

Effects of Short-Course Oral Corticosteroid Therapy in Early Dengue Infection in Vietnamese Patients: A Randomized, Placebo-Controlled Trial

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(See the Editorial Commentary by Barrett on pages 1225–6.)

Background. Patients with dengue can experience a variety of serious complications including hypovolemic shock, thrombocytopenia, and bleeding. These problems occur as plasma viremia is resolving and are thought to be immunologically mediated. Early corticosteroid therapy may prevent the development of such complications but could also prolong viral clearance.

Methods. We performed a randomized, placebo-controlled, blinded trial of low-dose (0.5 mg/kg) or high-dose (2 mg/kg) oral prednisolone therapy for 3 days in Vietnamese patients aged 5–20 years admitted with dengue and fever for ≤ 72 hours, aiming to assess potential harms from steroid use during the viremic phase. Intention-to-treat analysis was performed using linear trend tests with a range of clinical and virological endpoints specified in advance. In addition to recognized complications of dengue, we focused on the area under the curve for serial plasma viremia measurements and the number of days after enrollment to negative viremia and dengue nonstructural protein 1 status.

Results. Between August 2009 and January 2011, 225 participants were randomized to 1 of the 3 treatment arms. Baseline characteristics were similar across the groups. All patients recovered fully and adverse events were infrequent. Aside from a trend toward hyperglycemia in the steroid recipients, we found no association between treatment allocation and any of the predefined clinical, hematological, or virological endpoints.

Conclusions. Use of oral prednisolone during the early acute phase of dengue infection was not associated with prolongation of viremia or other adverse effects. Although not powered to assess efficacy, we found no reduction in the development of shock or other recognized complications of dengue virus infection in this study.

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Dengue is the most common vector-borne viral infection of humans, with around 50 million infections estimated to occur annually and some 2.5 billion people living in areas of risk [1]. A broad spectrum of disease manifestations is seen, ranging from asymptomatic infection to a systemic plasma leakage syndrome typically accompanied by thrombocytopenia and coagulation derangements. Severe plasma leakage may progress to life-threatening dengue shock syndrome (DSS). Globally the burden of severe disease falls primarily on children, and dengue ranks among the

leading causes of hospitalization and death for pediatric populations in Asia [1]. Currently neither vaccines nor effective antiviral agents are available and treatment remains supportive with particular emphasis on careful fluid management during the period of plasma leakage [2].

Relatively little is known about the specific mechanisms responsible for the microvascular dysfunction that results in plasma leakage. Although occasionally reported during primary infections, complications such as DSS are strongly associated with sequential or secondary infections [3–5]. Antibody-dependent enhancement resulting in increased viral replication is one factor thought to underlie this phenomenon [6–8], although a variety of other mechanisms likely also contribute to the final disease phenotype [9–13]. Severe complications rarely develop until 4–5 days after fever onset, at a time when intrinsic viral clearance mechanisms have reduced measurable plasma viremia to low levels [14], suggesting that immunopathogenic responses triggered by the virus are crucial [8, 15]. Although no specific pathway has been identified linking such immunopathogenic events with definitive effects on the microvasculature, preliminary evidence points to transient disruption of the surface glycocalyx, a matrix of proteoglycans and glycosaminoglycans lining the vascular endothelium that regulates microvascular filtration [16–18].

Corticosteroids are highly effective anti-inflammatory agents, frequently employed as adjunctive therapy in disease states where the host immune response is thought to contribute significantly to disease pathogenesis. In the 1980s parenteral steroids were used to treat patients with DSS in several small studies, but without evidence of benefit [19–21]. However, for established shock, recent research indicates that steroid use is only helpful in patients with inadequate cortisol responses [22, 23]. Among patients with less severe sepsis there is some evidence that early administration of corticosteroids improves outcome and/or reduces the need for hospitalization [24, 25]. There is also a growing body of work indicating that steroids modulate the function of the endothelial glycocalyx and may prevent damage to this layer [26, 27]. Extensive experience has been gained using short-course oral steroid therapy to suppress the inflammatory response associated with acute asthma, and children now commonly receive 1–2 mg/kg oral prednisolone for 3 days at the onset of an exacerbation [28–30]. Side effects are rarely reported, although there have been concerns regarding behavioral disturbance with 2 mg/kg doses, and the trend is now toward using lower doses [31–33].

