



ELSEVIER

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: <https://www.journals.elsevier.com/eclinicalmedicine>

Investigation of the efficacy and safety of eltrombopag to correct thrombocytopenia in moderate to severe dengue patients - a phase II randomized controlled clinical trial

Sajib Chakraborty^{a,*}, Saruar Alam^{a,#}, Mohammad Sayem^{b,#}, Mousumi Sanyal^{c,#}, Tonmoy Das^a, Piyal Saha^b, Mohammad Sayem^d, Bartholomia Keya Byapari^e, Chowdhury Tamanna Tabassum^c, Ahmedul Kabir^c, Md.Robed Amin^c, A.H.M. Nurun Nabi^{b,*}

^a Translational Systems Biology Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh

^b Laboratory of Population Genetics, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh

^c Department of Medicine, Dhaka Medical College, Dhaka, Bangladesh

^d Department of Medicine, Aichi Medical College, Dhaka, Bangladesh

^e Department of Hematology and Pathology, Square Hospitals Limited, Dhaka, Bangladesh

ARTICLE INFO

Article History:

Received 12 September 2020

Revised 17 October 2020

Accepted 21 October 2020

Available online xxx

ABSTRACT

Background: The dengue-infected patients with or without hemorrhagic manifestations, typically exhibit moderate to severe thrombocytopenia. A thrombopoietin receptor agonist – eltrombopag has been efficacious in correcting thrombocytopenia in patients with various pathological conditions including immune thrombocytopenia, chronic liver disease, and severe aplastic anemia. This study investigated the efficacy and safety of eltrombopag to correct dengue-mediated thrombocytopenia.

Methods: In this open-label, randomized controlled phase-II trial, patients with dengue fever (DF) and dengue hemorrhagic fever (DHF) having platelet (PLT) count lower than $100 \times 10^9/L$ without comorbidity, pregnancy, and liver abnormalities were enrolled in Dhaka Medical College Hospital, Better Life Hospital and AMZ hospital, Dhaka, Bangladesh. Between October 10, 2019, and December 30, 2019, 123 DF and DHF patients were assessed for eligibility to be enrolled in the trial. Fourteen patients were excluded as they failed to fulfill the inclusion criteria ($N = 6$) or refused to participate in the trial ($N = 8$). Finally, 109 patients were randomly assigned to either Group 1, ($N = 36$), Group 2 ($N = 37$), or Control-group ($N = 36$) in a 1:1:1 ratio. Two doses of eltrombopag - 25 mg/day and 50 mg/day were administered to Group-1 and Group-2 patients, respectively whereas the control-group patients received standard dengue treatment without eltrombopag. The management of all enrolled patients was according to WHO guidelines. The randomization procedure was performed by using a computerized system (STATA Inc.). CBC and immature platelet fraction (IPF) were monitored from Day-0 to Day-7. Absolute immature platelet count (A-IPC) was calculated from PLT count and IPF for each patient. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured on Day-0 and Day-4 and an Ultrasonogram (USG) of the abdomen was performed on Day-4 and Day-7 for each patient. The efficacy of eltrombopag as the primary outcome of the trial was investigated by the proportion of patients with recovered platelet count receiving eltrombopag with corrected platelet count (platelet count above the lower normal limit: $150 \times 10^9/L$) on Day-7 of the enrollment as compared to the Control-group. As the secondary outcomes, the reduction of bleeding tendency in response to eltrombopag as well as the safety of eltrombopag in dengue patients were assessed. The safety was evaluated in case of adverse events, liver function enzymes AST/ALT levels and USG. This trial is registered with the international clinical trial registry, number SLCTR/2019/037.

Results: A total of 101 patients including 77 DF and 24 DHF patients completed the trial as eight patients left the trial without completing the follow-up. Patients of the different groups were compared with respect to mean age (26 ± 8 , 30 ± 10 and 30 ± 9 years for, Group-1,-2 and Control-group, respectively) (p -value= 0.23) and basal PLT count (Group-1: $58 \pm 24 \times 10^9$; Group-2: $52 \pm 29 \times 10^9$ and control-group: $55 \pm 30 \times 10^9$) (p -value= 0.63). The mean PLT counts for Group-1 ($332 \times 10^9/L \pm 92$) and Group-2 ($371 \times 10^9/L \pm 111$) were significantly higher than control-group ($194 \times 10^9/L \pm 96$) on Day-7 (adjusted p -value= 1.15×10^{-06} for

* Corresponding authors.

E-mail addresses: sajib@du.ac.bd (S. Chakraborty), nabi@du.ac.bd (A.H.M.N. Nabi).

Contributed equally

Group-1 vs. Control-group, and adjusted p-value= 1.82×10^{-08} for Group-2 vs. Control-group). On Day-7, 91% of Group-1 ($N = 30$) and Group-2 ($N = 32$) patients who received eltrombopag achieved primary endpoint of PLT count above than lower normal limit ($150 \times 10^9/L$) (Group-1: 91%, OR: 8.33, 95% CI: 2.11 to 32.80, p-value: 0.0024 and Group-2: 91%, OR: 8.89, 95% CI: 2.26 to 34.89, p-value: 0.0017) compared to 55% ($N = 18$) of control-group patients who did not receive eltrombopag. The bleeding manifestations for thirteen out of fourteen grade-II DHF patients were subsided within Day-7 who received eltrombopag, whereas four out of ten grade-II DHF patients with PLT counts lower than the lower normal limit in the control group showed intermittent bleeding symptoms throughout the trial period. Mean A-IPC but not IPF was significantly higher for eltrombopag-treated groups in comparison to the Control-group. The frequency of the most common adverse events (vomiting and diarrheal tendencies) was similar in the treated-and control-groups ($N = 5$, 15%, and $N = 3$, 9% for Group-1 and -2, respectively vs. $N = 4$, 12% in the Control-group). Ten (30%) patients of Group-1 and, fourteen (40%) patients of Group-2 showed increased AST (U/L) as opposed to nine patients (27%) in the Control-group. Increased ALT levels were observed for three (9%), nine (26%), and seven (21%) patients belonging to the Group-1, -2, and Control-group, respectively. PLT counts higher than the upper normal limit ($450 \times 10^9/L$) on Day-7 were observed for seven patients who were administered the higher dose (50 mg/day) in contrast to the three patients receiving the lower dose (25 mg/day). USG reports did not show thrombosis events in any of the patients.

Interpretation: The trial revealed that the administration of eltrombopag in a short regimen for three days was efficacious to restore the PLT count in DF and DHF patients. The higher number of A-IPCs in eltrombopag treated patients underscored the possible mode of action of eltrombopag through stimulating megakaryopoiesis in dengue patients. The trial hints toward the positive effect of eltrombopag in the cessation of bleeding manifestation. Administration of the lower dose (25 mg/day) of eltrombopag was shown to be safer and equally efficacious to the higher dose (50 mg/day) in treating dengue-infected patients.

© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

Research in Context

Evidence before this study

The increased incidence of dengue fever (DF) and dengue hemorrhagic fever (DHF) has become a major health problem in the dengue-endemic regions. The reduced platelet count ($<100 \times 10^9/L$) – thrombocytopenia, is a major clinical symptom of dengue and is considered as an indicator of the severity of the dengue. So far there is no effective drug to correct thrombocytopenia in dengue patients. Platelet transfusion to correct dengue-induced thrombocytopenia remains controversial since it failed to have a positive impact on reducing the incidence of bleeding in patients exhibiting low platelet count consequently platelet transfusion is not routinely performed in the management of dengue. Eltrombopag – a thrombopoietin (TPO) receptor agonist is efficacious in correcting thrombocytopenia in patients with various pathological conditions including Immune thrombocytopenia (ITP), chronic liver disease (CLD), and severe aplastic anemia (SAA). Eltrombopag binds with the transmembrane domain of the thrombopoietin receptor (MPL) and does not compete with endogenous TPO. As a consequence, eltrombopag and TPO exhibit mutual additive effects. Since the pathogenesis of DF and DHF is associated with the manifestation of thrombocytopenia, we hypothesized that eltrombopag can potentially be beneficial to correct dengue mediated thrombocytopenia.

Added value of this study

So far the administration of thrombopoietin-receptor agonists to correct low platelet count in dengue patients has not been attempted. No cases were reported where dengue induced thrombocytopenia was corrected with the administration of eltrombopag. The trial revealed that the administration of eltrombopag for three days was efficacious to restore the lower normal limit of platelet count in DF and DHF patients. The higher number of absolute immature platelet count in eltrombopag treated patients underscored the possible mode of action

of eltrombopag through stimulating megakaryopoiesis in dengue patients.

Implications of all the available evidence

The trial hints toward the positive effect of eltrombopag on the increase of platelet count and cessation of bleeding manifestation in patients with persisting thrombocytopenia. Administration of the lower dose (25 mg/day) of eltrombopag was shown to be safer and equally efficacious to the higher dose (50 mg/day) in treating dengue-infected patients.

1. Introduction

The incidence of dengue fever (DF) – one of the most prevalent mosquito-borne infectious diseases, has increased at a rapid rate with an astounding 400% increase over just a span of the last thirteen years [1]. The emergence of DF as one of the fastest spreading infectious diseases worldwide, with substantial morbidity and mortality, has largely been attributed to growing urbanization and climate change [2]. The causative agents of the DF are a group of genetically similar but serotypically distinct dengue viruses (DENV 1–4) which are transmitted primarily by the mosquito – *Aedes aegypti* [3]. The prevalence of DF is high in the tropical and sub-tropical regions including South-East Asian, Western Pacific, Eastern Mediterranean, American and African regions [2]. Dengue fever has three distinct phases – acute or febrile phase, critical phase, and recovery or convalescent-phase [2]. The acute phase is defined by the high viral loads causing a high fever period that typically lasts 3 to 7 days from the onset of infection. The acute phase is followed by a critical phase in which the pathological manifestations are the thrombocytopenia, plasma leakage into the peritoneal/pleural cavities, and bleeding that becomes clinically detectable between days 4 to 6 of illness. The convalescent phase is characterized by the cessations of plasma leakage and reabsorption of leaked fluids [2,3]. According to the WHO classification, dengue infected patients can be classified into 2 major categories: dengue (symptomatic/asymptomatic) and severe dengue⁴. The patients infected with a distinct DENV serotype who are already

primed with a different DENV serotype are most susceptible to develop severe dengue⁵. Severe dengue patients exhibit combined manifestations of plasma leakage, coagulopathy, and platelet (PLT) count $<100 \times 10^9/L$, and a marked rise of hematocrit (hct) followed by bleeding that may result in the development of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. Based on the severity, DHF is further classified into four grades: Grade-I (fever with positive tourniquet test), Grade-II (spontaneous bleeding in addition to Grade-I symptoms), Grade-III (presence of weak and rapid pulse), and Grade-IV (profound shock with an undetectable pulse) [6]. Diagnosis of dengue virus infection includes the detection of viral genomic RNA, secreted NS1 protein, or the detection of virus-specific host immunoglobulins (IgM/IgG) [3]. The current clinical management of symptomatic DF includes but is not limited to intravenous hydration therapy, cautious monitoring of the PLT count, and hematocrit (hct) for the patients suffering from significant vascular leakage [6].

The reduced platelet count ($<100 \times 10^9/L$), thrombocytopenia is typically associated with DHF and DF and is considered as an indicator of the severity of the DF as reported by several studies [7,8]. Although the correlation between the bleeding manifestation of DF and platelet count has not been entirely established, one study involving 225 dengue patients showed that bleeding was more frequent in patients having platelet counts below $20 \times 10^9/L$ [9]. Although the underlying mechanisms involved in the manifestation of thrombocytopenia and bleeding during DF are not fully understood, several hypotheses have emerged. One hypothesis includes the direct or indirect role of DENV to inhibit the function of bone marrow progenitor cells thereby causing bone-marrow suppression in the acute febrile phase of DF [10]. Although, the underlying mechanisms of DENV-mediated bone marrow suppression during the acute phase have not been fully established several factors have suggested including direct damage of progenitor cells, infection stromal cells, and lastly the changes in bone marrow regulation by DENV [11].

