ORIGINAL ARTICLE

DEVELOPMENT OF A CLINICAL RISK SCORE TO DIAGNOSE CONCURRENT BACTERIAL INFECTIONS IN CHILDREN WITH DENGUE

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

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ABSTRACT

Background: The clinical course of dengue can be adversely affected by bacterial coinfection. Because of this, clinical manifestations may be severe and may lead to morbidity and mortality. Little is known about this dual infection in the pediatric population.

Objectives: This study was conducted to evaluate the clinical characteristics and risk factors of patients with dengue infection and coinfection and subsequently develop a scoring system to diagnose bacterial coinfection in patients with dengue.

Methods: A prospective cross-sectional observational study was conducted among hospitalized pediatric patients with confirmed dengue infection between January 2019 to August 2019. Baseline characteristics, risk factors, clinical parameters, laboratory findings, management and outcomes were recorded. Cases with concurrent bacterial infections were further analyzed. A scoring system was created which assigned 1 point each for the following risk factors - age ≤9 years, fever >5 days, dengue severe, and 2 points for CRP >12 mg/l)

Results: A total of 154 pediatric dengue patients were enrolled with a mean age of 8.54 ± 4.15 years, and 99 patients (64%) had bacterial coinfection. Patients with coinfection were A total of 154 pediatric dengue patients were enrolled with a mean age of 8.54 ± 4.15 years, and 99 patients (%) had bacterial co-infection. Patients with co-infection were younger, have prolonged fever (>5 days), and were more frequently observed to have hypotension, tachycardia, desaturations and bleeding. Patients with coinfection also had higher white blood cell counts (>8 x109 cells/L), higher neutrophil counts (58.80 ± 18.42 % count), and elevated CRP (>12 mg/l) and procalcitonin (>4.01 ng/L). Utilizing the scoring system developed, a score of ≥3 had a sensitivity of 66.67% and specificity of 76.36%, in diagnosing concurrent bacterial infection in children with dengue.

Conclusions: Patients with dengue and bacterial coinfections were younger with comorbidities. They presented with significantly abnormal vital signs, physical examination findings, and elevated acute phase reactants. Using age ≤ 9 years, fever >5 days, dengue severe, and CRP >12mg/l, a scoring system was developed to diagnose bacterial coinfection in patients with dengue. A score of ≥3 can help diagnose patients with dengue and bacterial coinfection who will most likely need early empiric antimicrobial therapy.

KEYWORDS: *Dengue, Concurrent Bacterial Infection, Risk Score*

INTRODUCTION

Dengue is a fast-emerging pandemic-prone viral disease affecting many parts of the world [1]. Globally, it is responsible for nearly 500,000 hospitalizations and 3.6 billion people remain at risk. In the Philippines, dengue illness is considered one of the country's eight pervasive infectious diseases [2]. Of the ten Association of Southeast Asian Nations (ASEAN) member countries, the Philippines ranks fourth in the number of dengue cases.

Dengue virus infection in humans is often inapparent[1] but can lead to a wide range of clinical manifestations that vary according to age and severity[3] and often with unpredictable clinical evolution and outcome [4]. The severity of infection depends on several factors related to the virus and host. Fluid management and antipyretic therapy with paracetamol is preferred during the febrile phase. Judicious fluid administration remains the mainstay of treatment during the critical phase [5]. However, optimal management of dengue may differ once it is confounded by bacterial coinfection. In addition, the clinical course of dengue infection can be adversely affected by bacterial coinfection. Due to complex interactions between pathogens [6], clinical manifestations may be severe and may lead to morbidity and mortality.

The problem in managing patients with dengue is identification of patients with concurrent bacterial infections. The clinical and laboratory presentation of dengue and some other bacterial infections such as leptospirosis, salmonellosis and bacteremia overlap [6, 7, 8], hence they are easily overlooked in a dengue endemic setting [9]. The diagnosis of coinfections proves to be challenging especially during dengue outbreaks [10]. This may lead to missed diagnosis due to unusual clinical presentations and may lead to delays in antibiotic therapy [6].

