Journal of Microbiology, Immunology and Infection (2017) 50, 314—320

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Risk factors for concurrent bacteremia in adult patients with dengue

Tun-Linn Thein [a,*,d], Ee-Ling Ng [a,d], Ming S. Yeang [b], Yee-Sin Leo [a,b,c], David C. Lye [a,b]

[a] *Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Department of Infectious Diseases, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore*
[b] *Yong Loo Lin School of Medicine, National University of Singapore, 10 Medical Drive, Singapore 117597, Singapore*
[c] *Saw Swee Hock School of Public Health, National University of Singapore, 16 Medical Drive, Singapore 117597, Singapore*

Received 6 October 2014; received in revised form 13 May 2015; accepted 30 June 2015
Available online 4 August 2015

KEYWORDS
Adult;
Concurrent bacteremia;
Dengue;
Mortality;
Severity;
Singapore

Abstract *Background/Purpose:* Bacteremia in dengue may occur with common exposure to pathogens in association with severe organ impairment or severe dengue, which may result in death. Cohort studies identifying risk factors for concurrent bacteremia among patients with dengue are rare.
Methods: We conducted a retrospective case—control study of adult patients with dengue who were admitted to the Department of Infectious Diseases at Tan Tock Seng Hospital, Singapore from 2004 to 2008. For each case of dengue with concurrent bacteremia (within the first 72 hours of admission), we selected four controls without bacteremia, who were matched on year of infection and dengue confirmation method. Conditional logistic regression was performed to identify risk factors for concurrent bacteremia.
Results: Among 9,553 patients with dengue, 29 (0.3%) had bacteremia. Eighteen of these patients (62.1%) had concurrent bacteremia. The predominant bacteria were *Staphylococcus aureus*, one of which was a methicillin-resistant strain. Dengue shock syndrome occurred more frequently and hospital stay was longer among cases than among controls. Three cases did not survive, whereas none of the controls died. In multivariate analysis, being critically ill at

* Corresponding author. Communicable Disease Center, Institute of Infectious Diseases and Epidemiology, Department of Infectious Diseases, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore.
E-mail address: Linn_Thein_Tun@ttsh.com.sg (T.-L. Thein).
[d] These authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.jmii.2015.06.008

hospital presentation was independently associated with 15 times the likelihood of a patient with dengue having concurrent bacteremia.
Conclusion: Concurrent bacteremia in adult patients with dengue is uncommon but presents atypically and results in more deaths and longer hospital stay. Given the associated mortality, collection of blood cultures and empiric antibiotic therapy may be considered in patients who are critically ill.

Introduction

Dengue is an arbovirus-borne infection that is widespread in tropical and subtropical regions.[1] A recent model by Bhatt et al[2] estimated that 390 million infections occur annually, with 96 million of these infections being clinically apparent. Despite the high rate of infection worldwide, there is no effective vaccine against dengue.[3,4] Clinical manifestations of dengue range from an asymptomatic infection to a mild, flu-like, self-limiting infection.[5] In adults, the infection can less commonly develop into the more severe and life-threatening forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Close monitoring and timely administration of fluid therapy have improved outcomes from DHF and DSS globally.[5,6] More recently, the World Health Organization (WHO) proposed a new classification that better guides clinical management: dengue with warning signs, dengue without warning signs, and severe dengue.[5,7]

Only a few case series of concurrent bacteremia and dengue infection have been reported. Bacteremia in dengue may occur with common exposure to pathogens[8,9] in association with severe organ impairment[10] or severe dengue, resulting in death.[11–13] In addition, nosocomial infection may result from prolonged hospitalization for severe dengue and its complications.[11] Hypotheses on the pathogenesis of concurrent bacteremia in patients with dengue include disintegration of endothelial cells by antibodies against dengue nonstructural protein 1[14,15] and/or relative immunosuppression in patients with dengue.[11]

A recent cohort study was carried out by Lee et al[16] to identify the clinical characteristics and risk factors of patients with DHF and concurrent bacteremia. However, large cohort studies identifying risk factors for patients with dengue and concurrent bacteremia are rare. In this large cohort study of adult patients with dengue who were admitted to the Department of Infectious Diseases at Tan Tock Seng Hospital (TTSH), Singapore from 2004 to 2008, we aimed to (1) determine the prevalence of concurrent bacteremia in adult patients with dengue; (2) describe the clinical characteristics of adult patients with dengue and concurrent bacteremia; and (3) identify clinical and laboratory risk factors for concurrent bacteremia at the time of hospital presentation. This information would be clinically useful for doctors performing appropriate microbiological investigations of bacteremia and starting early empiric antibiotic therapy.