Whereas in the late febrile or critical phase, a different mechanism including platelet consumption due to disseminated intravascular coagulation (DIC), leading to platelet destruction employing apoptotic mechanism, destruction by the complement system and/or anti-platelet antibodies has been observed [11,12]. Previously, a study involving 372 DF patients with platelet count $\leq 20 \times 10^9/L$ and without mild or severe bleeding, concluded that the impact of prophylactic platelet transfusion was not better than supportive care in preventing bleeding, and might be associated with adverse events [13]. In light of the notion that platelet transfusion may not be beneficial to dengue patients, several studies raised the argument that platelet transfusion should not be routinely performed in the management of dengue, and indeed WHO does not recommend platelet transfusion for dengue patients [14].

Thrombopoietin (TPO) is the key endogenous regulator of platelet production that acts through binding with the TPO receptor (MPL). To harness the physiological activity of human TPO as a therapeutic agent to correct the low platelet count, two distinct forms of human TPOs –a full-length glycosylated form of the recombinant human TPO (rhTPO) and pegylated megakaryocyte growth and development factor (PEG-rHuMGDF) were initially tested in clinical trials [15] [16]. However, in one study involving PEG-rHuMGDF administration in healthy volunteers, reported the development of auto-antibodies in thirteen persons that cross-reacted with endogenous TPO and consequently resulted in the discontinuation of all clinical studies with rhTPO and PEG-rHuMGDF [17]. To overcome this early setback, two commercially available thrombopoietin-receptor agonists that did not resemble endogenous TPO - romiplostim and eltrombopag have been developed and proved beneficial to correct the low platelet levels [18]. Eltrombopag has been proved to be equally effective but less costly than romiplostim [19].

Eltrombopag is efficacious in correcting thrombocytopenia in patients with various pathological conditions including Immune

thrombocytopenia (ITP) [20], chronic liver disease (CLD) [21], and severe aplastic anemia (SAA) [22]. Eltrombopag binds to the transmembrane domain of thrombopoietin receptor - MPL, which leads to the activation of downstream signaling pathways- JAK/STAT and MAPK pathways [23]. As Eltrombopag binds with the transmembrane domain of MPL, it does not pose a competition to endogenous TPO which binds to the extracellular domain. Consequently, eltrombopag and TPO should in theory exhibit mutual additive effects [24].

Since the pathogenesis of DF and DHF is associated with the manifestation of thrombocytopenia, we hypothesized that eltrombopag can potentially be beneficial to correct dengue mediated thrombocytopenia. However, the administration of thrombopoietin-receptor agonists to correct low platelet count in dengue patients has not been attempted systematically. There is only one report to correct dengue-induced thrombocytopenia of a 57 years female patient with multiple myeloma by the administration of romiplostim [25]. No cases were reported where dengue induced thrombocytopenia was reverted with the administration of eltrombopag. The major concern is that thrombopoietin-receptor agonists including eltrombopag are only applicable for chronic disorders with the long-term treatment regimen and may not have the same efficacy in acute conditions. Another major issue is that earlier reports have confirmed that thrombopoietin-receptor agonists may take at least one week to produce a response in patients with ITP [26]. Moreover, around one-third of the patients receiving thrombopoietin-receptor agonists do not respond and consequently discontinue the drug [27]. The underlying mechanism of unresponsiveness to thrombopoietin-receptor agonists remains unresolved. One last but important issue involves the safety of eltrombopag administration to dengue patients. To answer these questions, a well-organized clinical trial is required to test the efficacy and safety of eltrombopag in dengue patients. The current study is the first-ever trial to test the efficacy, optimal dose, and safety of eltrombopag to correct the DF induced thrombocytopenia. The trial was designed in an open-labeled, randomized, controlled trial involving more than a hundred DF and grade II DHF patients aged 15 to 65 years.

2. Methods and materials

2.1. Patients

The study was designed as a phase II open-label, randomized, controlled trial to compare the doses, efficacy, and safety of eltrombopag to correct thrombocytopenia in moderate to severe dengue patients. Patients with an age range between 15 and 65 years and clinical presentation of dengue virus infection including but is not limited to fever, leucopenia ($WBC \leq 5000$ cells/ mm^3), increased hematocrit (Hct) 5–10% from baseline, platelet count $\leq 150 \times 10^9/L$, headache, retro-orbital pain, myalgia, arthralgia/bone pain, rash were considered as suspected dengue patients in accordance with the world health organization (WHO) guideline [6]. Briefly, moderate dengue was defined for patients with a high fever ($40^\circ C/104^\circ F$) is accompanied by two of the warning signs including abdominal pain and tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, and liver enlargement, increase in hematocrit (Hct) and rapid decrease in platelet count [28]. Severe dengue including the DHF patients was characterized by one of the following symptoms: plasma leakage leading to shock or respiratory distress, severe bleeding, or organ failure (eg, elevated liver enzyme levels, impaired consciousness, or heart failure) [28]. The suspected patients were subjected to dengue specific antigen (NS1) and antibody (IgM/IgG) tests. Patients exhibiting positive results for NS1 or dengue specific IgM/IgG were considered as dengue positive patients. Patients diagnosed with dengue virus infection were screened for eligibility to enter the trial. Among the eligible candidates, patients with pregnancy, receiving immunosuppressive

therapy, thrombocytopenia caused by other factors such as severe aplastic anemia (SAA), chronic liver disease (CLD) and Immune thrombocytopenia (ITP), aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels higher than 5 times of upper normal limit, history of portal vein thrombosis and HBV/HCV infection were excluded from the trial. Additionally, patients with any severe comorbidity such as chronic kidney disease were excluded. According to the World Health Organization, Regional Office for South-East Asia (WHO SEARO) 2011, one of the warning signs of severe dengue and dengue hemorrhagic fever, has been characterized by the decreased platelet count below $100 \times 10^9/L$ [29]. Based on this characterization, eligible patients exhibiting platelet (PLT) count below $100 \times 10^9/L$ were enrolled in the trial.

2.2. Study settings

Dengue infected patients from three different hospitals- Dhaka Medical College Hospital (DMCH), Better Life Hospital, and AZM Hospital in Dhaka, Bangladesh were enrolled. Suspected dengue patients with or without hemorrhagic manifestations were enrolled by following the above-mentioned criteria. Management of dengue patients was carried out according to the WHO protocol [6,29] in all three hospitals.

2.3. Randomization and intervention protocol

Upon enrollment and signing of the informed consent, the eligible patients were randomly assigned to a particular treatment arm. The simple randomization procedure based on a single allocation ratio was performed by using a computerized system (STATA Inc.). The patients were randomly assigned to one of the three groups including two treatment groups (Group-1 and -2) and one Control-group in a 1:1:1 ratio. The treatment groups Group-1 and Group-2 patients received 25 mg/day and 50 mg/day of eltrombopag, respectively for three days in addition to standard treatment. In the Control-group, patients received standard treatment without eltrombopag. The management of dengue patients including the standard treatment procedures was carried out according to WHO guideline [6,29], and was essentially identical in all three groups. The only difference was that the randomly assigned patients in Group-1 and -2 were administered eltrombopag while the control-group patients did not receive the drug. Briefly, the standard treatment included the fluid allowance (oral and IV) for maintenance (for one day) and 5% deficit (oral and IV fluid together), which was administered over 48 h. The rate of IV replacement was adjusted according to the rate of plasma loss, guided by the clinical condition, vital signs, urine output, and hematocrit levels.

2.4. Procedures and study design

25 mg and 50 mg eltrombopag tablets were administered orally once for three consecutive days to Group-1 and -2 patients, respectively. The overall strategies for patient admission, enrollment, treatment, and follow-up are summarized in Supplementary Figure 1. In brief, the trial encompassed eight days from the day of enrollment (Day-0) to Day-7. The total duration of the trial (8 days) was divided into three phases - enrollment phase, treatment phase, and follow-up phase (Supplementary Figure 1). The days from onset of fever for each patient were taken into account. The enrollment phase included the day of enrollment of the patients to the trial (Day-0) when their PLT count falls below $100 \times 10^9/L$. Patients were monitored daily during the whole trial period. The course of their ailment during the trial period including any adverse effect was recorded. The patients enrolled in the trial were subjected to a wide range of tests including complete blood count (CBC) and immature platelet fraction (IPF) during the intervention (Day-0 to Day-2) and follow-up (Day-3 to Day-7)

phases (Supplementary Figure 1). Serum AST/ALT levels were measured on Day-0 and Day-4. Additionally, on Day-4 and Day-7, patients receiving eltrombopag (Group-1 and -2) underwent USG of the abdomen (Supplementary Figure 1). Furthermore, patients were stratified based on the classification dengue phases (acute/febrile, critical, and recovery) according to the World Health Organization [29] and Yacoub et al. [30]. We utilized the day of defervescence of each patient to differentiate between acute and critical phases. The day of defervescence was considered as the end of the acute phase and the next two days were classified as the critical phase followed by a recovery phase.

All the blood samples taken from patients (enrolled in three different hospitals) were transported as per WHO guideline [31] to a centralized laboratory (Biochemistry laboratory of Square Hospital) for CBC and serum ALT/AST measurements. The centralized laboratory testing system was introduced to reduce laboratory-specific variability in the measurements. For the laboratory test, whole blood was collected in tubes pre-filled with anticoagulant (EDTA) to prevent platelet activation prior to its use. Three ml blood was used for CBC analysis including the measurement of the IPF by Sysmex (XN:2000) automatic hematology analyzer. The IPF was identified by the flow cytometric operation of the Sysmex XN-2000 instrument with the use of a nucleic acid-specific fluorescent dye oxazine in the reticulocyte channel. Oxazine dye penetrates the cell membrane and stains residual RNA of red blood cells (reticulocytes) and platelets. A computer algorithm based on the fluorescence intensity finally distinguishes mature and immature platelets, which is expressed as a percentage of total platelets (IPF%). Absolute immature platelet count (A-IPC) was calculated from PLT count and IPF for each patient from Day-0 to Day-7 as described by Bat et al. [32]. Serum extracted from 3 ml of the blood sample was used to perform liver function tests. For this purpose, two of the liver enzymes - AST and ALT concentrations were determined by the autoanalyzer in the serum.

2.5. Outcomes

The primary objective of the study was to assess the efficacy of different doses of eltrombopag in correcting the dengue-induced thrombocytopenia. In order to determine the efficacy, the proportion of patients with a platelet count above the lower normal limit ($150 \times 10^9/L$) receiving eltrombopag was compared to the control group. The mean platelet counts at Day-0 to Day-7 were calculated for each patient. As a secondary outcome, the safety and tolerability of eltrombopag in dengue patients were assessed as measured by the incidence of adverse events and clinical laboratory parameters (including liver function enzymes AST/ALT and ultrasonogram of the abdomen). Additionally, the abatement of bleeding manifestation in response to eltrombopag was considered as a secondary outcome parameter.

2.6. Statistical analyses

The primary analysis compared the significant difference of eltrombopag response between two treated and control groups. The sample size was calculated by the method described by Bussel et al. [33]. In brief, the sample size was estimated for three groups (two treated and control groups) to ensure 90% power at the 5% significance level for the primary outcome based on the assumption that 70% of the patients in two treated groups receiving eltrombopag will respond (PLT count $>150 \times 10^9/L$) whereas 20% patients in the control group will exhibit a response. The odds ratio (OR) of the response was calculated with two-sided 95% CI. Hypotheses were tested based on the odds of achieving a response (PLT count above the lower normal limit $150 \times 10^9/L$) between treatment and control groups. We used MedCalc software for the calculation of the ORs of the response. To explore the effects of multiple variables (time and treatment) on

the variance of platelet count data, we performed a two-way ANOVA test with multiple correction post-hoc tests (Bonferroni). For evaluating the effect of treatment on platelet count, IPF and A-IPN, unpaired t-tests were done followed by multiple correction tests and false discovery rate (FDR) adjusted p-values were calculated. Also, the frequency of adverse effects among treated and control-group was compared using the Chi-square test. These statistical analyses were done using GraphPad Prism (version 8.0).