To identify concurrent bacterial infection among patients with confirmed dengue infection, serum inflammatory markers such as C-reactive protein (CRP) and Procalcitonin (PCT) may be utilized. Studies on the use of these inflammatory markers in pediatrics are still limited compared to studies performed in adults. The normal serum value of PCT is <0.1 ng/mL. The greatest elevation of serum PCT are seen in bacterial infections. In a study by Chen et. al. in adult patients with dengue and bacterial coinfections admitted in the ICU, they found the sensitivity and specificity of procalcitonin to be 81.5% and 59.5% respectively using a cutoff value of 1.14 ng/mL [11]. The NPV can be up to 89.8% in these situations, and this finding suggest that procalcitonin can be used for excluding concomitant bacteremia among dengue patients in the ICU.

On the other hand, CRP is a non-specific, acute-phase protein that increases 4-6 hours after exposure to an inflammatory trigger (infectious or not) and has an 8-hour doubling time, peaking from 36 to 50 hours after trigger stimulus. [12]. It is not a specific biomarker for differentiating infection from inflammation or for identifying specific infectious agents. [13]. Due to the limited specificity of CRP, the combined use of CRP with other biomarkers such as procalcitonin is being done [13].

There are several studies on the clinical characteristics and risk factors of patients with dengue infections and concurrent bacteremia [6, 9] however these studies were exclusively done in adults. Little is known about the incidence and risk factors for this dual infection in the pediatric population, thus this study was done to evaluate the clinical characteristics of patients with dengue infection and concurrent bacterial infections and to identify risk factors for these dual infections. It intended to create a scoring system to diagnose concurrent bacterial infection in patients with dengue to help clinicians start timely antibiotic therapy in patients with dengue infection.

MATERIALS AND METHODS

This was a prospective cross-sectional observational study conducted from July 2018 to August 2019 at a tertiary hospital in Metro Manila. The study participants were pediatric patients 1

month to 18 years old and 365 days admitted for a period of <72 hours. Those who were clinically diagnosed with dengue based on the 2009 WHO Dengue Case Classification *or* has laboratory confirmation of dengue through a positive anti-dengue immunoglobulin M (IgM) antibody (enzyme-linked immunosorbent assay [ELISA]) *and/or* dengue non-structural protein 1 (NS1) antigen *and* fever of >7 days and/*or* clinical deterioration despite treatment based on standardized dengue care pathways and/*or* alterations in laboratory parameters such as hyponatremia, elevated leukocyte count for age, high neutrophil counts for age, and elevated creatinine (from kidney failure due to shock) were included. Patients were excluded if they were previously hospitalized in another institution in the last 10 days or was a clinically and laboratory confirmed dengue case but admitted for more than 72 hours in our institution.

Subject Enrollment and Collection of Patient Data

Subjects who were eligible were enrolled after the informed consent process. A complete history was obtained and thorough physical examination was done. Patient's age, sex, height, weight, education, co-morbidities, and dengue vaccine history, were recorded on the data collection form. Clinical data collected included signs and symptoms such as presence and duration of fever, abdominal pain, persistent vomiting, mucosal bleeding, rash, aches and pains, difficulty of breathing, headache, loose stools, dysuria, cough and chest pain. Vital signs (systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate, temperature, oxygen saturation), presence or absence of fluid accumulation, spontaneous bleeding, and liver enlargement were also recorded. Laboratory data collected were hemoglobin, hematocrit, white blood cell count, neutrophils, lymphocytes, platelet count, albumin, alanine transaminases (ALT) and aspartate transaminases (AST), sodium, potassium, calcium, chloride, creatinine, blood urea nitrogen (BUN), CK-MB, and glomerular filtration rate (GFR).

Concurrent bacterial infection was defined as any clinical diagnosis of bacterial infection (e.g. pneumonia) and/or any bacteremia or bacteriuria from cultures taken within 72 hours from admission. Conventional blood and urine cultures were done, with the former supplemented by an automated BacT/Alert System (bioMerieux SA, Durham NC, USA). Patients with blood or urine cultures positive for coagulase-negative staphylococci were considered to have concurrent bacterial infection if the following were conditions were met: (1) with two or more positive blood cultures from different anatomic sites, (2) a positive culture from blood and another usually sterile site with identical antimicrobial susceptibility patterns, (3) growth in continuously monitored blood culture system within 15 hours of incubation, (4) clinical findings of infection, (5) an intravascular catheter has been in place for 3 days or more, and (6) similar or identical genotypes among isolates[14].