Because DSS occurs around the fifth day of illness, intervention with steroids early in infection may prevent or ameliorate the severity of this serious complication. Preliminary evidence indicates that corticosteroids do not increase dengue viral replication in cell culture (M. Hibberd, PhD, Genome Institute of Singapore), and no important adverse effects were reported in

the original studies of steroid therapy for DSS. However, before considering a large-scale efficacy trial we wished to assess whether immunomodulation during the viremic phase interferes with viral clearance mechanisms. Thus the primary objective of this study was to assess the safety of short-course oral corticosteroid therapy used early in the evolution of acute dengue infection. In addition we investigated potential dose-response effects of corticosteroids in relation to the clinical and virological safety parameters examined.

METHODS

We performed a randomized, placebo-controlled, partially blinded trial of early corticosteroid therapy in Vietnamese children and young adults with suspected dengue virus infection. The study took place on designated infectious diseases wards at the Hospital for Tropical Diseases of Ho Chi Minh City, with approval from the Ethical Committee of the Ministry of Health of Vietnam and the Oxford Tropical Research Ethics Committee.

Study Population, Patient Enrollment, and Study Procedures

Patients aged 5–20 years admitted to the Hospital for Tropical Diseases with clinically suspected dengue, supported by a positive rapid test for dengue nonstructural protein 1 (NS1 Ag-STRIP, Bio-Rad), and fever for ≤ 72 hours were eligible for enrollment provided the patient or their parent/guardian gave written informed consent and children 12–17 years gave assent. The following patients were excluded: those weighing < 20 kg; those with evidence for any dengue-related complications, symptoms suggesting another infectious disease, or a past history of serious illness or chronic disease including psychiatric/behavioral problems; and those taking regular medications. A negative pregnancy test was required for all postpubertal female participants.

A structured case report form was completed to document the history and detailed examination findings. Sequential participants were randomly allocated to receive low-dose (0.5 mg/kg) or high-dose (2 mg/kg) regimens of oral prednisolone or placebo once daily for 3 days using a 1:1:1 allocation. We did not blind the dose allocation, but the placebo group was additionally 1:1 randomized to low- or high-dose placebo to maintain blinding of the drug. The first dose was administered with food immediately after enrollment, with subsequent doses given each morning to minimize disturbance to the normal diurnal rhythm. All study drug treatments were directly observed by study staff. Once a patient was enrolled, all doses were given unless the study physician considered there to be a contraindication to corticosteroid therapy or the patient or family withdrew consent.

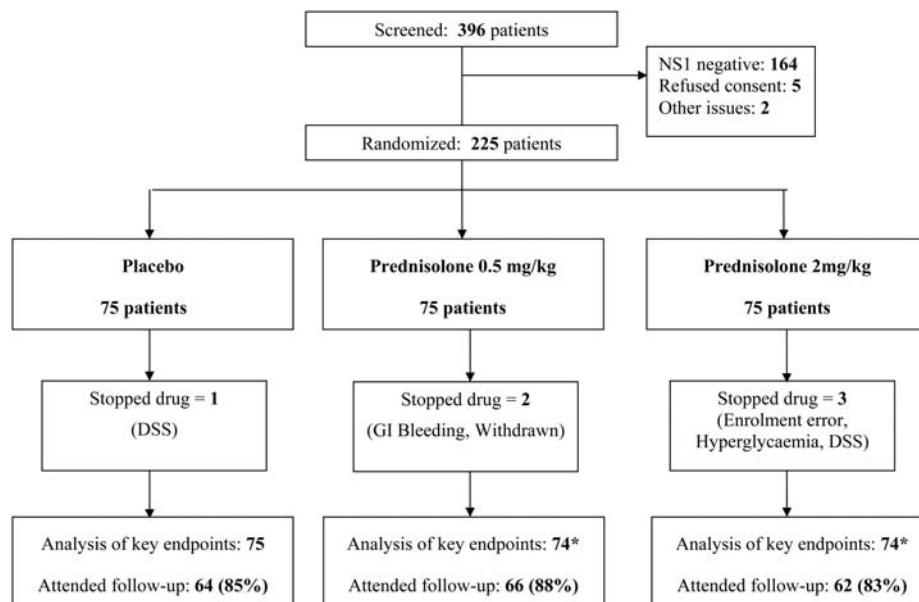


Figure 1. Trial profile. The patient withdrawn within the first 24 hours in the low-dose prednisolone group and the patient enrolled in error in the high-dose prednisolone group were excluded from the analysis of key endpoints. Abbreviations: DSS, dengue shock syndrome; GI, gastrointestinal.

