop Med Int Health 2008; 13:1328–40. https://doi.org/10.1111/j.1365-3156.2008.02151.x PMID: 18803612
7. Schexnieder KI, Reedy EA. Thrombocytopenia in dengue fever. Curr Hematol Rep 2005; 4:145–8. PMID: 15720964
8. Geoge T, Ho-Tin-Noe B, Carbo C, Benarafa C, Remold-O'Donnell E, Zhao BQ, et al. Inflammation induces hemorrhage in thrombocytopenia. Blood 2008; 111:4958–64. https://doi.org/10.1182/blood-2007-11-123629 PMID: 18256319
9. Iannacone M, Stiia G, Isogawa M, Whitmire JK, Marchese P, Chisari FV, et al. Platelets prevent IFN-alpha/beta-induced lethal hemorrhage promoting CTL-dependent clearance of lymphocytic choriomeningitis virus. Proceedings of the National Academy of Sciences of the United States of America 2008; 105:629–34. https://doi.org/10.1073/pnas.0711200105 PMID: 18184798
10. Gupta S, Konradt C, Corken A, Ware J, Nieswandt B, Di Paola J, et al. Hemostasis vs. homeostasis: Platelets are essential for preserving vascular barrier function in the absence of injury or inflammation.
Proceedings of the National Academy of Sciences of the United States of America 2020; 117: 24316– 25. https://doi.org/10.1073/pnas.2007642117 PMID: 32929010

11. Dimano EM, Saito M, Honda S, Miranda EA, Alonzo MT, Valerio MD, et al. Lack of efficacy of high-dose intravenous immunoglobulin treatment of severe thrombocytopenia in patients with secondary dengue virus infection. Am J Trop Med Hyg 2007; 77:1135–8. PMID: 18165536

12. Tam DT, Ngoc TV, Tien NT, Kieu NT, Thuy TT, Thanh LT, et al. Effects of short-course oral corticosteroid therapy in early dengue infection in Vietnamese children: a randomized, placebo-controlled trial. Clin Infect Dis 2012; 55:1216–24. https://doi.org/10.1093/cid/cis655 PMID: 22865871

13. Lye DC, Archuleta S, Syed-Omar SF, Low JG, Oh HM, Wei Y, et al. Prophylactic platelet transfusion plus supportive care versus supportive care alone in adults with dengue and thrombocytopenia: a multi-centre, open-label, randomised, superiority trial. Lancet 2017; 389:1611–8. https://doi.org/10.1016/S0140-6736(17)30269-6 PMID: 28283266

14. De Azeredo EL Monteiro RQ, de-Oliveira Pinto LM. Thrombocytopenia in dengue: interrelationship between virus and the imbalance between coagulation and fibrinolysis and inflammatory mediators. Mediators Inflamm 2015; 2015: 313842. https://doi.org/10.1155/2015/313842 PMID: 25999666.

15. Hoffmeister KM, Falelt H. Platelet clearance by the hepatic Ashwell-Morrell receptor: mechanisms and biological significance. Thrombosis research 2016; 141:S68–S72. https://doi.org/10.1016/S0049-3848(16)30370-X PMID: 27207430

16. Riswari SF, Tunjungputri RN, Kullaya V, Garishah FM, Utari GS, Farhanah N, et al. Desialylation of platelets induced by Von Willebrand Factor is a novel mechanism of platelet clearance in dengue. PLoS pathogens 2019; 15:e1007500. https://doi.org/10.1371/journal.ppat.1007500 PMID: 30849118

17. Puerta-Guardo H, Glasner DR, Harris E. Dengue virus NS1 disrupts the endothelial glycocalyx, leading to hyperpermeability. PLoS pathogens 2016; 12:e1005738. https://doi.org/10.1371/journal.ppat.1005738 PMID: 27416066

18. Espinosa DA, Beatty PR, Puerta-Guardo H, Islam MN, Belisle JT, Perera R, et al. Increased serum sialic acid is associated with morbidity and mortality in a murine model of dengue disease. The Journal of general virology 2019; 100:1515–22. https://doi.org/10.1099/jgv.0.001319 PMID: 31526452.