Methods

Patients

We conducted a retrospective study of laboratory-diagnosed dengue in adult patients who were admitted to the Department of Infectious Diseases at TTSH from January 1, 2004, to December 31, 2008.

Patients were diagnosed as having probable dengue based on the 1997 and 2009 WHO guidelines.[5,17] Dengue Real-time Reverse Transcription-Polymerase Chain Reaction, as described earlier,[18] or Panbio Rapid Dengue Duo Rapid Strip Test (Panbio Diagnostic, Queensland, Australia)[19,20] was performed at the Department of Laboratory Medicine, TTSH, according to the manufacturer's instructions, to confirm the diagnosis. Diagnosis of bacteremia was confirmed by a positive bacterial culture in the patient's blood sample obtained after admission to the hospital. In this study, "concurrent bacteremia" was defined as a positive bacterial blood culture within 72 hours of a patient's admission.[16] Blood culture was taken in the event of clinical deterioration despite treatment based on a standardized dengue care path.

For each case of dengue with concurrent bacteremia, we selected four controls among patients with dengue without clinical suspicion of bacteremia or without blood cultures collected or reported as negative by the Department of Laboratory Medicine, TTSH, matched on year of infection (a surrogate for predominant circulating dengue serotype) and dengue confirmation method. The selection generated all controls without collection of blood cultures. In brief, 29 patients had bacteremia from 2004 to 2008, among which 18 had blood samples collected for bacterial culture within 72 hours of admission. Although the other 11 patients yielded positive results for bacterial growth, blood samples were collected >72 hours after admission. Therefore, it was not possible to determine whether bacteremia was concurrent with dengue infection or was acquired nosocomially. An analysis identifying risk factors was carried out with 72 controls and 18 cases.

Demographics (age, sex, and ethnicity), comorbidities (diabetes mellitus, hypertension, hyperlipidemia, and cardiac diseases), dengue diagnosis classification, symptoms and signs at presentation (Table 1), parameters for laboratory investigation at presentation, types of intervention, dengue severity classification at hospital discharge, and clinical outcomes (Table 2) were extracted for comparison between cases and controls. The laboratory parameters

Table 1 Demographics, comorbidities, dengue diagnosis, and signs and symptoms of cases and controls at presentation[a]