2.7. Hierarchical clustering

Hierarchical clustering based on Euclidean distance was utilized to cluster the patients (rows) and trial-days (columns) according to their variability of platelet count. The results of the clustering were presented as heatmap illustrating the PLT count dynamics of patients across the treated and control groups. R-software package was used for hierarchical clustering analysis.

2.8. Role of the funding source

Contributions of the authors include the conceptualization, study design, data-collection, data-generation, data storage, data analysis, interpretation, and writing of the manuscript. The study funder provided the funding for the trial and provided the drug. The funder had no role in study design, data collection, and analysis, or writing the

manuscript. All authors had full access to all the study data and shared final responsibility for the decision to submit for publication.

2.9. Ethics statement

The trial protocols were approved by the institutional ethics committee at each participating center and complied with International Conference on Harmonisation Guideline for Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent according to local guidelines. The manuscript conforms with the CONSORT guidelines.

3. Results

Between October 10, 2019, and December 30, 2019, 123 dengue-patients were assessed for eligibility from three different hospitals (Dhaka Medical College Hospital, Better Life Hospital, and AMZ hospital) to be recruited in the trial. Fourteen patients were excluded as they either did not fulfill the inclusion criteria ($N = 6$) or refused to participate in the trial ($N = 8$). The rest of the 109 patients, were randomly assigned to receive either 25 mg eltrombopag per day (Group-1, $N = 36$), 50 mg eltrombopag per day (Group-2, $N = 37$), or assigned to the Control-group, ($N = 36$). Control-group patients received all the standard treatments like Group-1 and Group-2 except any doses of eltrombopag (Fig. 1). Eight patients did not complete the entire

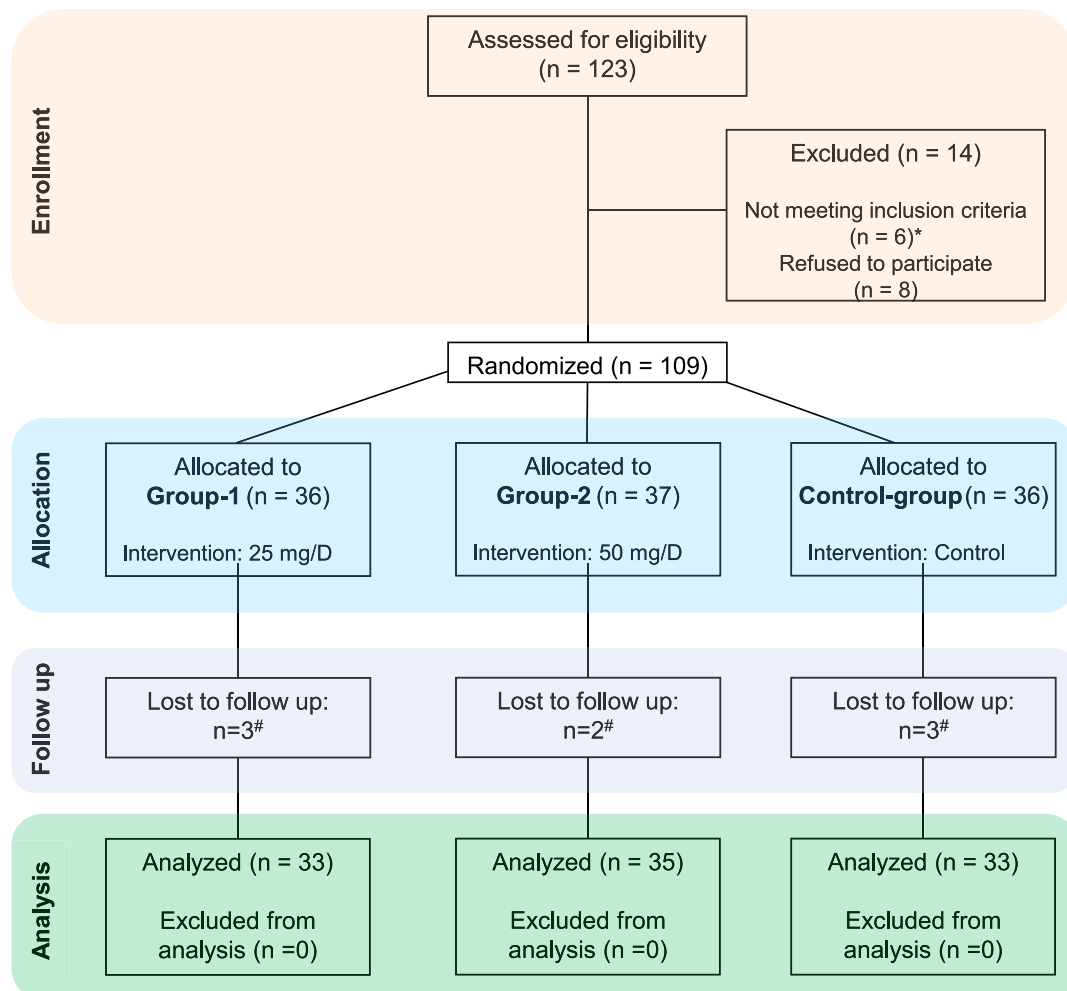


Fig. 1. CONSORT diagram - Trial profile. * Three patients had platelet (PLT) count above $100 \times 10^9/L$. One patient had an AST level higher than five times of upper normal limit. One patient had comorbidity (chronic kidney disease). One patient was infected with the hepatitis B virus. # Patients refused to complete the trial when their PLT count was above $200 \times 10^9/L$.

Table 1
Baseline characteristics of patients randomized into the different treatment arms.

Groups	Group 1	Group 2	Control-group
Eltrombopag	25 mg/D	50 mg/D	Nil
Age (Years)			
Median (Interquartile range)	25 (20–35)	26 (23–35)	28 (23–33)
Mean (SD)	26 (8)	30 (10)	30 (9)
Sex			
Male	26 (79%)	22 (63%)	26 (79%)
Female	7 (21%)	13 (37%)	7 (21%)
Baseline PLT × 10⁹/L			
Mean (SD)	58 (24)	52 (29)	55 (30)
Baseline IPF (%)			
Mean (SD)	10.71 (4.25)	12.82 (5.31)	13.08 (4.58)
Baseline A-IPN			
Mean (SD)	5.74 (2.62)	6.10 (3.68)	6.64 (3.65)
Baseline Hct (%)			
Mean (SD)	40 (4)	41 (5)	42 (6)
Baseline BP (mmHg)			
Systolic Mean (SD)	104.54 (5.05)	106.28 (19.14)	102.87 (8.38)
Diastolic Mean(SD)	73.63 (6.76)	73.14 (13.93)	72.27 (4.85)
Bleeding Manifestations (%)			
	9 (27%)	5 (14%)	7 (30%)
Days from onset of fever			
Median (range)	4 (2–8)	4 (2–9)	4 (2–8)
Mean (SD)	4.15 (1.50)	4.28 (1.50)	4.303 (1.23)

trial period (Day-0 to Day-7), hence the clinical data were available for 101 patients from Dhaka Medical College ($N = 50$), Better Life Hospital ($N = 28$), and AMZ hospital ($N = 23$) who participated throughout the trial period. The standard management protocols for dengue patients as recommend by WHO [6,29] were followed in all three hospitals thereby reducing any hospital-based variabilities. The mean age of the 101 randomized patients was 29 ± 9 years with almost one-third of female patients. There was no significant difference in mean and median ages among the three groups (p -value=0.23). The majority of the randomized patients ($N = 82$) were NS1 positive, while only twelve and seven patients were IgM and IgG positive, respectively. Among the randomized patients, twenty-four (24%) were categorized as grade-II DHF with moderate to severe bleeding manifestations during the study. The most predominant bleeding site was the rectum ($N = 8$) followed by gum ($N = 6$), skin ($N = 5$), vagina ($N = 3$), and nose ($N = 4$). Bleeding manifestation in the eye (Subconjunctival bleeding) was observed for one patient. Nine, five, and ten grade-II DHF patients were in Group-1, -2, and Control-group, respectively (Table 1). The mean basal platelet count (at Day-0) was $55 \times 10^9 \pm 27$ /L. No significant difference was observed among the mean of baseline platelet count among three groups (Group-1: $58 \times 10^9/L \pm 24$; Group-2: $52 \times 10^9/L \pm 29/L$ and control-group: $55 \times 10^9/L \pm 30$) (p -value=0.63) (Table 1). The baseline hematocrit (Hct) of the enrolled patients was 40 ± 9 . The mean baseline Hct for Group-1, -2, and Control-group patients were 40 ± 4 , 41 ± 5 , and 42 ± 6 , respectively (Table 1). In addition, for each patient, the days from the onset of fever were analyzed, and the results showed, that the median and average days from onset of fever on Day-0 in all three groups were similar across all three groups (Table 1). Fig. 2 shows the days from the onset of fever in a patient-specific manner across the treated and control groups. In Fig. 2 the day on which the dengue-specific NS1, IgM/IgG tests were carried out for each patient was shown.

3.1. Platelet count dynamics in response to eltrombopag administration

Platelet (PLT) counts were measured for each patient from Day-0 to Day-7. In order to determine the overall group (treatment and control) and time effect, a Two-way ANOVA test was performed followed by multiple correction tests (Bonferroni). The ANOVA results indicated that both time and groups (two treatment and one control groups) were significant ($p < 0.0001$) contributing factors to the overall variance of the PLT count data. The investigation of the

combined effect of time and treatment-groups on platelet count showed the combined effect is also significant ($p < 0.0001$) and constant in all three groups. This analysis reveals a significant effect of time on the platelet count but importantly highlighted the uniformity of time-effect in all groups. This uniformity of time effect in both treated and control group facilitated to dissect the impact of the treatment from the time on the PLT variability. From Day-0 to -3, the mean PLT counts among the three groups were not significantly different. After the completion of the intervention phase (Day-0 to -2), the mean PLT counts of Group-1 ($141 \times 10^9/L$) and -2 ($134 \times 10^9/L$) were although higher compared to the control-group ($95 \times 10^9/L$), but not statistically significant due to the large variability of PLT counts among the patients on Day-3 (Fig. 3A). On Day-4, the mean PLT counts (PLT $\times 10^9/L$) were significantly higher in Group-1 ($182 \times 10^9/L \pm 106$) and -2 ($182 \times 10^9/L \pm 98$) compared to Control-group ($112 \times 10^9/L \pm 75$) (adjusted p -values were 0.016 for Group-1 vs. Control and 0.008 for Group-2 vs. Control) (Fig. 3A and Supplementary Table 1). In the latter days (Day-5 to Day-7), mean PLT counts for Group-1 and Group-2 patients continued to be significantly higher than Control-group (Fig. 3A, Supplementary Table 1). At Day-7, mean PLT counts were the highest for all the three groups; however, the mean PLT count for Group-1 ($332 \times 10^9/L \pm 92$) and Group-2 ($371 \times 10^9/L \pm 111$) were significantly higher than the control-group ($194 \times 10^9/L \pm 96$) (Fig. 3A and Supplementary Table 1). Subsequently, to determine the efficacy, the proportion of patients with PLT count above the lower normal limit ($150 \times 10^9/L$) was considered as “platelet (PLT)-recovered”. The odds ratio (OR) of eltrombopag success was calculated by comparing the proportion of PLT-recovered patients for Group-1 vs. Control-group and Group-2 vs. Control-group. On Day-4, more than half of the patients were PLT-recovered in Group-1 (60%, OR: 4.1, 95% CI: 1.45 to 11.56, adjusted p -value: 0.016) and Group-2 (54%, OR: 3.16, 95% CI: 1.14 to 8.73, adjusted p -value: 0.04) (Fig. 3B and 3C, Table 2). In contrast, only 27% of patients were PLT-recovered in Control-group (Fig. 3D) on the same day. The proportion of PLT-recovered patients gradually increased up to Day-7 and eventually the highest proportion of the recovered patients was observed on Day-7 for all three groups. The proportion of the PLT-recovered patients were significantly higher in Group-1 (91%, OR: 8.33, 95% CI: 2.11 to 32.80, adjusted p -value: 0.008), and Group-2 (91%, OR: 8.89, 95% CI: 2.26 to 34.89, adjusted p -value: 0.005) in comparison to Control-group (55%) on Day-7 (Fig. 3B-D). The confidence interval limit of the OR was observed although as wider, the estimated CI limit does not include the value 1, and therefore nullifies the uncertainty in the calculated odds of eltrombopag success on dengue patients [34]. Three patients of Group-1 and seven patients of Group-2 had PLT count above the upper normal limit ($450 \times 10^9/L$) at Day-7 (Fig. 3B-D). Under the assumption (based on ANOVA results) that the impact of time was similar in all groups, the t -test with multiple correlation tests showed that the platelet count differs significantly between the treated (Group-1 and Group-2) and control groups. This difference can only be attributed to the effect of treatment considering the effect of time is uniform in all groups. According to WHO, one of the criteria for discharging dengue patients is platelet counts higher than $50 \times 10^9/L$. Therefore, we performed an additional analysis taking into account the platelet count of $50 \times 10^9/L$. In brief, we divided the patients of each group (two treated and control groups) according to the platelet count higher or lower than $50 \times 10^9/L$. Results revealed that, on Day-3, 73% of Group-1 and 80% of Group-2 patients showed platelet count higher than $50 \times 10^9/L$ in contrast to 67% of control group patients (Supplementary Table 2). On Day-4, 85% ($N = 28$) and 97% ($N = 34$) of patients in Group-1 and Group-2 respectively, had platelet count higher than $50 \times 10^9/L$ compared to 70% ($N = 23$) of patients in the Control group (Supplementary Table 2). All Group-2 patients exhibited a higher platelet count than $50 \times 10^9/L$ for the rest of the days (Day-5 to Day-7). On Day-5, 94% ($N = 31$) of Group-1 and 82% ($N = 27$) of Control-group