Randomization and Blinding

Block randomization was performed using a block length of 9. Participants were allotted the next consecutive study number and the study pharmacist was informed of the participant's weight. The dose was calculated in 10-kg weight bands up to a maximum daily dose of 60 mg. Identical prednisolone and placebo were available in 25-mg and 5-mg tablets; according to the participant's weight and the next randomization, the pharmacist calculated the total dose and number of each tablet to be given and made up a blind treatment pack containing sufficient tablets for 3 doses. An emergency pack was also provided containing 1 further dose of study drug in case of vomiting. All participants and study staff aside from the pharmacist were blind to the treatment allocation.

Clinical and Laboratory Evaluation

Standardized clinical information was recorded daily throughout the disease course by trained study physicians. A full blood count and random glucose level were checked daily, with a fasting glucose performed if the random level was high. Biochemistry and coagulation profiles were carried out at enrollment, on day 5–6 of illness (the critical period for complications), and at follow-up 2–3 weeks after discharge. Heparan sulfate (HS) levels were measured at the same time points using commercial enzyme-linked immunosorbent assay (ELISA) kits (Seikagaku, Japan). An ultrasound scan was performed on day 6 to assess plasma leakage.

Clinical management decisions were made by the attending ward doctors. If shock or other serious complications developed, the patient was transferred to the intensive care unit (ICU)

and managed according to standard hospital guidelines. Details of all adverse events, and their severity and likely relatedness to the study medication, were recorded on standardized forms by the study physicians and reported regularly to the Data and Safety Monitoring Board (DSMB).

Dengue Diagnostics

Capture immunoglobulin M (IgM) and immunoglobulin G (IgG) ELISA assays were performed using paired specimens and reagents provided by Venture Technologies (Sarawak, Malaysia) [34]. An indirect ELISA to pooled recombinant E proteins of the 4 dengue virus (DENV) serotypes (Hawaii Biotech, Hawaii) was also performed on the enrollment specimen [14]. A negative indirect ELISA within the first 72 hours with no rise in dengue-reactive IgG by day 7 defined a primary infection, while a positive indirect ELISA within 72 hours and/or an equivocal response, with a clear rise in dengue-reactive IgG greater than the IgM response by day 7, defined a secondary infection.

DENV plasma viremia levels were measured using an internally controlled, serotype-specific, real-time reverse-transcription polymerase chain reaction (RT-PCR) assay, with serial samples from each patient assayed at the same time [35]. Qualitative soluble NS1 assays were performed using Bio-Rad Platelia Dengue NS1 Antigen kits following the manufacturer's instructions.

Endpoints and Statistical Methods

This was an exploratory study focusing on potential adverse effects. After satisfactory review of the first 50 patients'

