Title	Severe Neutropenia in dengue patients : prevalence and significance(Main article)
Author(s)	Thein, Tun-Linn; Lye, David C.; Leo, Yee-Sin; Wong, Joshua G. X.; Hao, Ying; Wilder-Smith, Annelies
Citation	Thein, T.-L., Lye, D. C., Leo, Y.-S., Wong, J. G. X., Hao, Y., & Wilder-Smith, A. (2014). Severe Neutropenia in dengue patients : prevalence and significance. American journal of tropical medicine and hygiene, 90(6), 984-987.
Date	2014
URL	http://hdl.handle.net/10220/24593
Rights	© 2014 American Society of Tropical Medicine and Hygiene. This paper was published in American Journal of Tropical Medicine and Hygiene and is made available as an electronic reprint (preprint) with permission of American Society of Tropical Medicine and Hygiene. The paper can be found at the following official DOI: [http://dx.doi.org/10.4269/ajtmh.14-0004]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law.

Am. J. Trop. Med. Hyg., 90(6), 2014, pp. 984–987
doi:10.4269/ajtmh.14-0004

Short Report: Severe Neutropenia in Dengue Patients: Prevalence and Significance

Tun-Linn Thein,* David C. Lye, Yee-Sin Leo, Joshua G. X. Wong, Ying Hao, and Annelies Wilder-Smith

Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore; Yong Loo Lin School of Medicine, National University of Singapore, Singapore; Saw Swee Hock School of Public Health, National University of Singapore, Singapore; Lee Kong Chian School of Medicine, Singapore

Abstract. Studies on severe neutropenia in dengue are scarce, and its clinical significance is uncertain. We analyzed a cohort of 1,921 reverse transcription polymerase chain reaction-confirmed adult dengue patients admitted to the Communicable Disease Center in Singapore between 2005 and 2008. Time trend analyses for daily absolute neutrophil counts (ANCs) were done using Bayesian hierarchical and Markov models. We found that severe neutropenia, defined as ANC $\leq 0.5 \times 10^9$/L, was found in 11.8% with a median duration of 1 day. ANC nadir occurred on illness day 5. Severe neutropenia was not predictive of more severe disease and not associated with secondary bacterial infections, prolonged hospital stay, prolonged fever, or fatal outcome. We concluded that prophylactic antibiotics are not indicated in patients with severe neutropenia without indication for bacterial infection.

Dengue is a mosquito-borne viral disease of increasing global health importance that mainly affects populations in tropical and subtropical countries. In recent decades, dengue has also emerged as a major problem in international travelers,[1] and the need for more training on the clinical management of dengue for clinicians in Western countries is well-recognized.

Dengue is characterized by thrombocytopenia and leucopenia.[2] Neutropenia in dengue infections has also been reported, although less frequently.[3–5] Neutrophils are important to initiate and maintain an immune response leading to destruction of microorganisms.[6] In general, persons with severe neutropenia as defined as less than 0.5×10^9/L are at higher risk of secondary bacterial infections, and clinicians often institute antibiotics prophylactically for patients presenting with severe neutropenia. However, studies on severe neutropenia in dengue are scarce, and its clinical significance is uncertain. In particular, it is unknown whether dengue patients with severe neutropenia are more prone to secondary bacterial infections and whether prophylactic antibiotics would be justified.

Singapore is a developed city-state in Southeast Asia where dengue is endemic. Given its high standard of medical care and easy access to diagnostic facilities, Singapore offers opportunities to study large dengue cohorts and rare events. In this study, we aim to describe the prevalence of severe neutropenia and investigate its associations with secondary bacterial infections and dengue disease severity.

We retrospectively analyzed a large cohort of 1,921 adult patients with dengue admitted to the Communicable Diseases Center in Singapore between 2005 and 2008. Only dengue cases confirmed by polymerase chain reaction (PCR) were included in the analysis. The study was approved by the National Healthcare Group Domain Specific Review Board.

Demographic baseline data as well as daily clinical and laboratory data were collected. Comorbidities included any of diabetes mellitus, hypertension, heart failure, cardiac, lung, liver, or renal problems, cancer, or stroke.

*Address correspondence to Tun-Linn Thein, Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433. E-mail: linn_thein_tun@ttsh.com.sg

We defined severe, moderate, and mild neutropenia as absolute neutrophil count (ANC) $\leq 0.5 \times 10^9$/L, 0.5–1 $\times 10^9$/L, and 1–1.5 $\times 10^9$/L, respectively.[7] Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) were defined according to the 1997 World Health Organization (WHO) dengue case classification.[8] Nosocomial infections were diagnosed by infectious disease physicians based on compatible clinical and microbiological criteria.