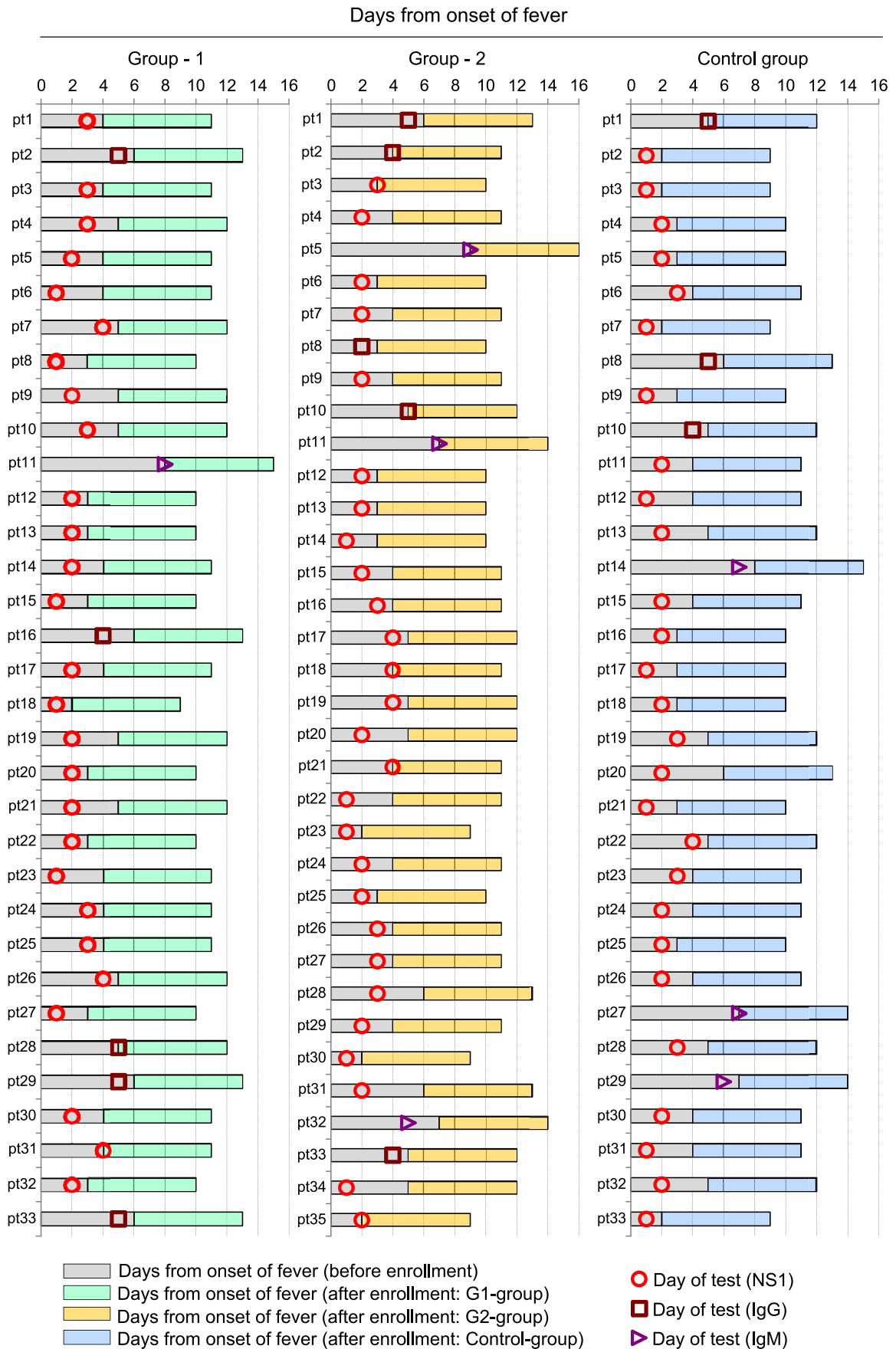


Fig. 2. Days from the onset of fever of each patient across treated- and control-groups

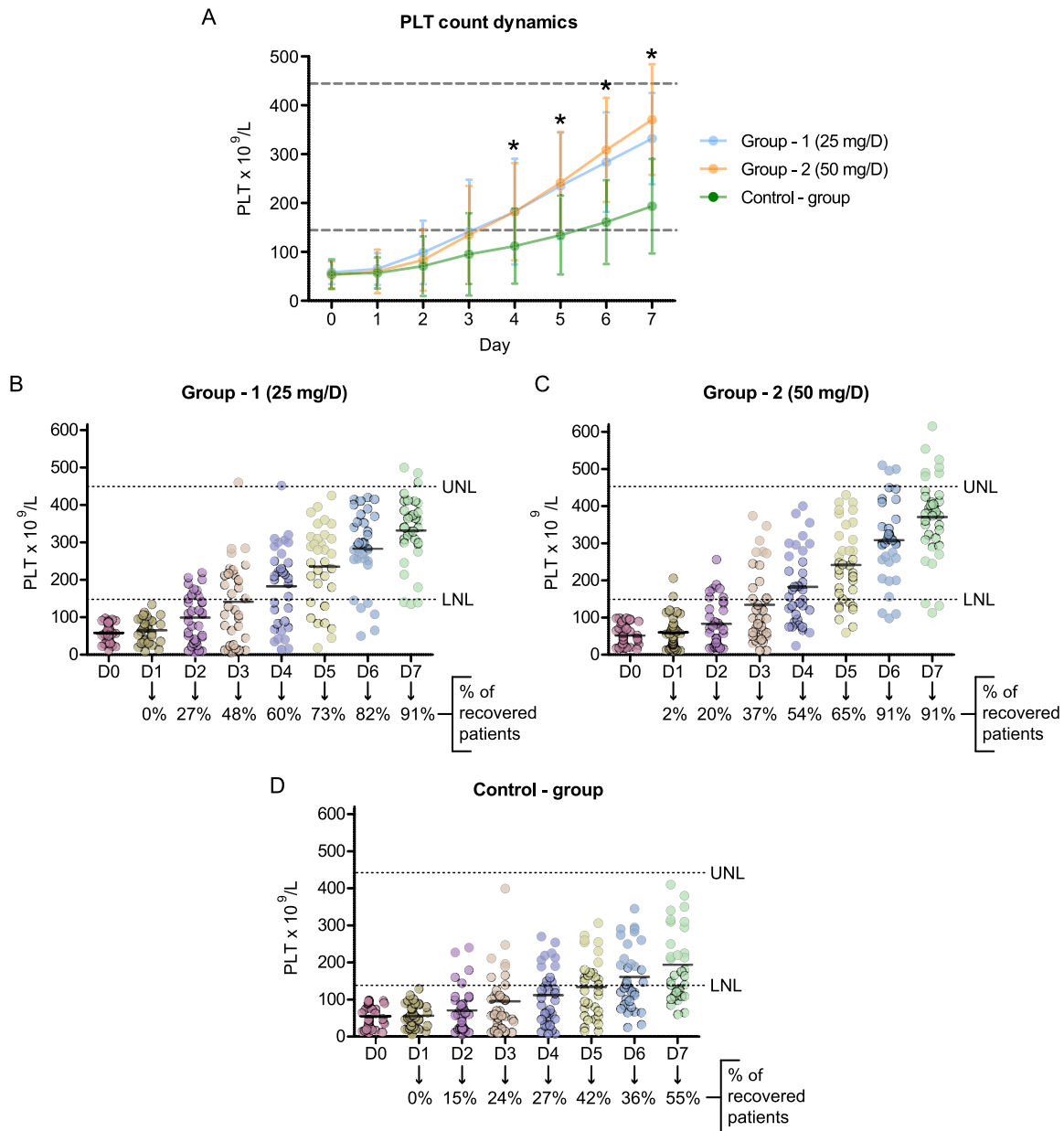


Fig. 3. Mean platelet count and dynamics of platelet count change in the treated and control group.

A. Mean platelet (PLT) count for each day between Day-0 and Day-7 of patients across all groups (treated – Group1/2 and Control-group) are shown. Y-axis shows the PLT count ($\times 10^9/L$). The error bars represent the standard deviation. Different line colors indicate different groups (blue: Group1, orange: Group2 and green: Control-group). Star indicates an adjusted p-value lower than 0.05 as calculated by *t*-test followed by multiple correction tests.

The Day-wise proportion of the platelet (PLT)-recovered patients (with PLT count above than lower normal limit) in Group1 (B), Group2 (C), and Control-group (D) are shown. Y-axis shows the PLT count ($\times 10^9/L$). The horizontal dash lines represent the lower normal limit, LNL ($150 \times 10^9/L$), and upper normal limit, UNL ($450 \times 10^9/L$) of PLT count. The patient was considered recovered if the PLT count was above the LNL. Mean PLT count as indicated by black bars.

patients, exhibited a higher platelet count than $50 \times 10^9/L$ (Supplementary Table 2). On Day-6 all the patients in Group-1 also achieved a platelet count higher than $50 \times 10^9/L$. All the control-group patients showed platelet counts above $50 \times 10^9/L$ on Day-7 (Supplementary Table 2). However, this difference on the different days of treatment was not statistically significant (Supplementary Table 2).

Furthermore, we stratified the patients based on the classification of dengue phases (acute/febrile, critical, and recovery) by World Health Organization [4] and Yacoub et al. [30] across all three groups. We utilized the day of defervescence of each patient to differentiate

between acute and critical phases. The day of defervescence was considered as the end of the acute phase and the next two days were classified as the critical phase followed by a recovery phase. Fig. 4A describes the number of patients in a particular dengue phase in a day-wise manner. The classification reveals that on the day of enrollment (Day-0) the number of patients distributed in different phases of dengue virus infection was uniform across the treated (Group-1 and –2) groups and Control-group (Fig. 4A). On Day-0, the proportion of patients in the recovery phase was 27% in Group-1 and Control-group, whereas in Group-2 the percentage of patients in the recovery

The Days from the onset of fever for each patient was indicated by the horizontal bar diagram. The treated (group-1 and –2) and control-group patients were shown separately. The gray color of the bar represents the days from onset of fever before enrolment whereas the colored bars indicate the days after enrolment (green: Group-1, yellow: Group-2, and blue: Control-group patients). The symbols represent the day of the test (red hollow circle, hollow rectangle, and hollow triangle indicate NS1, IgG, and IgM tests respectively).

Table 2
Odds ratio (OR) of response to eltrombopag.