Variable	Cases ($n = 18$)	Controls ($n = 72$)	p
Demographics			
Age (y)	38.5 (21–93)	36 (17–59.05)	NS
Male	11 (61.1)	50 (69.4)	NS
Chinese ethnicity	14 (77.8)	55 (76.4)	NS
Comorbidities			
Diabetes mellitus	1 (5.6)	4 (5.6)	NS
Hypertension	4 (22.2)	8 (11.1)	NS
Hyperlipidemia	3 (16.7)	6 (8.3)	NS
Cardiac disorder	2 (11.1)	3 (4.2)	NS
Charlson comorbidity index score > 3	1 (5.9)	1 (1.5)	NS
Dengue diagnosis			
Probable dengue 1997	11 (61.1)	66 (91.7)	0.005
Probable dengue 2009 with warning signs	15 (83.3)	54 (75.0)	NS
Signs & symptoms			
Fever	17 (94.4)	68 (94.4)	NS
Lethargy	7 (41.2)	22 (32.4)	NS
Bleeding gums	1 (5.6)	13 (18.1)	NS
Anorexia	10 (55.6)	47 (65.3)	NS
Nausea	7 (38.9)	39 (54.2)	NS
Vomiting	7 (38.9)	35 (48.6)	NS
Diarrhea	7 (38.9)	25 (34.7)	NS
Hemorrhagic manifestations[b]	4 (22.2)	37 (51.4)	0.032
Any rash	6 (33.3)	40 (55.6)	NS
Mucosal bleeding	2 (11.1)	19 (26.4)	NS
Gastrointestinal bleeding	0 (0)	3 (4.2)	NS
Severe bleeding[c]	1 (5.6)	4 (5.6)	NS
Petechiae	2 (11.8)	23 (33.8)	NS
Abdominal pain	8 (44.4)	27 (37.5)	NS
Headache	8 (44.4)	32 (44.4)	NS
Eye pain	1 (5.6)	1 (1.4)	NS
Myalgia	13 (72.2)	49 (68.1)	NS
Arthralgia	3 (16.7)	13 (18.1)	NS
Duration of fever at presentation (d)[d]	5 (0–14)	5 (3–7)	NS
Pitt bacteremia score ≥ 4	5 (29.4)	1 (1.5)	0.006
Temperature (°C)	38.4 (36.2–40.8)	37.6 (36.8–39.4)	0.026
Blood pressure (mmHg)	103 (74–140)	108 (90.65–131.8)	NS
Pulse rate (/min)	92 (55–166)	88 (63–116.9)	NS

[a] Conditional logistic modeling was performed (only $p \leq 0.05$ was indicated).
[b] Bleeding gums, petechiae, ecchymoses, purpura, or bruises.
[c] Hematemesis, rectal bleeding, or menorrhagia.
[d] Geometric means are indicated.
Data are presented as n (%) or median (5th–95th percentiles).
NS = not significant.

extracted included white blood cell count, absolute neutrophil count (ANC), lymphocyte count, platelet count, albumin, urea, aspartate aminotransferase, and alanine aminotransferase. DHF and DSS were defined according to the 1997 WHO guidelines,[17] whereas severe dengue was defined according to the 2009 WHO guidelines.[5] Charlson comorbidity index[21,22] and Pitt bacteremia score (PBS)[23] were calculated for cases and controls.

Ethics approval

This study was approved by the Domain-Specific Review Board of the National Healthcare Group, Singapore. Informed consent from patients was waived.

Statistical analysis

Categorical variables were described in numbers and percentages. Medians and 5^{th}–95^{th} percentiles were used in the descriptive analysis of continuous variables. Univariate and multivariate logistic regressions were used to calculate odds ratios (ORs).[24] Confounding effect was removed by performing multivariate logistic regression, adjusted for potential cofounders identified in univariate analysis. All statistical analyses were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) at a 5% level of significance, with adjusted ORs and corresponding 95% confidence intervals reported where applicable.

Table 2 Laboratory investigations, treatment modalities, and clinical outcomes of cases and controls[a]

Variable	Cases ($n = 18$)	Controls ($n = 72$)	p
Laboratory investigations at presentation			
Leukocyte count (10^9 cells/L)	4.1 (1.3–28.5)	3.2 (1.46–7.9)	NS
Neutrophil count (10^9 cells/L)	2.9 (0.6–26.4)	1.7 (0.56–5.74)	0.005
Lymphocyte count (10^9 cells/L)	0.7 (0.2–1.7)	0.9 (0.3–2.94)	0.014
Hematocrit change $\geq$ 20%	43.4 (23.6–53.5)	44.8 (34.82–53.3)	NS
Hematocrit change $\geq$ 20% & platelet count $<$ 50,000 cells/L	8 (44.4)	11 (15.3)	0.015
Platelet count (10^9 cells/L)	55 (17–148)	56 (14.1–150.5)	NS
Albumin (g/L)	30 (20–44)	36 (29.2–42)	0.001
Urea (mmol/L)	4.2 (1.3–15.5)	3 (1.38–6.0)	NS
Aspartate transaminase (U/L)	135.5 (22–1370)	119 (31–727)	NS
Alanine transaminase (U/L)	76 (18–669)	76 (20–445.5)	NS
Dengue severity at discharge			
Dengue hemorrhagic fever	8 (44.4)	30 (41.7)	NS
Dengue shock syndrome	3 (16.7)	1 (1.4)	0.031
Severe dengue	13 (72.2)	13 (18.1)	NS
Clinical interventions & outcomes at discharge			
Antibiotics	15 (88.2)	8 (11.8)	0.032
Intravenous fluids	18 (100.0)	61 (84.7)	NS
Total volume of fluids (mL)	9000 (3040–25,215)	3760 (1000–11,500)	0.002
Duration of fluid therapy (d)	5 (3–12)	3 (1.1–6)	0.016
Blood transfusion	2 (11.1)	2 (2.8)	NS
Platelet transfusion	4 (22.2)	9 (12.5)	NS
Length of hospitalization (d)	11 (4–88)	4 (2.65–7)	0.002
Intensive care	3 (16.7)	0 (0)	NS
Death	3 (16.7)	0 (0)	NS