Acute phase reactants such as procalcitonin and CRP were also taken upon enrollment of patients. Quantitative determination of procalcitonin was measured using a homogenous immunoassay method (Thermo Scientific B·R·A·H·M·S PCT sensitive KRYPTOR, Hennigsdorf, Germany) with the procedure performed according to manufacturer's instructions. The detection limit for the PCT assay was 0.02 ng/mL. C-reactive protein (CRP) was determined semi-quantitatively using latex agglutination (rheumajet CRP, Biokit). The detection limit was 6 mg/l of C-reactive protein.

Candidate variables that were reliably measured and readily available at the time of presentation were selected for the diagnostic model.

ETHICAL CONSIDERATIONS

The research protocol was approved by the Institutional Review and Ethics Committee of the Philippine Children's Medical Center. The study adhered to ethical considerations and principles set out in relevant guidelines, including the Declaration

of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012, and National Ethics Guidelines for Health Research.

STATISTICAL ANALYSIS

The minimum computed sample size was 279 subjects. This value gives 90% power to detect an effect size of 0.417 at 0.05 α-level of significance. The value used for this sample size computation was based on a study by See et. al. in 2013 [15]. However, the sample size achieved was only 154 subjects.

Descriptive statistics was used to summarize the general and clinical characteristics of participants. Frequencies and proportion were used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables. Independent T-test, Mann-Whitney U test, and Fisher's exact/Chi-square test was used to determine the difference of mean, median, and frequencies between patients with concurrent bacterial infection versus those without, respectively.

Multivariate analysis and formulation of the scoring system

Crude and adjusted odds ratio and the corresponding 95% confidence intervals from binary logistic regression were computed to determine predictors of concurrent bacterial infection. The corresponding coefficients in the regression were used to create a scoring system to assess the risk of having infection, following the method described by Tai and Machin in 2014 [16]. The regression coefficients were used as a basis for the scoring system. First, the constant term was dropped then the coefficients were divided to the least figure. Next, all coefficients were rounded off to the nearest integer. A constant value equivalent to the sum of all negative points was then added to avoid a negative point (e.g. for a point system $y = -4x_1 + 3x_2 + -2x_3$, a constant equal to +6 was added). For this data set, there were no negative coefficients and thus did not require a constant value.

All valid data were included for analysis while missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 15.0 was used for data analysis.

RESULTS

We included in our study a total of 154 pediatric patients comprising 76 males and 78 females, with a median age of 8 years old. Most patients (55.19%) are in elementary (Table 1). Thirty-three percent were positive for Dengue NS1, while 94% of the patients who were tested with dengue immunoglobulin M (IgM) were positive. Most of the patients (87.66%) were classified as dengue severe.

Table 1. Demographic and clinical profile of patients (n= 154)

	Total (n = 154)	With co-infection (n = 99)	Without co-infection (n = 55)	*p*
	Frequency (%); Median (Range); Mean ± SD			
Age, years	8.54 ± 4.15	7.76 ± 3.82	9.94 ± 4.37	**0.002***
<12 months	5 (3.25)	4 (4.04)	1 (1.92)	
1 – 5 years	31 (20.13)	23 (23.23)	8 (14.55)	
6 – 9 years	58 (37.66)	41 (41.41)	17 (30.91)	
10 – 12 years	33 (21.43)	22 (22.22)	11 (20)	
13 – 15 years	16 (10.39)	5 (5.05)	11 (20)	
16 – 17 years	11 (7.14)	4 (4.04)	7 (12.73)	
Sex				0.962†
Male	76 (49.35)	49 (49.49)	27 (49.09)	
Female	78 (50.65)	50 (50.51)	28 (50.91)	
Education				**0.031‡**
Out of school	4 (2.60)	4 (4.04)	0	
Elementary	85 (55.19)	57 (57.58)	28 (50.91)	
High school	31 (20.13)	14 (14.14)	17 (30.91)	
College	1 (0.65)	0	1 (1.82)	
Others	33 (21.43)	24 (24.24)	9 (16.36)	
Co-morbidities				
CHD	7 (4.55)	7 (07.07)	0	0.051‡
Asthma	1 (0.65)	1 (1.01)	0	1.000‡
CKD	1 (0.65)	1 (1.01)	0	1.000‡
Others	4 (2.60)	3 (3.03)	1 (1.79)	1.000†
Symptoms				
Fever, days	5.60 ± 1.51	5.94 ± 1.62	4.98 ± 1.03	**<0.001***
Abdominal pain	106 (68.83)	67 (67.68)	39 (70.91)	0.678†
Persistent vomiting	102 (66.23)	63 (63.64)	39 (70.91)	0.360†
Aches and pains	53 (34.42)	33 (33.33)	20 (36.36)	0.704†
Headache	39 (25.32)	22 (22.22)	17 (30.91)	0.235†
Cough	30 (19.48)	26 (26.26)	4 (7.27)	**0.004†**
Mucosal bleeding	28 (18.18)	20 (20.20)	8 (14.55)	0.383†
Weakness	19 (12.34)	14 (14.14)	5 (9.09)	0.361†
Rash	13 (8.44)	8 (8.08)	5 (9.09)	1.000‡
Decreased urine output	11 (7.14)	7 (7.07)	4 (7.27)	1.000‡
Others	36 (23.38)	25 (25.25)	11 (20)	0.461†
Dengue vaccine	7 (4.55)	3 (3.03)	4 (7.27)	0.249‡
Previous dengue infection	1 (0.65)	1 (1.01)	0	1.000‡