Table 1. Baseline Characteristics According to Treatment Allocation

	Placebo (n = 75)	Low-Dose Prednisolone (n = 75)	High-Dose Prednisolone (n = 75)	All Patients (N = 225)
A. Demographic data and virological information. Plasma viremia values were not available for the patient who withdrew or the patient incorrectly enrolled.				
Age, years	13 (12–15)	12 (11–14)	12 (10–14)	13 (11–14)
Sex, male	56 (75)	54 (72)	51 (68)	161 (72)
Temperature, °C	38.8 (38.5–39.4)	39.0 (38.6–39.6)	38.9 (38.5–39.4)	39.0 (38.5–39.5)
Day of illness at enrollment				
1	0 (0)	1 (1)	1 (1)	2 (1)
2	28 (37)	23(31)	29 (39)	80 (34)
3	47 (63)	51(68)	45 (60)	143 (64)
Serotype				
DENV-1	41 (55)	46 (61)	49 (65)	136 (60)
DENV-2	29 (39)	17 (23)	11 (15)	57 (25)
DENV-3	4 (5)	9 (12)	10 (13)	23 (10)
DENV-4	1 (1)	2 (3)	4 (5)	7 (3)
Unknown	0 (0)	1 (1)	1 (1)	2 (1)
Immune status				
Primary infection	22 (29)	21 (28)	25 (33)	68 (30)
Secondary infection	43 (57)	39 (52)	35 (47)	117 (52)
Unknown	10 (13)	15 (20)	15 (20)	40 (18)
Plasma viremia, log ₁₀ copies/mL	n = 75; 8.81 (7.98–9.23)	n = 74; 8.77 (8.23–9.47)	n = 74; 8.96 (8.08–9.57)	N = 223; 8.81 (8.05–9.39)
B. Hematology and biochemistry profiles for all subjects. Data were missing for no more than 3 subjects for all laboratory parameters except coagulation screening tests. Coagulation results were only accepted for samples without hemolysis or clot formation that were separated and frozen within 6 hours; for these tests, data were missing in up to 7 subjects.				
Hematocrit, %	40 (39–43)	39 (37–41)	39 (37–42)	39 (38–42)
Platelet count, 10 ⁹ /L	143 (108–172)	140 (100–186)	143 (113–201)	142 (107–188)
WBC, 10 ⁹ /dL	3.8 (2.7–5.0)	3.7 (3.0–5.4)	4.2 (3.0–5.2)	3.7 (2.9–5.2)
Neutrophils, %	67 (59–75)	68 (58–75)	70 (59–79)	69 (59–76)
Lymphocytes, %	18 (13–26)	19 (13–26)	17 (11–29)	18 (12–27)
Hemoglobin, g/dL	13.6 (13.0–14.4)	13.2 (12.3–14.0)	13.1 (12.3–13.9)	13.4 (12.5–14.1)
Albumin, g/L	43.5 (41.7–45.0)	43.3 (42.0–45.3)	43.9 (42.0–45.3)	43.4 (41.9–45.3)
Total bilirubin, µmol/L	7 (6–11)	8 (5–10)	7 (6–9)	7 (6–10)
AST, IU/L	48 (34–81)	39 (30–55)	43 (31–74)	41 (31–65)
ALT, IU/L	22 (15–43)	21 (15–33)	21 (14–39)	21 (15–36)
Sodium, mmol/L	136 (134–138)	136 (134–138)	136 (134–138)	136 (134–138)
Potassium, mmol/L	3.6 (3.3–3.8)	3.5 (3.3–3.8)	3.6 (3.4–3.9)	3.6 (3.3–3.8)
Urea, mmol/L	4.0 (3.3–4.6)	4.3 (3.5–4.9)	4.4 (3.4–5.0)	4.2 (3.4–4.9)
Creatinine, µmol/L	67 (59–85)	66 (58–80)	65 (58–75)	66 (58–80)
Random blood sugar, mg/dL	106 (92–122)	100 (91–113)	103 (93–119)	103 (92–119)
Heparan sulfate, µg/mL	59 (39–101)	51 (34–77)	48 (32–82)	51 (35–88)
PT, sec	15.4 (14.4–16.7)	15.7(14.2–16.9)	15.3 (14.2–17.2)	15.4 (14.3–16.9)
APTT, sec	37.2 (34.4–40.8)	36.5 (33.8–40.2)	37.6 (34.0–41.7)	37.1 (34.0–40.7)
Fibrinogen, g/L	3.2 (2.6–4.0)	3.1 (2.2–3.7)	3.0 (2.2–4.0)	3.1 (2.4–3.9)

Data are presented as No. (%) for categorical variables and median (interquartile range) for continuous variables.

Abbreviations: ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; DENV, dengue virus; PT, prothrombin time; WBC, white blood cell count.

data by the independent DSMB, we proceeded with enrollment as planned to 75 patients in each study arm.

The following clinical endpoints of interest were specified in advance: development of DSS or need for ICU admission;

clinical bleeding; hyperglycemia; the platelet nadir; the maximum hematocrit between day 3 and day 8 of illness; the percentage increase of the maximum hematocrit from baseline between day 3 and day 8 (a measure of the severity of plasma

Table 2. Summary of Adverse Events in the 3 Treatment Groups

Adverse Events	Placebo (n = 75)	Low-Dose Prednisolone (n = 75)	High-Dose Prednisolone (n = 75)
Any adverse events			
Patients with any adverse event	22 (29)	16 (21)	23 (31)
Total No. of adverse events	26	24	34
Any serious adverse event			
Patients with any serious event	10 (13)	6 (8)	14 (18)
Total No. of serious events	11	7	17
Common dengue-related events			
Dengue shock syndrome ^c	5 (7)	5 (7)	8 (11)
Severe thrombocytopenia ^c	2 (3)	1 (1)	5 (7)
Significant bleeding	1 (1) ^a	3 (4)	1 (1) ^a
Severe abdominal pain	0 (0)	0 (0)	2 (3)
Increased liver enzymes	10 (13) ^a	7 (9)	6 (8)
Possible drug-related events			
Hyperglycemia (any)	3 (4)	5 (7)	9 (12) ^b
Hypertension ^c	1 (1)	0 (0)	0 (0)
Pneumonia ^c	1 (1)	1 (1)	0 (0)
Upper respiratory infection	1 (1)	2 (3)	2 (3)
Other reported events			
Urticaria	1 (1)	0 (0)	0 (0)
Diarrhea	1 (1)	0 (0)	0 (0)
Febrile convulsion ^c	0 (0)	0 (0)	1 (1)

All data are presented as No. (%). One patient each in the low-dose and high-dose prednisolone groups was withdrawn within 24 hours of treatment allocation, but no adverse events were recorded during the period of observation.