[a] Conditional logistic modeling was performed (only $p \leq 0.05$ was indicated).
Data are presented as n (%) or median (5th–95th percentiles).
NS = not significant.

Results

Among 9,553 patients with dengue, 29 (0.3%) had bacteremia. Fifteen patients were subjected to dengue Real-time Reverse Transcription-Polymerase Chain Reaction, whereas the remaining 14 patients met the criteria for probable dengue and had positive results for dengue serology test: 13 were immunoglobulin M-positive and one was immunoglobulin G-positive. We found four patients, nine patients, five patients, five patients, and six patients from 2004 to 2008, respectively. The predominant isolated bacteria in these patients were *Staphylococcus aureus* [14 of 29 (48.3%)], one of which was a methicillin-resistant strain. The other bacteria isolated included *Salmonella* Typhi ($n = 5$), *Escherichia coli* ($n = 3$), *Klebsiella pneumoniae* ($n = 2$), *Streptococcus* species (alpha-hemolytic *Streptococcus* and *Streptococcus milleri*; $n = 2$), *Pseudomonas aeruginosa* ($n = 1$), *Acinetobacter baumannii* ($n = 1$), and an unspecified anaerobe. Eighteen (62.1%) cases had their blood sampled for bacterial growth less than 72 hours after admission: *Staphylococcus aureus* (5 of 14), *Salmonella* Typhi (4 of 5), and all of the other bacteria except *A. baumannii*. Further analyses were performed for these 18 cases versus 72 controls.

Cases and controls were similar in age, sex, and comorbidities, including Charlson comorbidity index and duration of fever before presentation to the hospital. At presentation, significantly fewer cases met the 1997 WHO criteria for dengue fever [11 (61.1%) cases vs. 66 (91.7%) controls], but significantly more cases [5 (29.4%)] than controls [1 (1.5%)] were critically ill (PBS $\geq$ 4). A significant proportion of cases had higher temperatures (median, 38.4°C vs. 37.6°C) but lower rates of hemorrhagic manifestations at presentation compared with controls [4 (22.2%) vs. 37 (51.4%); Table 1].

For common laboratory results, cases were significantly more likely than controls to have higher neutrophil counts and lower lymphocyte counts. In addition, cases were more likely than controls to have hemoconcentration (hematocrit change $\geq$ 20%) in association with lower platelet counts (<50,000 cells/L) and lower serum albumin levels. At the end of hospitalization, cases were significantly associated with DSS [3 (16.7%) cases vs. 1 (1.4%) control]. Cases and controls had similar rates of severe dengue according to the 2009 WHO classification. Not surprisingly, cases received more antibiotics, received more fluids (total volume and duration of fluid therapy), and stayed in the hospital longer than controls (median, 11 days vs. 4 days; $p < 0.01$). Three cases required intensive care admission and subsequently died, whereas none of the controls needed intensive care or died (Table 2). Two fatal cases experienced severe dengue shock (the *Klebsiella* case had elevated liver enzymes and the *Pseudomonas* case had pneumonia). The last fatal case experienced *Salmonella* septicemia with acute renal involvement.