CHD- congenital heart disease; CKD- chronic kidney disease
*Statistical Tests Used: * - Independent t-test; † - Chi-square Independent test; ‡ - Fisher's Exact test*

Patients presented with fever after a median of 5.6 days (sd ± 1.51). Of the 154 pediatric patients

who were included, 99 (64.29%) were classified as having bacterial co-infections (Table 2).

Table 2. Dengue features of patients (n = 154)

	Frequency (%)
Dengue fever testing	
Dengue NS1	51 (33.12)
Dengue IgM	119 (77.27)
Negative	7 (5.88)
Positive	112 (94.12)
Dengue classification	
Dengue Severe	135 (87.66)
Dengue with Warning Signs	19 (12.34)
Without concurrent bacterial infection	55 (35.71)
With concurrent bacterial infection	99 (64.29)
Pneumonia	88 (88.89)
LCBSI	11 (11.11)
UTI	9 (9.09)
Leptospirosis	1 (1.01)
Cellulitis	1 (1.01)
Meningitis	1 (1.01)

LCBSI – Laboratory-confirmed bloodstream infections;
UTI – Urinary tract infection

Of these 99 patients with bacterial co-infections, 88 (88.89%) had pneumonia, 11 (11.11%) had laboratory confirmed blood stream infection (LCBSI), 9 (9.09%) had culture confirmed urinary tract infection (UTI), and the rest had either leptospirosis, cellulitis, or meningitis. Pneumonia cases were diagnosed based on the presence of radiological features and accompanying symptoms (eg. cough, tachypnea, rales, chest pain, etc.). Leptospirosis was diagnosed based on molecular detection (Real-Time PCR) of pathogenic *Leptospira spp*. DNA from the blood of the patient. The diagnosis of meningitis was based on the presence of seizures and decreased sensorium and cerebrospinal fluid (CSF) findings of low sugar level along with an increased white blood cell count and increased protein. Lastly the diagnosis of cellulitis was based on physical examination findings of the skin or soft tissue which showed swelling, erythema, tenderness and warmth. There were no other infections noted. Of the patients with positive blood cultures, *Escherichia coli* was isolated in 2 patients, and one of each grew Methicillin-sensitive *Staphylococcus aureus, Streptococcus mitis, Salmonella sp., Klebsiella pneumoniae, Acinetobacter baumanii, Sphingomonas paucimobilis* and *Aeromonas hydrophilia* and were isolated singly from different patients. In those with positive urine cultures, Escherichia *coli* was isolated in seven patients, and *Acinetobacter baumanii* and *Morganella morganii, were isolated one* from each patient.

On the average, dengue patients with coinfection were significantly younger (7.78 $\pm$ 3.83 vs 9.87 $\pm$4.37, p = 0.002). Only 13 patients (8.4%) have a comorbidity and seven had congenital heart disease. Other comorbidities noted were asthma (2), chronic kidney disease (2), abnormal uterine bleeding (1), hypoxic ischemic encephalopathy (1), and neurogenic bladder (1). Fever duration was also longer in patients with coinfection, 5.9 days versus 4.9 days (p<0.001).