Definitions for selected adverse events (AEs) or serious adverse events (SAEs): Dengue shock syndrome was defined according to the World Health Organization 2009 guidelines.

Severe thrombocytopenia: platelet nadir <10 000/ μ L = SAE. Increased liver enzymes: aspartate aminotransferase or alanine aminotransferase \geq 300 IU/L = AE and \geq 1000 = SAE. Hyperglycemia: random blood sugar \geq 160 mg/dL = AE, and true fasting sugar \geq 120 mg/dL = SAE.

^a Includes 1 SAE.

^b Includes 2 SAEs.

^c All considered SAEs.

leakage); and the proportion of patients experiencing any adverse event or serious adverse event. The following virological parameters were also defined a priori for formal comparisons: the area under the curve (AUC) for log-transformed serial measurements of plasma viremia obtained during days 3–6 of illness; the number of days from enrollment until the RT-PCR first became negative; and the number of days from enrollment to negative NS1 status.

Analysis was by intention-to-treat but excluded 2 patients for whom endpoints could not be evaluated. The primary comparison between the 3 groups was a linear trend test based on linear regression for continuous endpoints, logistic regression for binary endpoints, and Cox regression for time-to-event endpoints. Comparisons for continuous laboratory parameters were adjusted for day of illness at enrollment and the enrollment value of the parameter. Comparisons of viremia and NS1 were also adjusted for serotype and immune status, factors known to influence these variables. All analyses were

performed with the statistical software R v2.13.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of 396 patients screened for eligibility between August 2009 and January 2011, 225 were enrolled and randomized to 1 of the 3 treatment arms (Figure 1). One patient was enrolled in error and another withdrew within 24 hours; no adverse events occurred in either patient while enrolled in the study. In 4 additional patients the study physician elected to stop the study drug because of complications (DSS in 2, gastrointestinal bleeding in 1, hyperglycemia in 1) but did not unblind the treatment allocation. All other patients completed their treatment course correctly and in only 1 instance was repeat drug administration necessary following vomiting.

Baseline characteristics were similar between the 3 treatment groups (Table 1). Considerably more boys than girls were

Table 3. Comparison of Predefined Clinical and Virological Endpoints Between the Treatment Groups

Endpoint	Placebo (n = 75)	Low-Dose Prednisolone (n = 74)	High-Dose Prednisolone (n = 74)	Overall Comparison (<i>P</i> Value)
DSS	5 (7)	5 (7)	8 (11)	.36
ICU admission	8 (11)	6 (8)	8 (11)	.98
Bleeding (any)	58 (77)	52 (70)	54 (73)	.54
Hyperglycemia (random)	3 (4)	5 (7)	9 (12)	.07
Platelet nadir days 3–8, 10 ⁹ /L	51 (25–83)	59 (33–89)	60 (29–93)	.36
Maximum hematocrit days 3–8, %	46 (42–49)	45 (42–47)	45 (42–48)	1.00
Percentage hemoconcentration	15 (10–23)	16 (8–22)	17 (10–27)	.28
AUC log viremia days 3–6, log ₁₀ copies/mL	20.96 (16.97–23.25)	21.56 (17.99–24.18)	21.29 (18.85–23.79)	.76
Time to undetectable viremia				
No. with undetectable viremia, %	32 (43)	28 (38)	25 (34)	.94
Days from enrollment, median (IQR)	7 (5–8)	7 (5–NE)	8 (5–8)	
Time to negative NS1				
No. with negative NS1, %	44 (59)	41 (55)	37 (50)	.08
Days from enrollment, median (IQR)	5 (3–8)	5 (3–11)	5 (3–NE)	
Patients with any adverse event	22 (29)	16 (22)	23 (31)	.81
Patients with any serious adverse event	10 (13)	6 (8)	14 (19)	.32

Data are presented as No. (%) for categorical variables, median (IQR) for continuous variables, and median (IQR) for time-to-event data estimated with the Kaplan-Meier method. Data were not available for the 2 patients withdrawn from the study.