After adjustment for statistically and clinically significant univariate risk factors, a PBS of ≥4 (being critically ill) was found to be independently associated with concurrent bacteremia in patients with dengue (adjusted OR 14.73, 95% confidence interval 1.30–167.28; Table 3).

Discussion

Despite the existence of an integrated dengue prevention program,[25] dengue remains endemic to Singapore. From 2004 to 2008, the number of notified dengue cases was 41,234. Of the notified cases, 28 adults were confirmed to have died of dengue. Among these confirmed cases of dengue-related deaths, 14.3% had bacteremia.[13] In a mortality study of dengue at the National University Hospital in Singapore, bacteremia was associated with 44.4% of dengue-related deaths.[11] In another mortality study of dengue at TTSH, bacteremia was associated with 42.9% of deaths.[12] A study in Taiwan showed that 5.5% of patients with DHF had bacteremia. Among these patients with bacteremia, 28.6% did not survive.[16] A separate Taiwanese study identifying clinical and laboratory manifestations of fatal DHF showed that 37.5% of patients with DHF and concurrent bacteremia did not survive.[10] In our study, notably, only 16.7% of cases did not survive. The observed difference in mortality may be attributable to the fact that Taiwanese patients with bacteremia were older and had DHF.

In our study, 29 patients [representing a very small proportion (0.3%) of our cohort from 2004 to 2008] had bacteremia; among these patients, 18 had concurrent bacteremia. A recent study at the National University Hospital in Singapore reported that 10 of 25 isolates from patients with concurrent bacteremia were primary bacteremia.[26] In our study, all 18 cases had bacteria isolated solely from the bloodstream (without other obvious sources), indicating primary bacteremia. Fifteen of 18 cases were given antibiotics on the day of blood culture collection or later during hospitalization. Seven of 18 cases (39%) were Gram-positive bacteria, and 10 cases (56%) were Gram-negative bacteria. Among Gram-negative bacteria, five were *Salmonella* Typhi cases, representing common exposure.[27] The remaining Gram-negative bacteria were gastrointestinal tract–commensal, which may be associated with DSS; however, three of 18 patients had DSS, and none had gastrointestinal bleeding, which may predispose to bacterial translocation. A study of the 2010 dengue outbreak in São Paolo, Brazil, documented immune activation and microbial translocation in patients with severe dengue.[28]

Table 3 Independent risk factors for concurrent bacteremia in patients with dengue at hospital presentation[a]

Variable	Adjusted OR (95% CI)	*p*
Hemorrhagic manifestations	0.35 (0.07–1.75)	0.200
Temperature (°C)	1.65 (0.74–3.64)	0.219
Pitt bacteremia score ≥ 4	14.73 (1.30–167.28)	0.030
Neutrophil count (10^9 cells/L)	1.29 (0.94–1.76)	0.115

[a] Conditional logistic regression modeling was performed.
CI = confidence interval; OR = odds ratio.

Several hypotheses on the pathogenesis of concurrent bacteremia in patients with dengue have been formulated. Antibodies against dengue virus nonstructural protein 1 have been observed to cross-react with endothelial cells,[14,15] resulting in disintegration of endothelial cells via caspase-dependent apoptosis.[14,29] The disintegration of endothelial cells may have facilitated the entry of bacteria into the bloodstream of patients with dengue. Another study revealed that the dengue virus could be isolated in human leukocytes[30] and that the titer of the virus correlated with the severity of the disease.[29,31] Lahiri et al[11] postulated that leukocyte infection could cause relative immunosuppression in patients with dengue, contributing to susceptibility to concurrent bacteremia and high mortality rates in patients with bacteremia, as observed in previous studies. Consistent with the abovementioned hypotheses, a significant proportion of cases in our study had more events of DSS at hospital discharge.