The group with coinfections had significantly higher cardiac and respiratory rates, and significantly lower blood pressure (Table 3). Abnormal physical examination findings were noted more frequently among patients with coinfection, such as fluid accumulation (68% versus 33%, p <0.001), spontaneous bleeding (44% versus 20%, p=0.002), liver enlargement (53% versus 24%, p=0.001), prolonged capillary refill time (CRT) (77% versus 62%, p=0.049), and poor pulses (71% versus 38%, p<0.001). Average WBC and neutrophil counts were also higher in those with coinfection (Table 3). In addition, among those with coinfections, 40 (40.40%) patients required blood transfusion (vs. 10.91%, p<0.001), and 51 (51.52%) patients required mechanical ventilator (vs. 16.36%, p<0.001) (Table 4).

Table 3. Physical examination/laboratory investigations upon enrollment of patients (n = 154)

	Total (n = 154)	With coinfection (n = 99)	Without coinfection (n = 55)	p
	Mean ± SD; Median (Range) Frequency (%)			
Vital signs				
CR	118.40 ± 22.07	122.92 ± 21.51	110.27 ± 20.85	**0.001***
RR	26 (10 – 58)	28 (10 – 58)	25 (18 – 45)	**0.012§**
SBP	85 (0 – 120)	80 (0 – 120)	90 (0 – 110)	**0.001§**
DBP	60 (0 – 95)	50 (0 – 90)	60 (0 – 95)	**0.002§**
Temperature	37.9 (35 – 40)	38 (35 – 40)	37 (36.2 – 39.7)	**0.011§**
Oxygen saturation	0.98 (0.8 – 1)	0.98 (0.8 – 1)	0.98 (0.9 – 1)	**0.019§**
Within normal ranges				
Blood pressure	24 (15.58)	16 (16.16)	8 (14.55)	0.791†
Cardiac rate	26 (16.88)	11 (11.11)	15 (27.27)	**0.010†**
Respiratory rate	31 (20.13)	15 (15.15)	16 (29.09)	**0.039†**
Fluid accumulation	86 (55.84)	68 (68.69)	18 (32.73)	**<0.001†**
Spontaneous Bleeding	55 (35.71)	44 (44.44)	11 (20)	**0.002†**
Liver enlargement	65 (42.21)	52 (52.53)	13 (23.64)	**0.001†**
Size (cm)	2 (2 – 6)	2.5 (2 – 6)	2 (2 – 6)	0.135§
CRT				**0.049†**
<2 secs	44 (28.57)	23 (23.23)	21 (38.18)	
>2 secs	110 (71.43)	76 (76.77)	34 (61.82)	
Pulse				**<0.001†**
Poor	91 (59.09)	70 (70.71)	21 (38.18)	
Full	63 (40.91)	29 (29.29)	34 (61.82)	
Complete blood count				
Hemoglobin	130.5 (53–208)	130 (53 – 208)	132 (99 – 186)	0.465§
Hematocrit	39.5 (14 – 63)	38 (14 – 63)	41 (32 – 56)	0.108§
WBC (10^9 cells/L)	6.45 (1.5 – 34)	8 (1.5 – 25)	4.7 (1.5 – 34)	**<0.001§**
Lymphocytes (% count)	34 (2 – 91)	30 (3 – 80)	41 (2 – 91)	**0.012§**
Neutrophils (%count)	55.48 ± 18.73	58.80 ± 18.42	49.51 ± 17.95	**0.003***
Platelet (10^9 cells/L)	31 (4 – 254)	29 (4 – 254)	36 (8 – 242)	0.062§
Within normal ranges				
Hemoglobin	44 (28.57)	26 (26.26)	18 (32.73)	0.458†
Hematocrit	64 (41.56)	36 (36.36)	28 (50.91)	0.079†
WBC	49 (31.82)	27 (27.27)	22 (40)	0.104†
Lymphocytes	30 (19.48)	20 (20.20)	10 (18.18)	0.762†
Platelet	11 (7.14)	4 (4.04)	7 (12.73)	0.056‡
Neutrophils	32 (20.78)	25 (25.25)	7 (12.73)	0.066†