Overall comparisons between the treatment groups correspond to linear trend tests. All comparisons for continuous laboratory parameters were adjusted for the day of illness at enrollment and the enrollment value of the parameter. Comparisons of viremia and NS1 were additionally adjusted for serotype and immune status.

Definitions are the same as for Table 2. Percentage hemoconcentration was defined as the percentage increase in hematocrit level comparing the maximum value between day 3 and day 8 of illness to a baseline value taken as the lowest result obtained on or before illness day 2 or after day 14 (provided the platelet count on the same sample was $\geq 200 \cdot 10^9/L$), or to a local population value matched for age and sex if no individual baseline was available (50 cases). Virological parameters are defined in the text.

Abbreviations: AUC, area under the curve; DSS, dengue shock syndrome; ICU, intensive care unit; IQR, interquartile range; NE, not estimable.

recruited, consistent with the generally observed male-to-female ratio among admissions to the pediatric ward. Dengue infection was confirmed in all subjects aside from the patient who withdrew (PCR not done) and the patient who was enrolled in error. Most infections were with DENV type 1 or DENV type 2, and approximately 30% were primary in each group.

Adverse events are reported in Table 2, with analysis of the predefined clinical and virological endpoints presented in Table 3. All patients recovered fully, although 22 required admission to the ICU, distributed across all treatment groups. DSS developed in 18 cases (all secondary infections). Three patients (2 primary and 1 secondary infection) were admitted to the ICU for close observation but did not develop overt DSS. One child with epistaxis (secondary infection) was transferred to the ICU but did not require intervention. Approximately 70% of patients across all treatment groups experienced some form of bleeding, in most cases minor petechiae or skin bruising, or mild to moderate mucosal bleeding. Additional to the child with epistaxis, 4 patients experienced significant bleeding: 2 patients with DSS, 1 patient with gross hematuria, and 1 patient with isolated upper gastrointestinal bleeding. Only 1 patient needed transfusion, a child with profound DSS who eventually made a good recovery.

Liver enzyme abnormalities were relatively common but no patient developed jaundice or reported specific symptoms; in 1 patient the aspartate aminotransferase (AST) level increased to >1000 IU/L, but he remained stable and at follow-up liver function was entirely normal.

We found no clear associations between treatment allocation and any of the predefined clinical endpoints. Although the association between steroid use and dysglycemia was not statistically significant ($P = .07$, trend test), raised random sugar levels were more frequent in the high-dose prednisolone group, and both cases with elevated fasting levels were in this group. Dysglycemia resolved after the study drug was stopped in one case, while in the other, investigation revealed an elevated HbA1c and a strong family history of diabetes. One child was clinically well but hypertensive for several weeks after discharge (placebo group). Two children developed radiologically confirmed pneumonia and 5 developed upper respiratory tract infections shortly after discharge; these superinfections were distributed across the treatment groups and likely reflect exposure of patients to other pathogens while in hospital. No instances of behavioral disturbance were identified through the daily assessments.