Group	Days	Number of patients PLT<LNL [#]	Number of patients PLT>LNL [#]	Total number of patients	% of Recovery	Odds ratio	95% CI	Z-stat	p-value	Adj. p-value
Group 1: 25 mg/day	Day 1	33	0	33	0	1	0.01 to 51.89	0	1	1
	Day 2	24	9	33	27.2727273	2.1	0.61 to 7.12	1.19	0.2339	0.272883
	Day 3	17	16	33	48.4848485	2.94	1.03 to 8.39	2.016	0.0438	0.06132
	Day 4	13	20	33	60.6060606	4.1	1.45 to 11.56	2.669	0.007	0.01633*
	Day 5	9	24	33	72.7272727	3.32	1.14 to 9.18	2.209	0.0272	0.0476*
	Day 6	6	27	33	81.8181818	7.12	2.27 to 22.39	3.369	0.0008	0.0056*
	Day 7	3	30	33	90.9090909	8.33	2.11 to 32.80	3.032	0.0024	0.0084*
Group 2: 50 mg/day	Day 1	33	2	35	5.71428571	5	0.23 to 108.13	1.026	0.3048	0.3556
	Day 2	28	7	35	20	1.4	0.39 to 4.94	0.523	0.6011	0.6011
	Day 3	22	13	35	37.1428571	1.84	0.64 to 5.28	1.144	0.2526	0.35364
	Day 4	16	19	35	54.2857143	3.16	1.14 to 8.73	2.227	0.0259	0.045325*
	Day 5	8	23	35	65.7142857	3.49	1.19 to 10.19	2.287	0.0222	0.045325*
	Day 6	3	32	35	91.4285714	16.89	4.22 to 67.58	3.995	0.0001	0.0007*
	Day 7	3	32	35	91.4285714	8.89	2.26 to 34.89	3.313	0.0017	0.00595*

[#] LNL=150 × 10⁹/L

* adjusted p-values below 0.05.

phase was 20% indicating that at the day of enrollment the proportion of patients in the recovery phase were similar ranging from 20 to 27% and more than 60% remaining patients were in acute and critical phases. The highest percentages of patients with critical phase were observed for Group-2 (69%) and Control-group (55%) on Day-1. For group-1 the highest percentage of critical patients was on Day-3. This implies that during the eltrombopag administration (Day-0 to Day-2) a significant fraction of patients were in acute and critical phases. All the patients were considered in the recovery phase from Day-4 to Day-7 in all three groups. Next, we analyzed the platelet count on the pre-stratified patients based on dengue-phases (acute, critical, and recovery) (Fig. 4B). Analysis of patients in the acute phase on Day-0 to Day-1 showed that the difference of platelet count between the treated (Group-1 and -2) was not statistically different from the Control-group. For patients with critical phase, the PLT count variation was not significant on Day-0 to Day-3. In the case of recovery phase patients, the PLT count variations were not significant on Day-0 to Day-3. In contrast, from Day-4 to Day-7 PLT count of patients with recovery phase was significantly higher in the treated groups in comparison to the Control-group (Group-1 vs. Control: Day-4, Day-5, Day-6 and Day-7 adjusted p-values were 0.016, 3×10^{-4} , 1×10^{-5} and 1×10^{-6} , respectively; Group-2 vs. Control: Day-4, Day-5, Day-6 and Day-7 adjusted p-values were 0.008, 7×10^{-5} , 2×10^{-7} and 1×10^{-8} , respectively) (Fig. 4B). Therefore a comparison of the platelet count from Day-4 to Day-7 among the treated and control groups revealed that eltrombopag contributes to a rapid increase of the platelet recovery in treated groups compared to the Control-group (Fig. 4B). All together these analyses highlight that the goal of eltrombopag treatment is to augment the platelet recovery in patients with moderate to severe thrombocytopenia rather than preventing thrombocytopenia in dengue-patients in the critical phase.

All the grade-II DHF patients in Group-1 ($N = 9$), exhibited PLT count higher than $150 \times 10^9/L$ (LNL) on Day-7 except one patient with a PLT count of $135 \times 10^9/L$ on the same day (Table 3). Interestingly, the bleeding manifestation of this patient did not subside throughout the trial period (Day-0 to Day-7), whereas all other Group-1 patients did not exhibit bleeding after Day-4. All five grade-II DHF patients in Group-2 showed much higher PLT counts than the LNL and did not show bleeding tendencies after Day-4. Six (60%) grade-II DHF patients in the Control-group did not achieve PLT counts higher than LNL on Day-7. Interestingly, four of these patients with the lowest PLT counts continued to show intermittent bleeding tendencies throughout the trial period, whereas the rest of the control-group patients did not show any sign of bleeding after Day-4 (Table 3).

3.2. Dynamics of immature platelet fraction and absolute immature platelet count in response to eltrombopag administration

Immature platelet fraction (IPF) was measured for each patient from Day-0 to -7. Baseline IPFs on Day-0 among the patients of Group-1 ($11\% \pm 4$), -2 ($13\% \pm 5$) and Control-group ($13\% \pm 5$) were not significantly different. After the initial increase of IPF in Group-1 and -2 patients during the intervention phase (from Day-0 to Day-2), it appeared to decrease in the follow-up phase (Day-3 to -7). Interestingly, control-group patients showed a steady level of IPF from Day-0 to Day-3 before decreasing throughout the later days. On Day-7, the mean IPF among the treated- and control-group patients was not significantly different (Group-1: $6\% \pm 3$, Group-2: $6\% \pm 2$ and Control-group: $7\% \pm 4$) (Group-1 vs. Control adjusted p-value= 0.46 and Group-2 vs. Control adjusted p-value= 0.38) (Fig. 5A and Supplementary Table 1). The A-IPC (representing the multiplied product of the IPF and the circulating platelet count divided by 100) was calculated for each patient from Day-0 to Day-7. In contrast to IPF, the mean A-IPC was significantly higher in Group-1 and -2 patients compared to the control-group from Day-5 to Day-7 (Fig. 5B and Supplementary Table 1). On Day-4 A-IPC was significantly higher in Group-1 patients compared to Control-group. Although not significant, the mean A-IPC of Group-2 patients remained relatively higher compared to that of Group-1 patients from Day-3 to Day-7 (Fig. 5B). For Group-1, -2, and control-group, the highest mean A-IPC was observed on Day-6, Day-5, and Day-7, respectively (Fig. 5B).

3.3. Identification of differential responses of patients to eltrombopag

Endpoint analysis involving a comparison of PLT count among the treatment groups on Day-7 may not always reflect the dynamics of PLT count from throughout the trial period. Moreover, variability in the PLT count of the patients within each group indicated patient-specific differential response to eltrombopag. Therefore to investigate the impact of patient-specific PLT dynamics in response to eltrombopag, we performed hierarchical clustering methods based on PLT counts of patients from Day-0 to Day-7. To investigate this response variability, patients in each group were clustered based on the PLT count across the trial period by applying supervised hierarchical clustering. For each group of patients, three distinct clusters were identified, representing high, intermediate, and low response clusters. In Group-1, 55% of the patients ($N = 18$) constituted the high-response cluster (C1) while only 18% ($N = 6$) patients clustered together as the low-response cluster (C3). Among the six patients with low

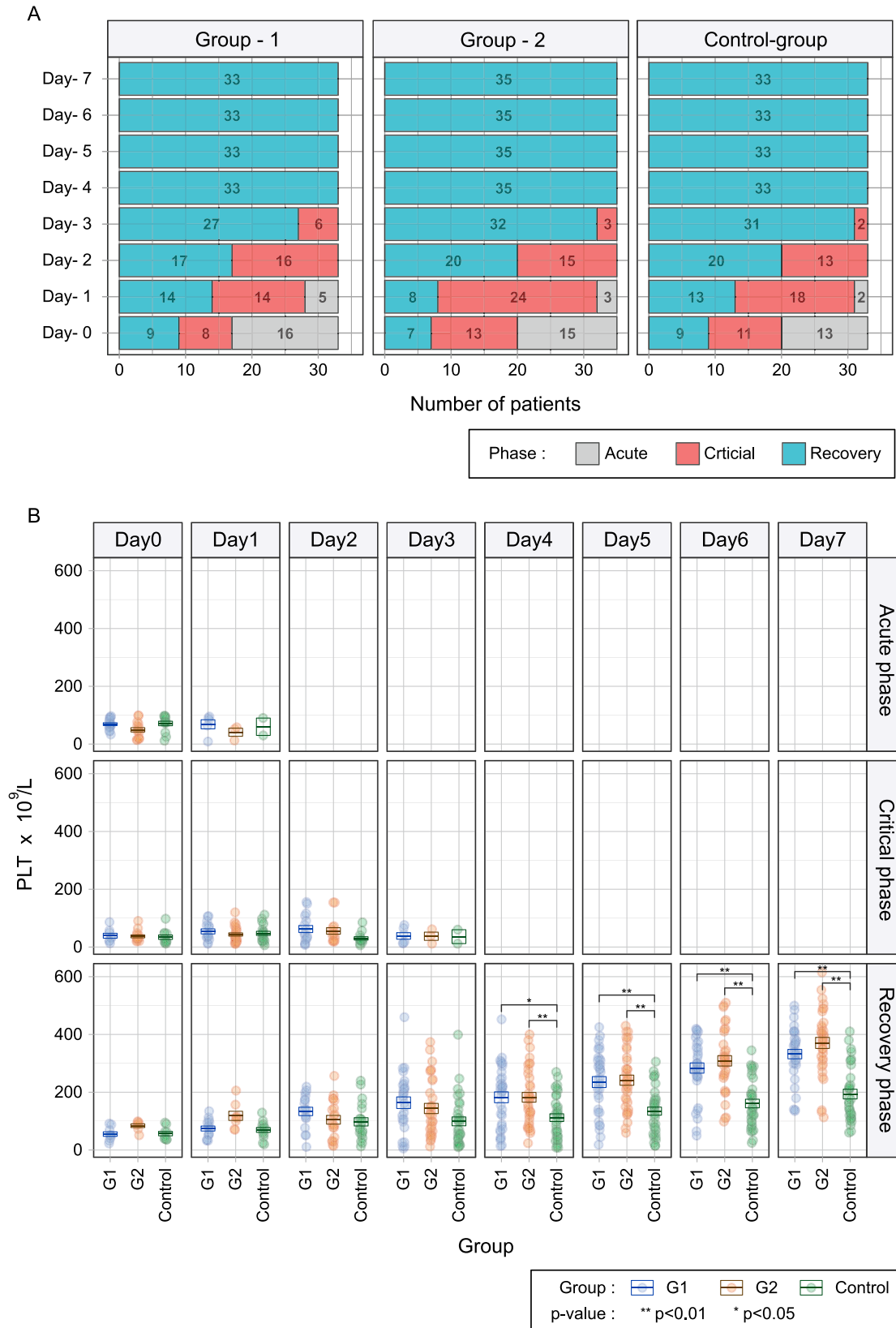


Fig. 4. Dengue-phase wise stratification of patients.

Dengue patients were stratified based on dengue clinical phases (Acute/febrile, Critical, and Recovery phases) (A). The day-wise proportions of patients in the treated (Group-1 and –2) and control groups are shown as a stacked bar diagram. Color codes represent different phases (gray: Acute phase, Red: Critical phase, and bluish-green: Recovery phase). The platelet (PLT) counts of patients in dengue-phase specific manner are shown for each day across the treated and control groups. Each dot represents the PTL count of one patient. Color codes represent different groups (blue: Group-1, Orange: Group-2 and Green: Control-group). The average PLT count is indicated as a crossbar. The statistical significance is indicated by start symbols were ** indicates adjusted p-value<0.01 and * indicates adjusted p-value<0.05.

Table 3
Platelet count and treatment outcome of grade II dengue hemorrhagic patients.