For descriptive analyses, median, percentiles (pctls), number, and percentage were used. The Kruskal–Wallis test was used to assess statistical significance of continuous variables, and the Fisher's exact test was used for categorical variables. Clinically relevant variables were put into the multivariate logistic regression model to identify the factors associated with severe neutropenia or nosocomial infection. In addition, the Bayesian hierarchical model and the Markov model were applied to estimate the daily means of neutrophil counts for DHF and non-DHF patients, and Z tests were used to compare the daily means of neutrophil counts for DHF and non-DHF patients.[9] All statistical analyses were performed in R15.3 and WinBUGS14.

The demographic characteristics of 1,921 patients with PCR-confirmed dengue were median age of 35 years (5th–95th pctl = 17–61), 70.1% males, 73.7% Chinese ethnicity, and 17.6% with comorbidities. Median day of illness (since onset of fever) on admission was 4 days (5th–95th pctl = 2–6). Median length of stay (LOS) in the hospital was 5 days (5th–95th pctl = 3–8). DHF was diagnosed in 533 (27.8%) patients in our cohort. Nine patients were admitted to the intensive care unit (ICU), where one patient died. Antibiotic therapy was given to 158 (8.2%) patients (Table 1).

In total, 40 patients were diagnosed with nosocomial infections (18 patients with pneumonia, 12 patients with urinary tract infections, 9 patients with bacteremia, and 1 patient with both pneumonia and bacteremia). In multivariate analysis, DHF status and longer hospital LOS were significantly associated with nosocomial infections after adjusting for age, sex, and ethnicity (Table 2). Severe neutropenia was not a predictor for nosocomial infections.

During hospitalization, 1,579 (82.2%) patients had ANC $< 1.5 \times 10^9$/L. Severe, moderate, and mild neutropenia were noted in 227 (11.8%), 856 (44.6%), and 496 (25.8%) patients, respectively. Four patients had ANC $< 0.2 \times 10^9$/L,

TABLE 1
Patient characteristics and clinical outcomes stratified by ANCs during hospitalization

Variables	Severe (N = 227)	Moderate (N = 856)	Mild (N = 496)	Others (N = 342)	Total (N = 1,921)	P value*
Age, years	29 (16–57)	34 (17–59)	35 (19–60)	38 (21–64)	35 (17–61)	< 0.0001
Male	122 (53.74)	610 (71.26)	349 (70.36)	267 (78.07)	1,348 (70.17)	< 0.0001
Chinese	204 (89.87)	696 (81.31)	342 (68.95)	174 (50.88)	1,416 (73.71)	< 0.0001
Comorbidity	25 (11.01)	125 (14.60)	105 (21.17)	84 (24.56)	339 (17.64)	< 0.0001
Year of infection						0.14
2005	153 (67.40)	539 (62.97)	29 (5.85)	198 (57.89)	919 (47.83)	
2006	13 (5.73)	68 (7.94)	29 (5.85)	21 (6.14)	131 (6.82)	
2007	36 (15.86)	142 (16.59)	77 (15.52)	63 (18.42)	318 (16.55)	
2008	25 (11.01)	107 (12.50)	80 (16.13)	60 (17.54)	272 (14.15)	
Day of illness on admission	4 (2–6)	4 (2–6)	4 (2–6)	4 (2–6)	4 (2–6)	0.13
Min white cell count (10^9/L)	13 (4–30.7)	20 (10–39)	28 (14–48.25)	38 (24–71.9)	2.30 (1.30–5.10)	< 0.0001
Min lymphocyte count (10^9/L)	0.39 (0.15–0.80)	0.41 (0.16–0.9)	0.47 (0.18–1.08)	0.57 (0.22–1.6)	0.45 (0.16–1.10)	< 0.0001
Any nosocomial infection	2 (0.88)	12 (1.40)	11 (2.22)	15 (4.39)	40 (2.08)	0.006
Hospital LOS	5 (3–8)	5 (3–8)	5 (3–8)	5 (3–8)	5 (3–8)	0.63
Antibiotics received	17 (7.49)	57 (6.66)	39 (7.86)	45 (13.16)	158 (8.22)	0.002
ICU	0 (0)	4 (0.47)	3 (0.60)	2 (0.58)	9 (0.47)	na
Death	0 (0)	1 (0.12)	0 (0)	0 (0)	1 (0.12)	na