CR- cardiac rate; RR- respiratory rate; SBP- systolic blood pressure;
DBP- diastolic blood pressure;
CRT- capillary refill time; WBC- white blood cell
*Statistical Tests Used: * - Independent t-test; † - Chi-square Independent test; ‡ - Fisher's Exact test; § - Mann Whitney U test*

Table 4. Management of patients with dengue (n = 154)

	Total (n = 154)	With coinfection (n = 100)	Without infection (n = 54)	p
	Frequency (%)			
Required blood transfusion	46 (29.87)	39 (39.80)	7 (12.50)	**<0.001†**
Required mechanical ventilator	60 (38.96)	50 (51.02)	10 (17.86)	**<0.001†**
Required inotropes	101 (65.68)	69 (70.41)	32 (57.14)	0.096†
Hemodialysis	13 (8.44)	10 (10.20)	3 (5.36)	0.377‡
Hemoperfusion	13 (8.44)	10 (10.20)	3 (5.36)	0.377‡

Statistical Tests Used: † - Chi-square Independent test; ‡ - Fisher's Exact test

Cephalosporins, specifically cefotaxime (Table 5), were the most common empiric antibiotics given to patients despite absence of a documented coinfection (69.09%). This was followed by aminoglycosides (24.03%) and penicillins (7.79%). In our institution the primary physician decides on the need for an antibiotic if there are abnormal laboratory results pointing to an infection or if patients are not responding to the usual dengue management.

Table 5. Antibiotics used in dengue patients (n = 154)

Cephalosporins	110 (71.43)	83 (84.69)	27 (48.21)	**<0.001†**
Cefotaxime	76 (69.09)	58 (69.88)	18 (66.67)	
Ceftriaxone	25 (22.73)	21 (25.30)	4 (14.81)	
Cefuroxime	9 (8.18)	4 (4.82)	5 (18.52)	
Aminoglycosides	34 (24.03)	27 (29.59)	1 (14.29)	**0.032†**
Gentamicin	33 (89.19)	26 (89.66)	7 (87.50)	
Amikacin	1 (2.70)	1 (3.45)	0	
Penicillin	12 (7.79)	9 (9.18)	3 (5.36)	0.538‡
Penicillin G	7 (58.33)	5 (55.56)	2 (66.67)	
Oxacillin	3 (25)	2 (22.22)	1 (33.33)	
Ampicillin	2 (16.67)	2 (22.22)	0	
Meropenem	8 (5.19)	7 (7.14)	1 (1.79)	0.259‡
Vancomycin	6 (3.90)	6 (6.12)	0	0.087‡
Azithromycin	1 (0.65)	1 (1.02)	0	1.000‡
Fluconazole	1 (0.65)	1 (1.02)	0	1.000‡
Other antibiotics	2 (1.30)	2 (2.0)	0	0.534‡

Statistical Tests Used: † - Chi-square Independent test; ‡ - Fisher's Exact test

As to clinical outcomes (Table 6), evidence was insufficient to make a conclusion as to length of hospital stay. However, those with coinfection had a higher mortality rate versus those without (35.35% vs 9.09%, p=0.001) with an overall mortality of 26%. All mortality were classified as severe dengue cases. (Table 7).

Table 6. Clinical outcomes of patients with dengue (n= 154)

	Total (n = 154)	With coinfection (n = 99)	Without co-infection (n = 55)	p
	Frequency (%); Median (Range); Mean ± SD			
Admission				0.062‡
Ward	24 (15.58)	11 (11.11)	13 (23.64)	
PICU	129 (83.77)	87 (87.88)	42 (76.36)	
IICU	1 (0.65)	1 (1.01)	0	
Length of hospital stay	6 (1 – 56)	6 (1 – 42)	5 (2 – 56)	0.784§
Mortality	40 (25.97)	35 (35.35)	5 (9.09)	**0.001†**

PICU- Pediatric Intensive Care Unit; Intermediate Intensive Care Unit
Statistical Tests Used: † - Chi-square Independent test; ‡ - Fisher's Exact test; § - Mann Whitney U test

Table 7. Survival rate according to Dengue classification (n=154)

	Non-survivor (n = 40)	Survivor (n = 114)	*p*
Dengue classification			**0.004**
DWS	0	19 (16.67)	
DS	40 (100)	95 (83.33)	

DS- Dengue Severe; DWS- Dengue with Warning Signs

Development of a Scoring System

Upon binary logistic regression analysis, the variables age of ≤9 years, fever of more than 5 days, dengue severity, WBC of ≥5 x 109/L, procalcitonin of >0.5 ng/mL, and CRP of >12mg/L, were found to be significantly associated with concurrent bacterial infection (Table 8).