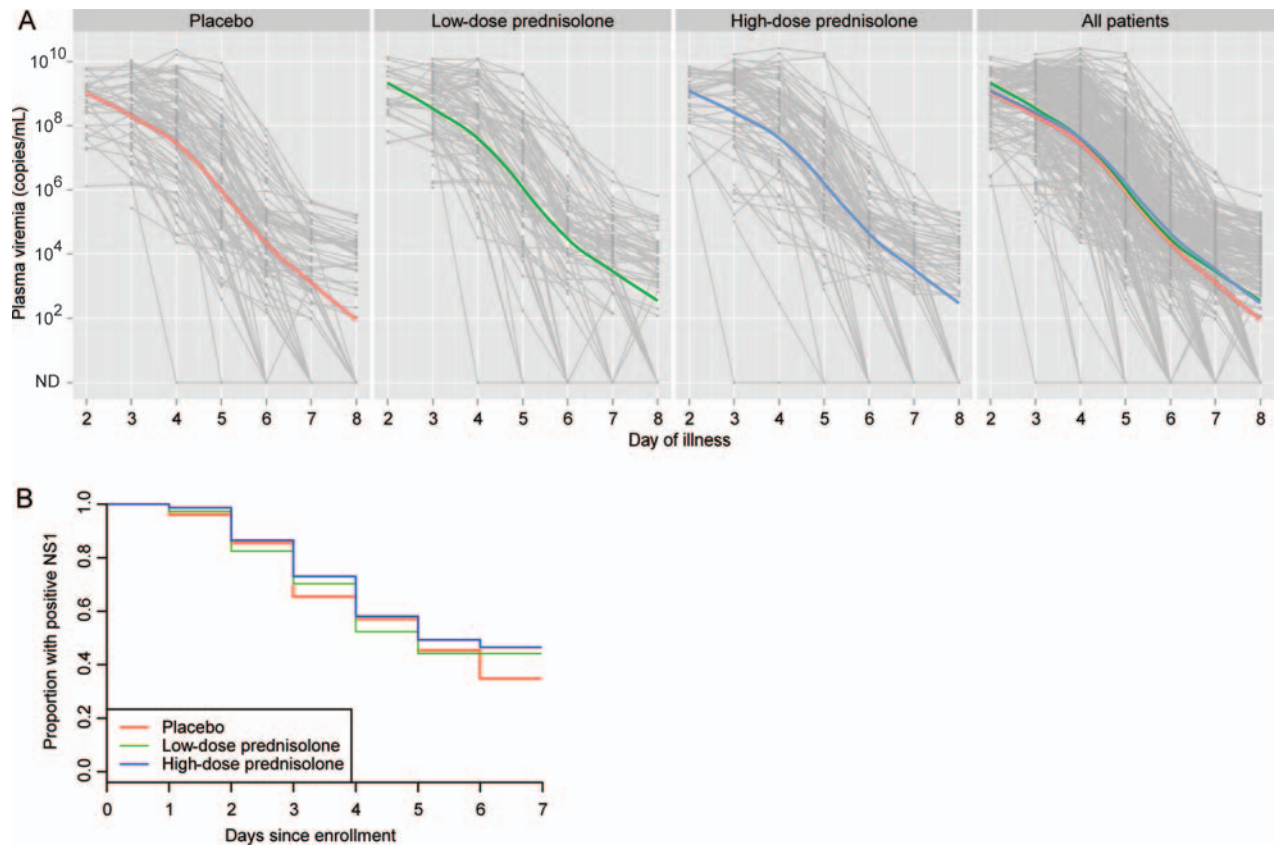


Figure 2. A, Dengue viremia kinetics for all serotypes by day of illness, in the 3 treatment arms separately and finally with all data combined. Gray lines represent individual patient data, and the colored lines correspond to Loess scatterplot smoothers. B, Kaplan-Meier plot indicating the proportion of patients remaining positive for NS1 in the 3 treatment groups, by day since study enrollment.

We also found no effect of corticosteroid therapy on the virological or hematological parameters of interest (Table 3, Figure 2). In particular, there was no evidence of significantly increased or prolonged viremia with prednisolone use, nor was there evidence of a beneficial effect on thrombocytopenia or percentage hemoconcentration. NS1 antigenemia was relatively prolonged and many patients remained positive at

discharge. Fever clearance times were similar across the treatment arms (median [interquartile range]: 5 [4–6], 4 [4–6], and 4 [4–6] days in the placebo, low-dose, and high-dose steroid groups, respectively).

Several exploratory analyses that were not part of the predefined analytical plan are presented in Table 4. Prednisolone use was significantly associated with less marked derangements in

Table 4. Comparison of Additional Selected Laboratory Parameters Between the 3 Treatment Arms

Laboratory Parameter	Placebo (n = 75)	Low-Dose Prednisolone (n = 74)	High-Dose Prednisolone (n = 74)	Overall Comparison (P Value)
Lowest recorded WBC, 10 ⁶ /dL	2.1 (1.6–2.5)	2.4 (1.9–2.7)	2.6 (2.2–3.3)	<.0001
AST, day 5–6 value, IU/L	128 (82–182)	84 (66–139)	85 (62–157)	.01
ALT, day 5–6 value, IU/L	63 (39–98)	43 (30–73)	45 (30–91)	.15
Heparan sulfate, day 5–6 value, µg/mL	146 (87–209)	102 (53–170)	108 (54–193)	.09

All data are presented as median (interquartile range). Data were not available for the 2 patients withdrawn from the study.

Overall comparisons between the treatment groups correspond to linear trend tests. All comparisons for continuous laboratory parameters were adjusted for the day of illness at enrollment and the enrollment value of the parameter. Comparisons of heparan sulfate concentrations were additionally adjusted for serotype and immune status.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell count.

total white blood count and AST levels during the illness course (trend tests $P < .001$ and $P = .01$, respectively), although there was no apparent effect on alanine aminotransferase levels. Observed HS levels increased less in steroid-treated patients during the critical period for complications, although the trend test across the 3 groups was not significant ($P = .09$). Other indices, including coagulation tests and plasma albumin levels, demonstrated similar profiles over time in the 3 treatment arms, consistent with the natural evolution of the illness and without any detectable steroid effect (data not shown).