Although most of the signs and symptoms at presentation were not significantly different between cases and controls, a significant proportion of our cases did not present with hemorrhagic manifestations. This was not reported in other dengue studies of concurrent bacteremia. Furthermore, patients with dengue and bacterial infection were more likely to be critically ill, as defined by the validated PBS.[23] PBS has been shown to be predictive of mortality in patients with intensive care unit–acquired sepsis.[32] Our results indicated that PBS can be used to detect the presence of concurrent bacteremia. Neutrophilia, lymphopenia, and hemoconcentration with significant thrombocytopenia may be suggestive as well. See et al[26] reported that patients with dengue and bacterial infection had higher ANC compared with patients with dengue without bacterial infection. However, See et al[26] reported that patients with dengue and bacterial infection had lower hematocrit and higher platelet count, contrary to our findings. These differences may be attributable to the inclusion of primary and secondary bacterial infections identified from blood or urine cultures in their cohort, whereas our study included cases with primary bacteremia only and our controls were matched on year of infection and dengue confirmation method. Cases required more medical resources. It is important to confirm dengue diagnosis in a timely manner and to triage patients who are likely to develop bacteremia for closer monitoring and early intervention.

Our study had several limitations. Its retrospective study design may have led to some inaccuracy, although our standardized dengue care path prospectively documented relevant symptoms and signs, and subjected all patients to full blood count, renal panel test, and liver panel test (which were repeated when the initial results yielded abnormal findings). Blood cultures were ordered as clinically indicated; most patients with confirmed dengue who did not undergo blood culture recovered without antibiotic therapy. The small sample size limited the number of variables assessed in the multivariate analysis. We studied adult patients with dengue who presented to a tertiary care infectious disease center in a developed country; thus, our findings and outcomes may not be generalizable to other

populations (such as children) and settings (community hospitals and developing countries).

In conclusion, adult patients with dengue and concurrent bacteremia and adult patients with dengue without concurrent bacteremia have few differentiating predictive factors. Patients with dengue and concurrent bacteremia are more likely to have DSS, are more likely to be critically ill, have higher neutrophil count, lower lymphocyte count, and hemoconcentration with thrombocytopenia at hospital presentation. A high index of suspicion for bacteremia among critically ill adult patients with dengue is warranted. Given the associated mortality, collection of blood cultures and empiric antibiotic therapy may be considered in patients who are critically ill.

Conflict of interest

All authors have no conflicts of interest.

Acknowledgements

We are grateful to the staff at the Communicable Disease Center, who looked after patients with dengue during epidemics, and members of the STOP Dengue Translational Clinical Research Program, who provided invaluable support in data processing, management, and administrative work. This work was supported by the National Research Foundation through the National Medical Research Council of Singapore (Grant NMRC/TCR/005/2008). The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