Table 8. Factors associated with concurrent bacterial Infection (n = 154)

	Crude Odds Ratio (95% CI)	*p*	Adjusted Odds Ratio (95% CI)	*p*
Age, years				
≤9	2.447 (1.24 – 4.82)	**0.010**	2.922 (1.30 – 6.59)	**0.010**
>9	Reference	-	Reference	-
Fever, days				
≤5	Reference	-	Reference	-
>5	3.333 (1.63 – 6.80)	**0.001**	**4.120 (1.80 – 9.42)**	**0.001**
Dengue classification				
DWS	Reference	-	Reference	-
DS	4.798 (1.71 – 13.49)	**0.003**	**3.876 (1.07 – 14.02)**	**0.039**
WBC				
≤5 (10^9 cells/L)	Reference	-		
>5 (10^9 cells/L)	2.074 (1.06 – 4.07)	**0.034**		
Procalcitonin				
≤0.5 ng/mL	Reference	-		
>0.5 ng/mL	7.530 (2.32 – 24.48)	**0.001**		
CRP				
≤12 mg/l	Reference	-	Reference	-
>12 mg/l	6.043 (2.48 – 14.72)	**<0.001**	6.028 (2.31 – 15.71)	**<0.001**

WBC- White Blood Cell; CRP- C-Reactive Protein
Adjusted R^2 = 23%; p<0.001

The adjusted model explained 23% in the variation of the prevalence of concurrent bacterial infection (p<0.001). A scoring system was derived based on the regression coefficients of the variables, using the method described by Tai and Machin in 2014 [16]. With this method, age of ≤9 years, fever of more than 5 days, dengue severe, CRP of >12mg/L were used for the final risk score. The item scores ranged from 1 to 2, and the total score ranged from 0 to 5 (Table 9). Overall, among 154 pediatric patients enrolled for the scoring, 16.2% scored 0 or 1, 27% scored 2, and 56.49% scored 3 or more.

Table 9. Proposed scoring system to determine concurrent bacterial infection among pediatric dengue patients

	Reference value	Regression Coefficient	Crude point	Final point
Age ≤9 years	No = 0	**1.072**	0	0
	Yes = 1		1	1
Fever >5 days	No = 0	1.416	0	0
	Yes = 1		1.32	1
Dengue Severe	No = 0	1.355	0	0
	Yes = 1		1.26	1
CRP >12 mg/l	No = 0	1.796	0	0
	Yes = 1		1.68	2

CRP- C-Reactive Protein

Figure 1 and Table 8 show the diagnostic performance at each cut-off points. The maximum Youden's index indicates the optimal cut-off point, and in this case, the optimal cut-off was at 3 points. This means that by using the scoring system defined earlier, a patient with a score of at least three has the optimal discriminative power to distinguish between those with and without bacterial infection, with a sensitivity of 66.67% and specificity of 76.36%.

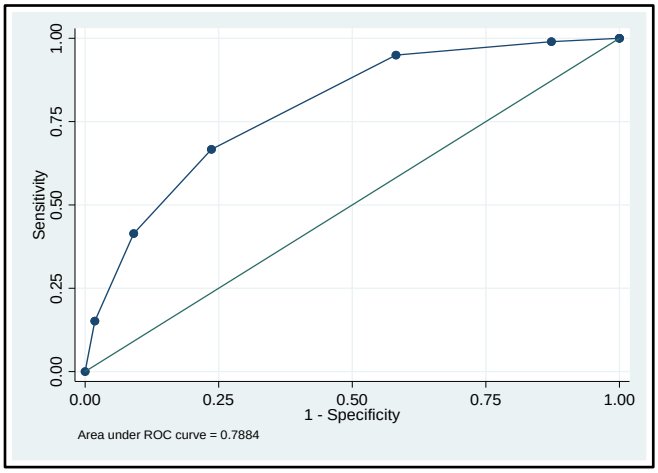

Figure 1. Receiver operating characteristic curve of the proposed scoring system

Optimal Cut-Off of Procalcitonin

Since procalcitonin was not included in the final risk scoring system, we computed for the optimal cut-off of procalcitonin in predicting coinfection among dengue patients. Figure 2 shows the ROC curve of procalcitonin in predicting coinfection among dengue patients. Based on the

highest J index, the suggested optimal cutoff of procalcitonin is > 2.5 ng/mL, with a sensitivity 67.68%, specificity 83.33%, accuracy 73.20%, and Youden's index 51.01%.