Group	Gender	Age	Bleeding site at day 0	DF/DHF*	Grade	PLT × 10 ⁹ /L Day0	PLT × 10 ⁹ /L Day7	Bleeding site at day 7	D7 PLT Recovery**
Group 1: 25 mg/Day	F	41	Skin	DHF	Grade II	33	180	No bleeding	Yes
	M	35	Conjunctiva	DHF	Grade II	69	360	No bleeding	Yes
	F	36	Vaginal, Gum	DHF	Grade II	91	330	No bleeding	Yes
	M	18	Rectal	DHF	Grade II	43	320	No bleeding	Yes
	M	25	Rectal	DHF	Grade II	63	310	No bleeding	Yes
	M	27	Gum, nose	DHF	Grade II	88	380	No bleeding	Yes
	M	21	Gum	DHF	Grade II	97	245	No bleeding	Yes
	M	29	Rectal	DHF	Grade II	48	385	No bleeding	Yes
	M	20	Skin	DHF	Grade II	45	135	Skin	No
	M	20	Skin	DHF	Grade II	45	135	Skin	No
Group 2: 50 mg/Day	F	47	Malena	DHF	Grade II	20	324	No bleeding	Yes
	F	43	Vaginal	DHF	Grade II	28	399	No bleeding	Yes
	F	18	Gum	DHF	Grade II	42	375	No bleeding	Yes
	M	33	Rectal	DHF	Grade II	99	245	No bleeding	Yes
	M	32	Skin	DHF	Grade II	41	270	No bleeding	Yes
	M	60	Rectal	DHF	Grade II	18	140	No bleeding	No
Control-group: No eltrombopag	M	39	Rectal	DHF	Grade II	30	165	No bleeding	Yes
	F	31	Vaginal	DHF	Grade II	92	120	Vaginal	No
	M	40	Nose	DHF	Grade II	74	98	Nose	No
	M	20	Nasal	DHF	Grade II	12	180	No bleeding	Yes
	F	30	Gum, Rectal	DHF	Grade II	25	125	No bleeding	No
	M	34	Gum	DHF	Grade II	20	410	No bleeding	Yes
	M	28	Nose	DHF	Grade II	63	175	No bleeding	Yes
	M	27	Skin	DHF	Grade II	77	65	Skin	No
	F	28	Rectal, nose	DHF	Grade II	98	60	Nose	No

* DF= Dengue fever, DHF= Dengue hemorrhagic fever.

** PLT-recover: Platelet count higher than lower normal limit (150 × 10⁹/L).

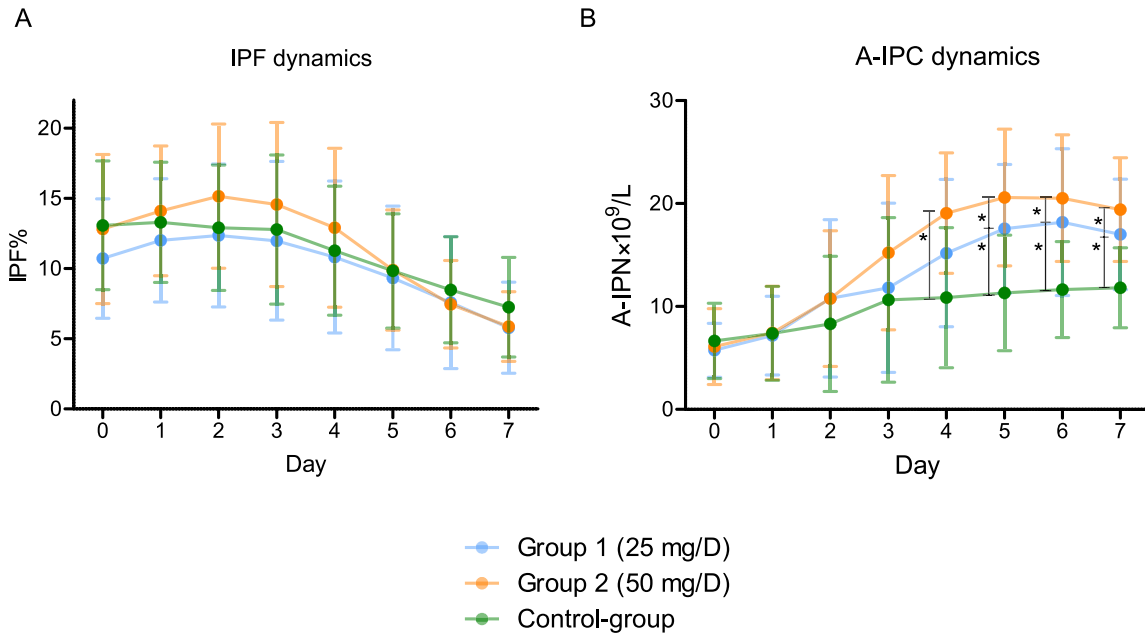


Fig. 5. Mean immature platelet fraction and absolute immature platelet count dynamics of platelet count change in the treated and control group.

Mean immature platelet fraction (IPF) (A) and absolute immature platelet count (A-IPC) (B) were shown for each day between Day-0 and Day-7 of patients across all groups. The error bars represent the standard deviation. The star symbols indicate the significance of the statistical tests where the adjusted p-values were below 0.05.

response, three had PLT count above the LNL. The remaining, 27% of the patients were considered as an intermediate-response cluster (C2) (Fig. 6A). Interestingly, in the case of Group-2 patients, a lower percentage of patients (34%, N = 12) were grouped as the high-response cluster (C1), whereas 40% of patients belonged to the low-response cluster (C3) (Fig. 6B). As opposed to Group-1 and Group-2, the majority of the Control-group patients (55%, N = 18) were clustered in the low-response category (C3) and only 18% of patients were found to belong to the high-response category (C1) (Fig. 6C).

3.4. Determination/Observation of adverse effects in eltrombopag treated patients

In total twelve patients exhibited vomiting, diarrheal episodes, and pain in lower extremities. Eleven patients had vomiting and diarrhea while only one patient showed pain in the lower extremities. It was unclear whether or not the eltrombopag treatment was responsible for these adverse effects (AEs) since the number of patients suffered from the AEs in the treatment groups (Group-1 and -2) was similar to that of the Control-group. The number of patients

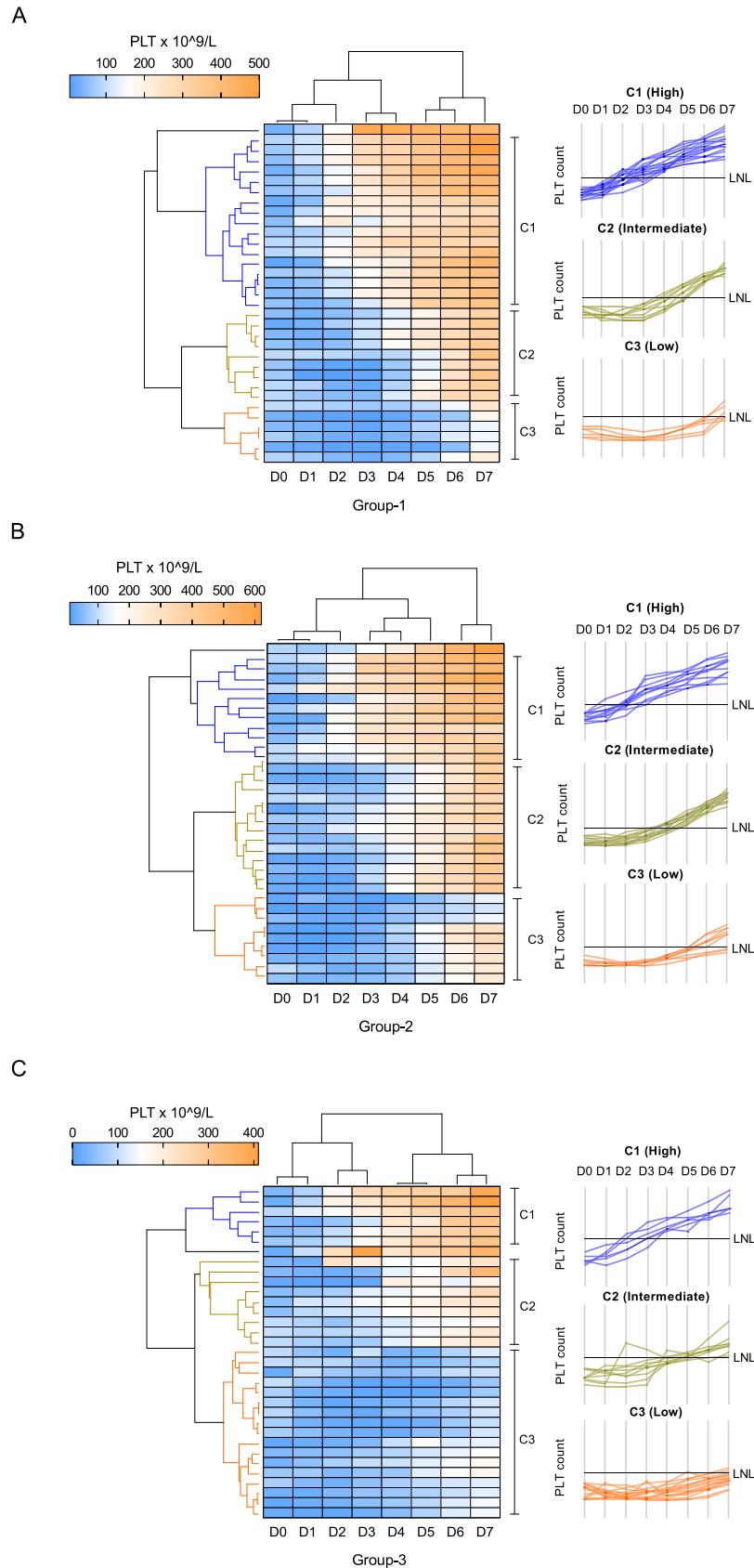


Fig. 6. Hierarchical clustering differentiate responders from non-responders to eltrombopag.

Heatmaps representing the supervised hierarchical clustering results for Group-1 (A), Group-2 (B), and control-group (C) patients are shown. In each group, three distinct clusters signifying high-, intermediate- and low-responsive patients. The color indicates the Day-wise PLT counts of each patient from Day-0 to Day-7 where blue and orange color indicate low and high PLT count, respectively. The platelet (PLT) counts of patients belonging to a particular cluster were shown as profile plot (right panel). The lower normal limit of PLT count was shown as a horizontal black line.