19. Moore ML, Chi MH, Zhou W, Goleniewska K, O’Neal JF, Higginbotham JN, et al. Cutting edge: oseltamivir decreases T cell GM1 expression and inhibits clearance of respiratory syncytial virus: potential role of endogenous sialidase in antiviral immunity. The Journal of Immunology 2007; 178:2651–4. https://doi.org/10.4049/jimmunol.178.5.2651 PMID: 17312105

20. Jansen AJ, Peng J, Zhao HG, Hou M, Ni H. Sialidase inhibition to increase platelet counts: A new treatment option for thrombocytopenia. Am J Hematol 2015; 90:E94–5. https://doi.org/10.1002/ajh.23953 PMID: 25615710

21. Shaim H, McCaffrey P, Trieu JA, DeAnda A, Yates SGJP. Evaluating the effects of oseltamivir phosphate on platelet counts: a retrospective review. Platelets 2020; 31:1080–4. https://doi.org/10.1080/09537104.2020.1714576 PMID: 31931672

22. Revilla N, Corral J, Miñana A, Mingot-Castellano ME, Campos RM, Velasco F, et al. Multrefractory primary immune thrombocytopenia; targeting the decreased sialic acid content. Platelets 2019; 30:743–51. https://doi.org/10.1080/09537104.2018.1513476 PMID: 30296193

23. Shao L, Wu Y, Zhou H, Qin P, Ni H, Peng J, et al. Successful treatment with oseltamivir phosphate in a patient with chronic immune thrombocytopenia positive for anti-GP Ib/IX antibody. Platelets 2015; 26:495–7. https://doi.org/10.3109/09537104.2014.948838 PMID: 25166956

24. Bigot P, Auffret M, Gautier S, Weinborn M, Ettahar NK, Perona A, et al. Unexpected platelet elevation in a patient with idiopathic thrombocytopenia treated with oseltamivir for influenza infection. Fundam Clin Pharmacol 2016; 30:483–5. https://doi.org/10.1111/fcp.12213 PMID: 27343486

25. Sun L, Wang J, Shao L, Yuan C, Zhao H, Li D, et al. Dexamethasone plus oseltamivir versus dexamethasone in treatment-naive primary immune thrombocytopenia: a multicentre, randomised, open-label, phase 2 trial. The Lancet Haematology 2021; 8:e289–e98. https://doi.org/10.1016/S2352-3026(21)00030-2 PMID: 33770484

26. Li MF, Li XL, Fan KL, Yu YY, Gong J, Geng SY, et al. Platelet desialylation is a novel mechanism and a therapeutic target in thrombocytopenia during sepsis: an open-label, multicenter, randomized controlled trial. J Hematol Oncol 2017; 10:104. https://doi.org/10.1186/s13045-017-0476-1 PMID: 28947777

27. Zhang XH, Wang QM, Zhang JM, Feng FE, Wang FR, Chen H, et al. Desialylation is associated with apoptosis and phagocytosis of platelets in patients with prolonged isolated thrombocytopenia after allo-HSCT. J Hematol Oncol 2015; 8:116. https://doi.org/10.1186/s13045-015-0216-3 PMID: 26497387

28. Glasner DR, Ratnasiri K, Puerta-Guardo H, Espinosa DA, Beatty PR, Harris E. Dengue virus NS1 cytokine-independent vascular leak is dependent on endothelial glycocalyx components. PLoS Pathog 2017; 13:e1006673. https://doi.org/10.1371/journal.ppat.1006673 PMID: 29121099
29. Gilmour AM, Abdulkhalek S, Cheng TS, Alghamdi F, Jayanth P, O'Shea LK, et al. A novel epidermal growth factor receptor-signaling platform and its targeted translation in pancreatic cancer. Cellular signalling 2013; 25:2587–603. https://doi.org/10.1016/j.cellsig.2013.08.008 PMID: 23993964

30. Hata K, Koseki K, Yamaguchi K, Moriya S, Suzuki Y, Yingsakmongkon S, et al. Limited inhibitory effects of oseltamivir and zanamivir on human sialidases. Antimicrobial agents and chemotherapy 2008; 52:3484–91. https://doi.org/10.1128/AAC.00344-08 PMID: 18694948