Values shown are median (5th–95th pctls) or number (%). Severe is ANC ≤ 0.5 × 10^9/L, moderate is ANC 0.5–1 × 10^9/L, mild is ANC 1–1.5 × 10^9/L, and others is ANC more than 1.5 × 10^9/L during hospitalization. Nosocomial infection is pneumonia, urinary tract infection, or any bacteremia. Comorbidities are defined as any of these diseases: diabetes mellitus, hypertension, heart failure, cardiac, lung, liver, or renal problems, cancer, or stroke. Min = minimum; na = not applicable.
* P values were calculated for trend between the groups of neutropenia.

whereas none had ANC < 0.1 × 10^9/L. The lowest ANC (nadir) occurred on illness day 5 (Figure 1). Median duration of severe neutropenia was 1 day. Among 227 patients with severe neutropenia, only 2 patients had documented nosocomial infections and 17 (7.5%) patients received antibiotic therapy, whereas zero patients were admitted to the ICU or died. In multivariate analysis, younger age, women, and Chinese ethnicity but not nosocomial infection were associated with severe neutropenia after adjusting for comorbidity and DHF (Table 3).

In terms of the temporal relationship of neutropenia and fever, ANC nadir was observed around the time of defervescence in 25% of patients on the day of defervescence, 19% of patients on the day after defervescence, and 17% of patients on the day before defervescence. Severe neutropenia was statistically non-different in those patients with fever versus those patients without fever at neutrophil nadir (P = 0.19) or prolonged fever (P = 0.301).

Figure 1 illustrates the time trend during hospitalization for ANC of the whole cohort and DHF versus non-DHF status. Time trend analyses revealed that daily ANC was similar for DHF and non-DHF.

In our cohort of hospitalized confirmed adult dengue inpatients, 82.2% of patients had some form of neutropenia, and 11.8% of patients had severe neutropenia. The neutrophil nadir was on day 4, and it coincided in 61% of the cases around the day of defervescence. Dengue-associated bone marrow suppression has been well-documented. A review

TABLE 2
Factors associated with nosocomial infection

Factors	No nosocomial infection (N = 1,881)	Nosocomial infection (N = 40)	Adjusted odds ratio	P value
Age, years	34 (17–61)	39 (18–70)	1.02 (0.98–1.05)	0.218
Female	558 (29.67)	15 (37.5)	1.59 (0.74–3.35)	0.228
Chinese	1,383 (73.53)	33 (82.5)	1.22 (0.51–3.36)	0.678
Comorbidity	332 (17.65)	7 (17.5)	0.44 (0.13–1.27)	0.157
DHF	505 (26.85)	28 (70)	4.43 (2.07–10.06)	< 0.001
LOS	5 (3–8)	8 (5–21)	1.52 (1.34–1.76)	< 0.001
Severe neutropenia	225 (11.96)	2 (5.0)	0.46 (0.07–1.6)	0.296

Values shown are median (5th–95th pctls) for continuous variables and n (%) for categorical variables. P values were calculated by logistic regression. Comorbidities are defined as any of these diseases: diabetes mellitus, hypertension, heart failure, cardiac, lung, liver, or renal problems, cancer, or stroke. DHF = dengue hemorrhagic fever; LOS = length of stay.