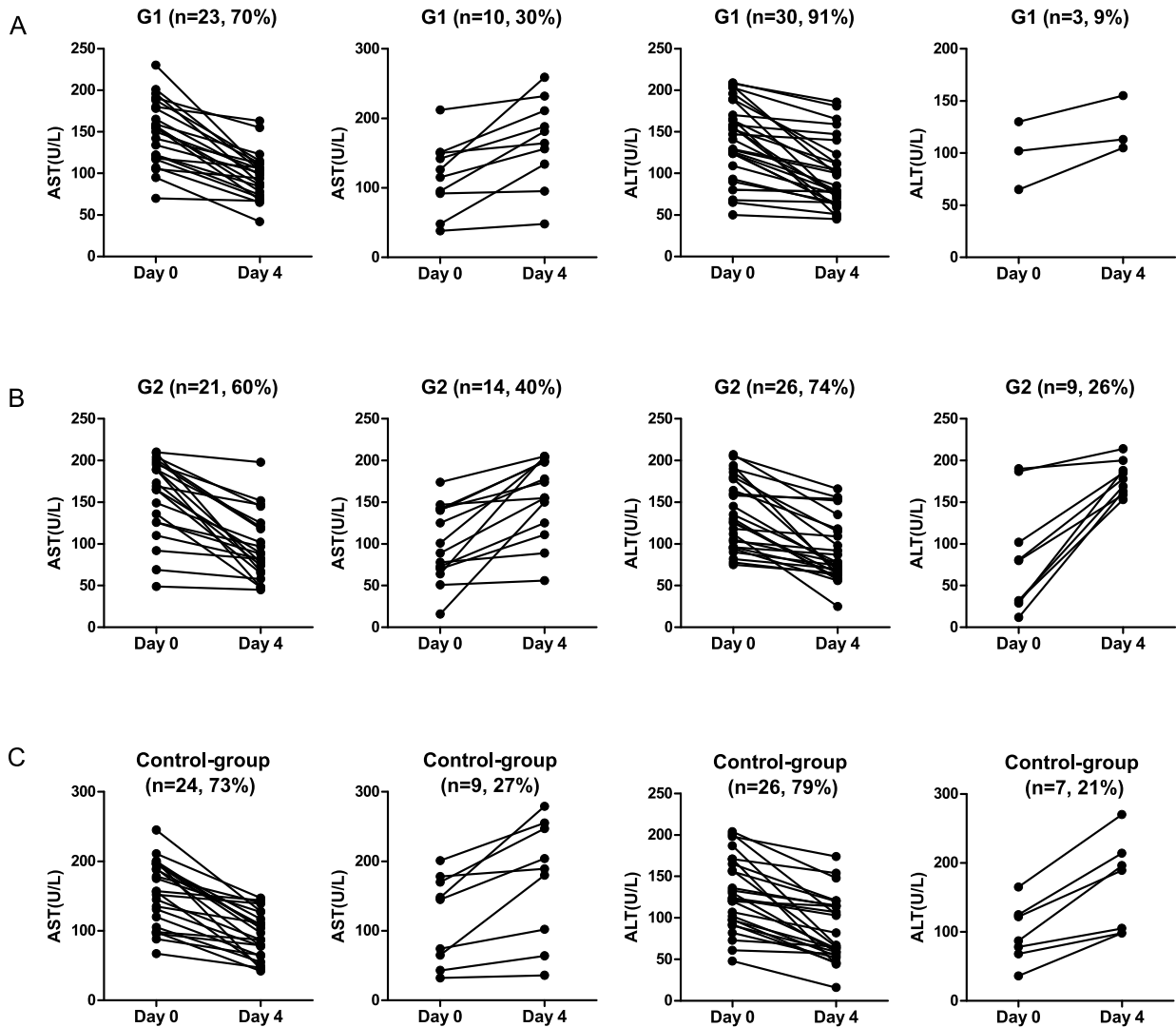


Fig. 7. AST and ALT levels of patients on Day-0 and Day-4

The levels of AST and ALT on Day-0 and Day-4 for Group-1 (A), Group-2 (B), and Control-group (C) patients are shown. The first and third columns represent the patients exhibiting decreased AST and ALT levels from Day-0 to Day-4. In contrast, the second and fourth columns represent patients with increased AST and ALT levels from Day-0 to Day-4.

exhibiting one or more adverse events during the trial period (Day-0 to Day-7) was 5 (15%) and 3 (9%) for Group-1 and -2, respectively. For the Control-group, four patients (12%) showed similar vomiting and diarrheal tendencies. Apart from vomiting and diarrhea, one patient receiving 25 mg/d eltrombopag showed pain in the lower extremities. Vomiting and diarrhea were the only two adverse events recorded for the patients in the treatment groups (Group-1 and -2) as well as in the control group (Control-group). Since the proportion of patients with the manifestation of the AEs was similar and not deviating significantly between treated and untreated groups, eltrombopag may not be the causal agent for these AEs. No deaths had occurred during the study. Ten patients (30%) in the Group-1 showed increased AST (U/L) (Fig. 7A) as opposed to 14 patients (40%) in Group-2 (Fig. 7B). Nine control-group patients (27%) showed increased AST levels (Fig. 7C) (Table 4). Increased ALT levels were observed in three (9%), nine (26%), and seven (21%) patients belonging to Group-1, -2, and Control-group, respectively (Fig. 7A-C, Table 4). These results suggested that eltrombopag may not have contributed to any abnormal hepatic function, as the proportion of patients with elevated AST/ALT levels was similar in all three groups. Ultrasonogram analysis on Day-4 and -7 showed no signs of abdominal thrombosis after the administration of eltrombopag (Supplementary Table 3). USG report showed that one patient in Group-1 had

non-alcoholic fatty liver disease. Edematous gall bladder with or without mild ascites was identified for two patients in Group-2. Renal cyst and edematous gall bladder were identified in two patients in Control-group. No thromboembolic complications were found in any patients indicating that eltrombopag administration for only three days did not cause any thromboembolic events.

4. Discussion

Severe thrombocytopenia - a major clinical manifestation of DF, may serve as a determinant of dengue severity and indicator for the higher probability of progression to DHF [8]. Since, PLT transfusion is not considered as a routine intervention to correct PLT counts, the treatment of low PLT count remains one of the major challenges in the clinical management of dengue. In this context, we present an open-labeled, randomized, controlled clinical trial where the administration of a thrombopoietin receptor agonist - eltrombopag was observed to be efficacious and safe in dengue patients. The primary outcomes of the clinical trial were fulfilled as a significant proportion of the patients (91%) receiving eltrombopag exhibited PLT count above the lower normal limit ($150 \times 10^9/L$) on Day-7 after the enrollment. In contrast, a lower proportion of the control-group patients (55%) achieved a PLT count above the lower normal limit on Day-7.

Table 4
Adverse events (AEs) statistics.

Eltrombopag	Group 1 25 mg/D	Group 2 50 mg/D	Control-group Nil	p-value
Total patients showing AEs individual events	5 (15%)	3 (9%)	4 (12%)	NS
diarrhea	3 (9%)	2 (6%)	3 (9%)	ns
Vomiting	4 (11%)	3 (9%)	3 (9%)	NS
Pain in lower extremity	1 (3%)	0	0	N/A
Aspartate aminotransferase (AST) increased	10 (30%)	14 (40%)	9 (27%)	NS
Alanine aminotransferase (ALT) increased	3 (9%)	9 (26%)	7 (21%)	NS

NS = Not statistically significant (p-value>0.05).

N/A = Not applicable.

AEs = Adverse effects.

The two eltrombopag doses (25 mg/day and 50 mg/day) used in the trial, were equally efficacious to restore the PLT count as no significant difference in response was observed between the doses. Stratification of patients into different dengue-phases (acute, critical, and recovery) showed that on the day of enrollment (Day-0) the proportion of recovery-phase patients was almost equally distributed across the treated- and Control-groups. On Day-4 and onwards all the patients were appeared to be on the recovery phase according to the classification. The results showed that the PLT count enhancing effect of eltrombopag from Day-4 to Day-7 may act synergistically with the endogenous platelet production thereby increasing the platelet count rapidly during the recovery phase of dengue in the treated-patients compared to Control-group patients. Another important finding that came out from the trial as a secondary outcome is that the bleeding manifestation was abated within Day-7 for all but one grade-II DHF patients (thirteen out of fourteen) who received eltrombopag, whereas four out of ten grade-II DHF patients with PLT counts lower than the lower normal limit in the Control-group showed intermittent bleeding symptoms throughout the trial period. This result indicated that PLT count may not be correlated with the onset of bleeding, however, lower PLT count may hinder the cessation of bleeding manifestation. The current trial did not find any evidence of impact originating from demographic factors such as age and gender on eltrombopag response. Therefore the study clearly shows that eltrombopag can be used irrespective of age and gender to patients suffering from severe dengue-induced thrombocytopenia.

As the secondary outcome, the trial showed the safety of eltrombopag administration for a short regimen of three consecutive days in dengue patients. However, the lower dose (25 mg/day) appears to be safer than the higher dose (50 mg/day) of eltrombopag as the higher dose showed the tendency to increase PLT count beyond the UNL ($450 \times 10^9/L$) for some patients. Additionally, higher dose administration resulted in an increase in both ALT and AST levels compared to lower dose and control (Fig. 7). Although the causal effect between the elevation of the liver enzymes and administration of eltrombopag was not clear as the number of patients with elevated ALT and AST did not differ significantly between the treated and Control-group.

The interesting aspect of the trial was the onset of action of eltrombopag within eight days of its administration. Previously, it was deemed that thrombopoietin receptor agonist – romiplostim requires at least one week to generate a viable response [26,35]. In contrast, the minimum time to observe a response to eltrombopag therapy was 2 weeks in ITP patients where eltrombopag administration increased the PLT count $>50 \times 10^9/L$ in 50% of the patients within two weeks of the onset of therapy [36].

In the current study, the major limitation was that the action of eltrombopag was clinically visible after Day-4 in terms of increased mature platelet count when the patients were in the recovery phase. Evidently, eltrombopag failed to increase platelet count significantly

immediately after administration on Day-0 compared to the Control-group. This time lag from Day-0 to Day-4 is not unusual for eltrombopag action [26]. The trial was open-labelled; thus the outcome might possess some bias due to the lack of blinding. Also the sample sizes on each groups were relatively small. This might have also affected the study estimates like the subgroup analysis. The results showed that, although not statistically significant, a higher fraction of patients in the Control-group (30% on Day-4, 18% on Day-5 and 6% in Day-6) compared to the treated group (15% on Day-4, 6% in Day-5 for Group-1 and 3% in Day-4 for Group-2) showed persisting thrombocytopenia (PLT count lower than $50 \times 10^9/L$) (Supplementary Table 2). Comparison between treated and control groups hinted that eltrombopag treatment may have beneficial effects on increasing platelet count and reducing the duration of hospital stay for a fraction of dengue patients with persisting thrombocytopenia.

Previously it has been shown that the time required to observe an eltrombopag response is highly variable among patients and may require 1–3 months for an ITP patient depending on starting and incrementing dose [26]. A clinical trial where eltrombopag was administered to healthy male subjects, showed a steady increase in PLT count after 8 days with a peak at day 16 and returned to baseline PLT count on day 22 [37]. The underlying mechanism for this time-lag to generate a response to eltrombopag remains unresolved in ITP patients. The most plausible hypothesis is that the time-lag for eltrombopag to generate a response involves the stimulation of hematopoietic stem cells to generate megakaryocyte (MK) precursors which ultimately leads to increased production of the platelet [26]. In the current model of thrombopoiesis, after TPO-driven endomitosis and maturation, MK cells migrate to the vascular niche where they begin the extension process of proplatelets and release them into vascular sinusoids [38]. In case of the urgent demand for circulating platelets, mature MK cells can undergo membrane rupture to release platelets to meet the acute demand under the influence of IL-1 α [39]. However, a recent study showed that migration of MK cells is surprisingly slow and put forward an alternative hypothesis stating that a pool of MK cells that localize at the sinusoids are distinct from the other pool of MK cells that resides at a distant periostic niche [40]. In light of this finding, it can be argued that heterogeneous MK pools may play an important role in determining the response of eltrombopag. Distinct MK pools consisting fast-responsive and quiescent response MK cells, where the former is localized at the vessel and can engage in the rapid production of platelets based on the demand, and the later represents the quiescent MK pools that are located at a distant site from the vasculature [40]. MK cells are evidently altered in ITP in the form of diminished maturation, lower ploidy, impaired platelet production, and release [41]. Moreover, a large proportion of ITP patients develop autoantibodies that hinder MK cell maturation [42]. These insights into the MK cells abnormality may explain the variable time for the onset of action and treatment outcomes of eltrombopag in ITP patients. The pathophysiology of DF and importantly the status of MK cells in dengue virus infection has not been fully established. However, it has been hypothesized that impairment of the megakaryopoiesis occurs in the bone marrow during the early acute phase of dengue virus infection and in the late febrile and convalescent-phase, peripheral destruction of platelets contributes to the manifestation of thrombocytopenia [43]. Kinetics study of dengue virus propagation suggested that dengue viral RNA was readily detectable on day 3 from MK cells but declined after day 5, suggesting that MK cells may be the early targets of DENV [43]. In light of these findings, it can be surmised that since the patients receiving eltrombopag during the current trial were in late febrile and convalescent-phase and therefore their megakaryopoiesis was most likely devoid of dengue-induced impairment. Since the pathophysiology of dengue and ITP are quite distinct, the rapid response of dengue patients to eltrombopag may be attributed to the different status of MK cells in dengue fever compared to ITP. Absolute immature PLT count (A-IPC)

was shown to be a surrogate marker for the platelet production and consumption [44]. In light of this finding, the impact of eltrombopag on the production of platelets can potentially be assessed by the A-IPC. Indeed the increase in the absolute immature PLT count (A-IPC) in the eltrombopag treated groups in comparison to the control-group hints towards the ability of eltrombopag to stimulate thrombopoiesis in the bone marrow of dengue patients.