References

1. World Health Organization. *Dengue and severe dengue*. 2013. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/index.html [accessed 11.08.15].
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature* 2013;**496**:504—7.
3. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 2012;**380**:1559—67.
4. Halstead SB. Dengue vaccine development: a 75% solution? *Lancet* 2012;**380**:1535—6.
5. World Health Organization. *Dengue: guidelines for diagnosis, treatment, prevention and control*. Geneva: World Health Organization; 2009.
6. Wills BA, Nguyen MD, Ha TL, Dong TH, Tran TN, Le TT, et al. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005;**353**:877—89.
7. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. *Trop Med Int Health* 2011;**16**:936—48.
8. Leelarasamee A, Chupaprawan C, Chenchittikul M, Udompanthurat S. Etiologies of acute undifferentiated febrile illness in Thailand. *J Med Assoc Thai* 2004;**87**:464—72.
9. Laoprasopwattana K, Pruekprasert P, Dissaneewate P, Geater A, Vachvanichsanong P. Outcome of dengue hemorrhagic fever—caused acute kidney injury in Thai children. *J Pediatr* 2010;**157**:303—9.
10. Lee IK, Liu JW, Yang KD. Fatal dengue hemorrhagic fever in adults: emphasizing the evolutionary pre-fatal clinical and laboratory manifestations. *PLoS Negl Trop Dis* 2012;**6**:e1532.
11. Lahiri M, Fisher D, Tambyah P. Dengue mortality: reassessing the risks in transition countries. *Trans R Soc Trop Med Hyg* 2008;**102**:1011—6.
12. Ong A, Sandar M, Chen MI, Leo YS. Fatal dengue hemorrhagic fever in adults during a dengue epidemic in Singapore. *Int J Infect Dis* 2007;**11**:263—7.
13. Leo YS, Thein TL, Fisher DA, Low JG, Oh HM, Narayanan RL, et al. Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study. *BMC Infect Dis* 2011;**11**:123.
14. Lin CF, Lei HY, Shiau AL, Liu HS, Yeh TM, Chen SH, et al. Endothelial cell apoptosis induced by antibodies against dengue virus nonstructural protein 1 via production of nitric oxide. *J Immunol* 2002;**169**:657—64.
15. Falconar AK. Monoclonal antibodies that bind to common epitopes on the dengue virus type 2 nonstructural-1 and envelope glycoproteins display weak neutralizing activity and differentiated responses to virulent strains: implications for pathogenesis and vaccines. *Clin Vaccine Immunol* 2008;**15**:549—61.
16. Lee IK, Liu JW, Yang KD. Clinical characteristics and risk factors for concurrent bacteremia in adults with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2005;**72**:221—6.
17. World Health Organization. *Dengue: guidelines for diagnosis, treatment, prevention and control*. Geneva: World Health Organization; 1997.
18. Barkham TM, Chung YK, Tang KF, Ooi EE. The performance of RT-PCR compared with a rapid serological assay for acute dengue fever in a diagnostic laboratory. *Trans R Soc Trop Med Hyg* 2006;**100**:142—8.
19. Blacksell SD, Bell D, Kelley J, Mammen MP, Gibbons RV, Jarman RG, et al. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. *Clin Vaccine Immunol* 2007;**14**:1458—64.
20. Cuzzubbo AJ, Endy TP, Nisalak A, Kalayanarooj S, Vaughn DW, Ogata SA, et al. Use of recombinant envelope proteins for serological diagnosis of dengue virus infection in an immunochromatographic assay. *Clin Diagn Lab Immunol* 2001;**8**:1150—5.
21. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;**40**:373—83.
22. Lesens O, Methlin C, Hansmann Y, Remy V, Martinot M, Bergin C, et al. Role of comorbidity in mortality related to *Staphylococcus aureus* bacteremia: a prospective study using the Charlson weighted index of comorbidity. *Infect Control Hosp Epidemiol* 2003;**24**:890—6.
23. Chow JW, Yu VL. Combination antibiotic therapy versus monotherapy for Gram-negative bacteraemia: a commentary. *Int J Antimicrob Agents* 1999;**11**:7—12.
24. Chan YH. Biostatistics 203—survival analysis. *Singapore Med J* 2004;**45**:249.
25. National Environment Agency. *Fight dengue*. 2013. Available from: http://www.dengue.gov.sg/ [accessed 11.08.13].
26. See KC, Phua J, Yip HS, Yeo LL, Lim TK. Identification of concurrent bacterial infection in adult patients with dengue. *Am J Trop Med Hyg* 2013;**89**:804—10.
27. Pancharoen C, Thisyakorn U. Coinfections in dengue patients. *Pediatr Infect Dis J* 1998;**17**:81—2.
28. van de Weg CA, Pannuti CS, de Araujo ES, van den Ham HJ, Andeweg AC, Boas LS, et al. Microbial translocation is associated with extensive immune activation in dengue virus infected patients with severe disease. *PLoS Negl Trop Dis* 2013;**7**:e2236.

29. Courageot MP, Catteau A, Despres P. Mechanisms of dengue virus—induced cell death. *Adv Virus Res* 2003;**60**:157—86.
30. Morens DM, Marchette NJ, Chu MC, Halstead SB. Growth of dengue type 2 virus isolates in human peripheral blood leukocytes correlates with severe and mild dengue disease. *Am J Trop Med Hyg* 1991;**45**:644—51.
31. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;**181**:2—9.
32. Rhee JY, Kwon KT, Ki HK, Shin SY, Jung DS, Chung DR, et al. Scoring systems for prediction of mortality in patients with intensive care unit—acquired sepsis: a comparison of the Pitt bacteremia score and the Acute Physiology and Chronic Health Evaluation II scoring systems. *Shock* 2009;**31**:146—50.