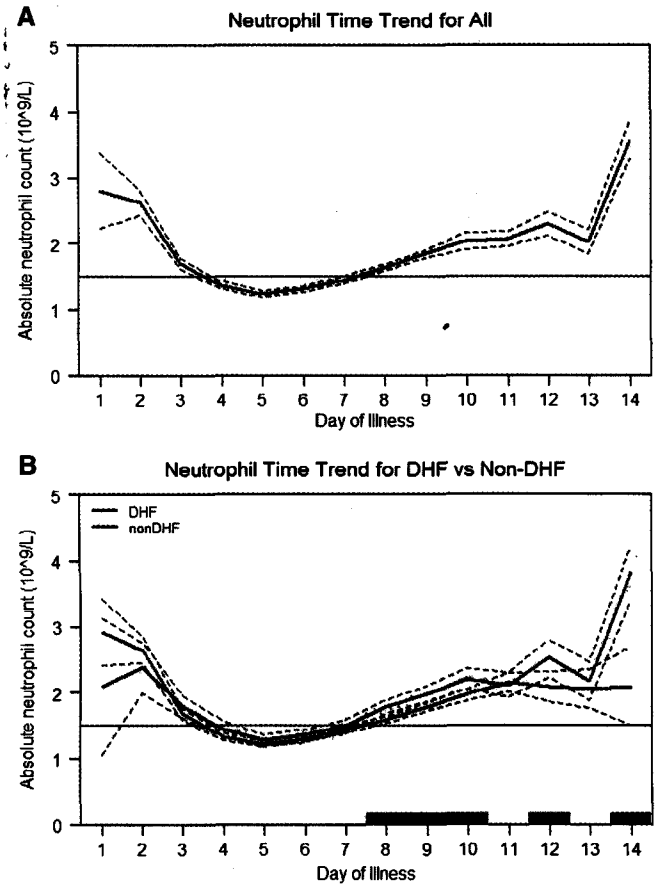

FIGURE 1. Time trend of absolute neutrophil count (ANC) for the (**A**) whole cohort and (**B**) DHF versus non-DHF. (Overall means are indicated as solid lines, with 95% credible intervals indicated as dashed lines. The red bar on the x axis indicates days with a significant difference between DHF and non-DHF by z test. The blue line indicates an ANC level of 1.5 × 10^9/L.)

TABLE 3
Factors associated with severe neutropenia

Factors	Non-severe neutropenia ($N = 1,694$)	Severe neutropenia ($N = 227$)	Adjusted odds ratio	P value
Age, years	35 (18–61)	29 (16–57)	0.96 (0.95–0.97)	< 0.001
Female	468 (27.63)	105 (46.25)	2.59 (1.93–3.49)	< 0.001
Chinese	1,212 (71.54)	204 (89.87)	3.97 (2.58–6.39)	< 0.001
Comorbidity	314 (18.54)	25 (11.01)	0.71 (0.44–1.11)	0.151
DHF	469 (27.69)	64 (28.19)	0.91 (0.66–1.25)	0.577

Values shown are median (5th–95th pctls) for continuous variables and *n* (%) for categorical variables. *P* values were calculated by logistic regression. Comorbidities are defined as any of these diseases: diabetes mellitus, hypertension, heart failure, cardiac, lung, liver, or renal problems, cancer, or stroke. DHF = dengue hemorrhagic fever.

of experimental dengue infections of volunteers and histopathological studies of bone marrow from patients with severe dengue virus infection suggests that marrow suppression evolves rapidly through several phases, with an onset of marrow suppression within 3–4 days of infection and occurrence of a neutrophil nadir on the fourth to fifth day after onset of dengue fever.[10]

Severe neutropenia in our study was not associated with more severe disease as measured by the incidence of DHF, prolonged hospitalization, and death. Failure of the neutrophil count to differentiate DSS from non-severe patients was also reported by Hoang and others[11] from Vietnam. A study from Thailand reported that primary dengue infection presented with significantly lower maximal percentage of neutrophils compared with secondary dengue infection.[12] However, Phan and others[13] reported a significant decrease in neutrophil counts, complement activity, and platelet counts in DHF/DSS patients.[13] Also, a study in Mexico in adult dengue patients found that neutropenia, prolonged partial thromboplastin time, and elevated transaminases were observed more often in DHF patients.[14]

Dengue patients with severe neutropenia did not have an increased risk for nosocomial infection or antibiotic use in our study. These findings suggest that severe neutropenia is not associated with an increased risk of secondary bacterial infections. This finding is also consistent with a study from Saudi Arabia, where 8.5% of hospitalized dengue patients had severe neutropenia, but none developed a secondary bacterial infection.[15] In our study, median duration of severe neutropenia was 1 day only. We postulate that the transient course of neutropenia is the main reason for the lack of risk of neutropenia-associated secondary bacterial infections.

No guidelines exist to give antibiotics to dengue patients presenting with severe neutropenia. Some studies suggest that antibiotics should be empirically used for patients with severe dengue,[16] but even those studies use the caveat that antibiotics should only be used for patients who are at risk for bacteremia and present with altered consciousness and leukocytosis. The evidence garnered from this large cohort study in Singapore suggests that antibiotics should not be given prophylactically on the basis of severe neutropenia alone.

In conclusion, in this large population of almost 2,000 hospitalized adult patients with laboratory-confirmed dengue, severe neutropenia was reported in 11.8% of patients, with a median duration of 1 day and a nadir on day 4 of illness. Severe neutropenia was not predictive for DHF or DSS. Severe neutropenia was not associated with secondary bacterial infections, prolonged hospital stay, prolonged fever, or fatal outcome. We conclude that prophylactic antibiotics are not indicated in patients with severe neutropenia who have otherwise no clinical or laboratory indication for bacterial infection.