Although the efficacy of eltrombopag to correct dengue-induced thrombocytopenia was high, within the patients a notable inter-patient variability was observed as evident by clustering analysis. The underlying reason for this variability may include but not be limited to different viral loads, platelet activation status, number of fast-responding MK pools, host-genetic variability, etc. For instance, the high copy number of DENV genome in the platelet pellet from dengue-infected patients positively correlates with the increased platelet activation as well as increased binding of complement factor C3 and IgG on their surface on day 4 from the onset of illness [45]. Moreover, restoring of the platelet counts on day 10 was inversely correlated with platelet activation markers [45]. In a seminal study, Soranzo et al. identified host-genetic factors - three independent loci that are associated with PLT count by employing genome-wide association study (GWAS) and meta-analysis [46]. In summary, all these aforementioned factors may account for the inter-patient variability of response to eltrombopag in dengue patients.

Eltrombopag was found to be safe to administer in a short regimen to correct dengue-induced thrombocytopenia. Between the two doses of eltrombopag, the lower dose (25 mg/day) was found to be safer compared to the higher dose (50 mg/day). 40% and 26% of patients who received a higher dose of eltrombopag showed elevated levels of AST and ALT, respectively, as opposed to 30% and 9% of patients who received a lower dose on day 4. In the Control-group, 27% and 21% of patients also demonstrated elevated AST and ALT levels, respectively on Day-4. It is unclear whether the elevated AST and ALT levels of the patients in Group-2 was due to the effect of eltrombopag or caused by hepatic manifestations due to dengue viral toxicity and immune-system mediated injury in response to DENV infection [47]. The seven patients receiving the higher dose showed PLT count above the upper normal limit ($450 \times 10^9/L$) on Day-7 in contrast to the three patients receiving the lower dose. Therefore, administration of 50 mg/day eltrombopag for three consecutive days may increase the risk of thrombocytosis in dengue patients. Taken together all these findings into account, the current clinical trial revealed that eltrombopag can be considered as a therapeutic option to increase the PLT counts in DF and DHF patients in the recovery phase. Eltrombopag may potentially be beneficial in treating severe dengue patients with persisting thrombocytopenia.

Data sharing statement

Individual participant data that underlie the results being reported, will be made available after de-identification (text, tables, figures, and appendices). Documents that will be shared include Study protocol, Statistical analysis plan, Informed consent form, Clinical study report, and Analytical code. This will be shared immediately following publication. Data will be shared with researchers who provide a methodologically sound proposal. Data will be shared with types of analyses which achieve the aims in an approved proposal. Proposals should be directed to nabi@du.ac.bd and sajib@du.ac.bd. To gain access, the data requester will need to sign a data access agreement.

Author contribution

Contributions of the authors include the conceptualization, study design, data-collection, data-generation, data storage, data analysis, interpretation, and writing of the manuscript. The study funder

provided the funding for the trial and provided the drug. The funder had no role in study design, data collection, and analysis, or writing the manuscript. All authors had full access to all the study data and shared final responsibility for the decision to submit for publication. SC, MS, and AHMNN conceived the idea. SC and AHMNN designed the experiments. SC, SA, and MS collected the blood samples and conducted the laboratory experiments. MS, MS, and CTT recruited the patients and administered the drug of interest. SC, TD and PS performed the statistical analyses. SC, SA and BKB measured the immature platelet fraction. SC, AK, MRA, and AHMNN analyzed and interpreted the data and, wrote the manuscript. All authors read and approved the manuscript.

Funding

Incepta pharmaceuticals limited, Bangladesh.

Declaration of Competing Interest

SC, MS, AK, MRA, and AHMNN report grants from Incepta Pharmaceutical Limited, Bangladesh, during the conduct of the study.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.eclinm.2020.100624](https://doi.org/10.1016/j.eclinm.2020.100624).

References

- [1] Wilder-Smith A, Ooi EE, Horstick O, Wills B. Dengue. *Lancet* 2019;393(10169):350–63.
- [2] Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. *Nat Rev Dis Primers* 2016;2:16055.
- [3] Muller DA, Depelenaire AC, Young PR. Clinical and Laboratory diagnosis of dengue virus infection. *J Infect Dis* 2017;215(suppl_2):S89–95.
- [4] World Health Organization ROFS-EAW, SEARO). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. 2011.
- [5] Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. *Nat Rev Microbiol* 2010;8(12 Suppl):S7–16.
- [6] Kalayanarooj S. Clinical manifestations and management of dengue/DHF/DSS. *Trop Med Health* 2011;39(4 Suppl):83–7.
- [7] Lam PK, Ngoc TV, Thu Thuy TT, et al. The value of daily platelet counts for predicting dengue shock syndrome: results from a prospective observational study of 2301 Vietnamese children with dengue. *PLoS Negl Trop Dis* 2017;11(4):e0005498.
- [8] Ralapanawa U, Alawattagama ATM, Gunrathne M, Tennakoon S, Kularatne SAM, Jayalath T. Value of peripheral blood count for dengue severity prediction. *BMC Res Notes* 2018;11(1):400.
- [9] Makroo RN, Raina V, Kumar P, Kanth RK. Role of platelet transfusion in the management of dengue patients in a tertiary care hospital. *Asian J Transfus Sci* 2007;1(1):4–7.
- [10] Vogt MB, Lahon A, Arya RP, Spencer Clinton JL, Rico-Hesse R. Dengue viruses infect human megakaryocytes, with probable clinical consequences. *PLoS Negl Trop Dis* 2019;13(11):e0007837.
- [11] 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. *Mediat Inflamm*. 2015;2015:313842.
- [12] Hottz ED, Oliveira MF, Nunes PC, et al. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost*: JTH 2013;11(5):951–62.
- [13] Lye DC, Archuleta S, Syed-Omar SF, et al. Prophylactic platelet transfusion plus supportive care versus supportive care alone in adults with dengue and thrombocytopenia: a multicentre, open-label, randomised, superiority trial. *Lancet* 2017;389(10079):1611–8.
- [14] Kaur P, Kaur G. Transfusion support in patients with dengue fever. *Int J Appl Basic Med Res* 2014;4(Suppl 1):S8–S12.
- [15] Kuter DJ, Goodnough LT, Romo J, et al. Thrombopoietin therapy increases platelet yields in healthy platelet donors. *Blood* 2001;98(5):1339–45.
- [16] Vadhan-Raj S, Murray LJ, Bueso-Ramos C, et al. Stimulation of megakaryocyte and platelet production by a single dose of recombinant human thrombopoietin in patients with cancer. *Ann Intern Med* 1997;126(9):673–81.
- [17] Li J, Yang C, Xia Y, et al. Thrombocytopenia caused by the development of antibodies to thrombopoietin. *Blood* 2001;98(12):3241–8.
- [18] Wormann B. Clinical indications for thrombopoietin and thrombopoietin-receptor agonists. *Transfus Med Hemother: Off Organ Deutsch Ges Transfus Med Immunhamatol* 2013;40(5):319–25.

- [19] Allen R, Bryden P, Grotzinger KM, Stapelkamp C, Woods B. Cost-effectiveness of eltrombopag versus romiplostim for the treatment of chronic immune thrombocytopenia in England and Wales. *Value Health: J Int Soc Pharmacoecon Outcomes Res* 2016;19(5):614–22.
- [20] Bussel JB, Cheng G, Saleh MN, et al. Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. *N Engl J Med* 2007;357(22):2237–47.
- [21] Afdhal NH, Giannini EG, Tayyab G, et al. Eltrombopag before procedures in patients with cirrhosis and thrombocytopenia. *N Engl J Med* 2012;367(8):716–24.
- [22] Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag added to standard immunosuppression for aplastic anemia. *N Engl J Med* 2017;376(16):1540–50.
- [23] Gill H, Wong RSM, Kwong YL. From chronic immune thrombocytopenia to severe aplastic anemia: recent insights into the evolution of eltrombopag. *Ther Adv Hematol* 2017;8(5):159–74.
- [24] Erickson-Miller CL, Delorme E, Tian SS, et al. Preclinical activity of eltrombopag (SB-497115), an oral, nonpeptide thrombopoietin receptor agonist. *Stem Cells* 2009;27(2):424–30.
- [25] Rodriguez-Mejorada SM, Rosel-Gomez CG, Rosado-Castro RA, Domingo-Padilla MA, Ruiz-Delgado GJ. Romiplostim reverts the thrombocytopenia in dengue hemorrhagic fever. *Hematol Oncol Stem Cell Ther* 2011;4(1):48–9.
- [26] Bussel JB, Lakkaraja M. Thrombopoietic agents: there is still much to learn. *Presse medicale* 2014;43(4 Pt 2):e69–78.
- [27] Ghanima W, Cooper N, Rodeghiero F, Godeau B, Bussel JB. Thrombopoietin receptor agonists: ten years later. *Haematologica* 2019;104(6):1112–23.
- [28] Srikiatkachorn A, Rothman AL, Gibbons RV, et al. Dengue-how best to classify it. *Clin Infect Dis: Off Publ Infect Dis Soc Am* 2011;53(6):563–7.
- [29] World Health Organization ROFS-EA. Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever. revised and expanded edition. WHO Reg Office South-East Asia 2011.
- [30] Yacoub S, Wertheim H, Simmons CP, Screation G, Wills B. Cardiovascular manifestations of the emerging dengue pandemic. *Nat Rev Cardiol* 2014;11(6):335–45.
- [31] World Health Organization ROFS-EA. Manual on the management, maintenance and use of blood cold chain equipment. WHO Reg Office South-East Asia 2005.
- [32] Bat T, Leitman SF, Calvo KR, Chauvet D, Dunbar CE. Measurement of the absolute immature platelet number reflects marrow production and is not impacted by platelet transfusion. *Transfusion* 2013;53(6):1201–4.
- [33] Bussel JB, de Miguel PG, Despotovic JM, et al. Eltrombopag for the treatment of children with persistent and chronic immune thrombocytopenia (PETIT): a randomised, multicentre, placebo-controlled study. *Lancet Haematol* 2015;2(8):e315–25.
- [34] Szumilas M. Explaining odds ratios. *J Can Acad Child Adolesc Psychiatry* 2010;19(3):227–9.
- [35] Bussel JB, Kuter DJ, George JN, et al. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. *N Engl J Med* 2006;355(16):1672–81.
- [36] Bussel JB, Provan D, Shamsi T, et al. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet* 2009;373(9664):641–8.
- [37] Jenkins JM, Williams D, Deng Y, et al. Phase 1 clinical study of eltrombopag, an oral, nonpeptide thrombopoietin receptor agonist. *Blood* 2007;109(11):4739–41.
- [38] Machlus KR, Italiano Jr. JE. The incredible journey: from megakaryocyte development to platelet formation. *J Cell Biol* 2013;201(6):785–96.
- [39] Nishimura S, Nagasaki M, Kunishima S, et al. IL-1 α induces thrombopoiesis through megakaryocyte rupture in response to acute platelet needs. *J Cell Biol* 2015;209(3):453–66.
- [40] Stegner D, vanEeuwijk JMM, Angay O, et al. Thrombopoiesis is spatially regulated by the bone marrow vasculature. *Nat Commun* 2017;8(1):127.
- [41] Zufferey A, Kapur R, Semple JW. Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). *J Clin Med* 2017;6(2).
- [42] McMillan R, Wang L, Tomer A, Nichol J, Pistillo J. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. *Blood* 2004;103(4):1364–9.
- [43] Noisakran S, Onlamoon N, Pattanapanyasat K, et al. Role of CD61+ cells in thrombocytopenia of dengue patients. *Int J Hematol* 2012;96(5):600–10.
- [44] Muronoi T, Koyama K, Nunomiya S, et al. Immature platelet fraction predicts coagulopathy-related platelet consumption and mortality in patients with sepsis. *Thromb Res* 2016;144:169–75.
- [45] Ojha A, Nandi D, Batra H, et al. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Sci Rep* 2017;7:41697.
- [46] Soranzo N, Spector TD, Mangino M, et al. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. *Nat Genet* 2009;41(11):1182–90.
- [47] Samanta J, Sharma V. Dengue and its effects on liver. *World J Clin Cases* 2015;3(2):125–31.