DISCUSSION

In this randomized controlled trial, apart from transient hyperglycemia in a small number of cases, use of oral prednisolone for 3 days during the early acute phase of dengue infection was not associated with significant clinical or virological adverse effects. We also found no evidence of an effect on the subsequent development of recognized complications of dengue virus infection. DSS, significant bleeding, thrombocytopenia, and coagulopathy occurred with similar frequencies across all the groups and at the expected rate for children with dengue in our setting. Although no intervention was required, the incidence of hyperglycemia in the high-dose group is of concern and indicates that glucose monitoring is important even for short-course prednisolone therapy at 2 mg/kg/day. Otherwise, the treatment was generally well tolerated. No behavioral disturbances were reported even in the high-dose treatment group; however, we did not perform formal behavioral testing and Asian parents may be reluctant to disclose minor concerns for cultural reasons.

The trial was focused on children, the group most at risk for DSS, and we did not include a pharmacokinetic assessment. We also chose an oral formulation because possible future large-scale efficacy studies are likely to be community-based, aiming to target the population at risk as early as possible. Thus one limitation of the study is that we cannot be sure that we achieved therapeutic drug concentrations. However signals such as the observed changes in total white blood cell count, AST, and blood glucose—all recognized responses to steroid therapy—suggest that reasonable drug levels were achieved. Another caveat is that the study was hospital based and the participants may have been sicker than their community counterparts; in general, however, the threshold for hospitalization of suspected dengue cases is low in Vietnam. It is possible that higher steroid dosage or longer duration of therapy might have influenced disease progression, but given the dysglycemia observed it is unlikely that such regimens would be considered safe for use in future community-based efficacy studies.

Although the trial was primarily designed to assess safety, we were unable to detect any reduction in the severity of vascular

leakage or other recognized complications of dengue. Taking DSS as the endpoint, these data indicate a relative risk for high-dose prednisolone vs placebo of 1.62 (95% confidence interval, .57–5.16). Thus, although it remains possible that high-dose steroids could reduce the risk of shock by up to 43%, a large trial powered to assess efficacy does not seem justified on this evidence. The absence of adverse effects of early immunomodulation on virological safety parameters is reassuring but underscores how little we understand the mechanisms responsible for the microvascular complications. Of note, however, two-thirds of all participants were enrolled on day 3 of illness and it may be that both the viral clearance mechanisms and the pathological process involving the microvasculature are so well established by this time that immunomodulation has little effect. Detailed studies will be published elsewhere, comparing a broad range of immunological parameters across the 3 treatment arms aimed at identifying true correlates of disease and elucidating the pathogenesis of the vasculopathy more clearly.

HS levels were raised at enrollment but increased less in the steroid-treated patients, although not reaching statistical significance. Disruption of the HS fraction of the endothelial glycocalyx is emerging as a plausible mechanism contributing to dengue vasculopathy, potentially via direct viral or NS1-mediated pathways [36, 37]. Alterations to the glycocalyx layer in the coronary microcirculation appear to contribute to myocardial ischemia and reperfusion injuries, and there is preliminary evidence that postischemic shedding of HS is inhibited by hydrocortisone with preservation of glycocalyx architecture [26]. Further study of the kinetics of HS during dengue infections, and of relationships with vascular leakage and bleeding severity, are in progress.

In conclusion we found that use of oral prednisolone during the acute stage of dengue infection was not associated with significant clinical or virological adverse effects, nor with a reduction in the incidence of recognized complications of dengue, although the study was not powered to assess efficacy. As the global burden of dengue increases, there is great interest in developing novel therapeutic strategies to improve outcome, focused on agents that target the virus and/or the immune response. This and other recent intervention studies suggest that any potential therapy will likely need to be initiated very early in the disease evolution [38]. Ongoing research efforts directed toward improving dengue rapid diagnostics and identifying robust early predictors for severe disease are crucial to ensure that if a simple, safe, and effective therapy does become available, prompt intervention targeted toward high-risk groups is a realistic possibility in dengue-endemic areas.

Notes

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Potential conflicts of interest C. P. S. performs consulting for Unither Virology LLC. Payments for these consulting services go to his institution (Oxford University). All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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