Received January 2, 2014. Accepted for publication March 7, 2014.

Published online April 14, 2014.

Financial support: This work was supported by the STOP Dengue Translational Clinical Research Programme funded by the National Research Foundation through the National Medical Research Council, Singapore (Grant NMRC/TCR/005/2008).

Authors' addresses: Tun-Linn Thein, Joshua G. X. Wong, and Ying Hao, Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore, E-mails: linn_thein_tun@ttsh.com.sg, Joshua_Gx_Wong@ttsh.com.sg, and ying_hao@ttsh.com.sg. David C. Lye, Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore, and Yong Loo Lin School of Medicine, National University of Singapore, Singapore, E-mail: david_lye@ttsh.com.sg. Yee-Sin Leo, Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore, and Yong Loo Lin School of Medicine, National University of Singapore, Singapore, and Saw Swee Hock School of Public Health, National University of Singapore, Singapore, E-mail: yee_sin_leo@ttsh.com.sg. Annelies Wilder-Smith, Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore, and Lee Kong Chian School of Medicine, Singapore, E-mail: awilder-smith@ntu.edu.sg.

REFERENCES

1. Wilder-Smith A, Schwartz E, 2005. Dengue in travelers. *N Engl J Med 353:* 924–932.
2. Simmons CP, Farrar JJ, Nguyen vV, Wills B, 2012. Dengue. *N Engl J Med 366:* 1423–1432.
3. Ali N, Usman M, Syed N, Khurshid M, 2007. Haemorrhagic manifestations and utility of haematological parameters in dengue fever: a tertiary care centre experience at Karachi. *Scand J Infect Dis 39:* 1025–1028.
4. Wilder-Smith A, Earnest A, Paton NI, 2004. Use of simple laboratory features to distinguish the early stage of severe acute respiratory syndrome from dengue fever. *Clin Infect Dis 39:* 1818–1823.
5. Chadwick D, Arch B, Wilder-Smith A, Paton N, 2006. Distinguishing dengue fever from other infections on the basis of simple clinical and laboratory features: application of logistic regression analysis. *J Clin Virol 35:* 147–153.
6. Nathan C, 2006. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol 6:* 173–182.
7. Beers MH, Berkow R, 1999. *The Merck Manual of Diagnosis and Therapy*. Whitehouse Station, NJ: Merck Research Laboratories.
8. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*. Geneva: World Health Organization.
9. Lee VJ, Chow A, Zheng X, Carrasco LR, Cook AR, Lye DC, Ng LC, Leo YS, 2012. Simple clinical and laboratory predictors of chikungunya versus dengue infections in adults. *PLoS Negl Trop Dis 6:* e1786.
10. La Russa VF, Innis BL, 1995. Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol 8:* 249–270.
11. Hoang LT, Lynn DJ, Henn M, Birren BW, Lennon NJ, Le PT, Duong KT, Nguyen TT, Mai LN, Farrar JJ, Hibberd ML, Simmons CP, 2010. The early whole-blood transcriptional signature of dengue virus and features associated with progression to dengue shock syndrome in Vietnamese children and young adults. *J Virol 84:* 12982–12994.
12. Pancharoen C, Mekmullica J, Thisyakorn U, 2001. Primary dengue infection: what are the clinical distinctions from

secondary infection? *Southeast Asian J Trop Med Public Health 32:* 476–480.
13. Phan DT, Ha NT, Thuc LT, Diet NH, Phu LV, Ninh LY, An VT, 1991. Some changes in immunity and blood in relation to clinical states of dengue hemorrhagic fever patients in Vietnam. *Haematologia (Budap) 24:* 13–21.
14. Murillo-Llanes J, Soto-Valenzuela H, Flores-Flores P, Peraza-Garay F, 2007. Clinical and epidemiological characteristic of dengue. *Rev Med Inst Mex Seguro Soc 45:* 485–491.
15. Khan NA, Azhar EI, El-Fiky S, Madani HH, Abuljadial MA, Ashshi AM, Turkistani AM, Hamouh EA, 2008. Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. *Acta Trop 105:* 39–44.
16. Lee IK, Liu JW, Yang KD, 2012. Fatal dengue hemorrhagic fever in adults: emphasizing the evolutionary pre-fatal clinical and laboratory manifestations. *PLoS Negl Trop Dis 6:* e1532.