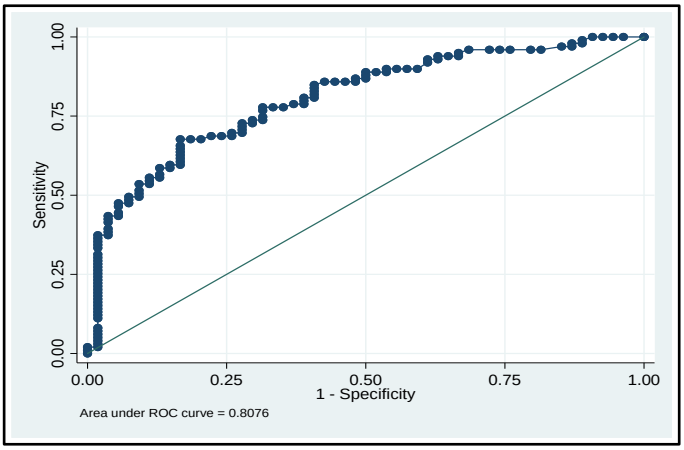

Figure 2. Receiver operating characteristic curve of procalcitonin in predicting concurrent infection

DISCUSSION

Concurrent bacterial infections in patients with dengue are uncommon yet important as they are also associated with mortality [6]. This occurrence was seldom discussed in the past and so hindered widespread awareness of concurrent bacterial infections in patients with dengue [17]. Only four studies investigated on the presence of concurrent bacterial infections in patients with dengue and these were all retrospective [6, 18, 19, 20]. In children, published studies have mostly been case reports. As to our knowledge, this is the first prospective study done to investigate concurrent bacterial infections in children with dengue.

Concurrent bacterial infections in our patients with dengue is associated with high mortality compared to those without concurrent bacterial infections. This is also true in previous studies, although done in adults.[6, 18, 19, 20]. In our study, 64% of enrolled patients were diagnosed with concurrent bacterial infection compared with other studies with a lower occurrence of concurrent bacterial infection at 4-7%. We predicted our data to be high because we did not include all patients with dengue who were admitted at our institution regardless of a suspicion of coinfection. Like others have shown, majority of laboratory confirmed blood stream infections were due to gram-negative organisms [7, 18, 20]. It is postulated that this occurs due to the disintegration of the mucocutaneous barrier due to vascular leakage [19, 21, 22] which results in seepage of bacteria or in microbial translocation (MT) into the bloodstream [22, 23, 24]. This then leads to aberrant cytokine cascade mostly mediated by gram-negative bacteria leading to a worse outcome in dengue patients with coinfection [25]. Other probable reasons for coinfections are rapid urbanization and increased population density, frequent travel, poor sanitation, changing season, and poor infrastructure and inadequate vector control measures [26]. These may explain the wide range of other infections that we found in our study such as pneumonia, meningitis and leptospirosis which have only been highlighted mostly in case reports.

We identified several clinical and laboratory risk factors in dengue patients with concurrent bacterial infection. In our study, younger age (≤9 y) was an important risk factor for concurrent bacterial infection. Age has been a well-established epidemiological risk factor when it comes to disease severity in dengue [27, 28] and has been associated with a poor prognosis [29]. This is probably because of increased microvascular fragility seen in younger children [27].

Similar to the findings of Premaratna, R. et al and Lee et al, our study showed that patients with prolonged fever (>5 days) are at high risk for possible coinfection. The febrile phase usually lasts for 5-7 days [23], however in those with prolonged fever > 5 days, it could be that at the time when they enter the critical phase, increased permeability of the epithelial lining may facilitate entry of microorganisms leading to sepsis [30]. Contrary to the findings of Premaratna, R. et al and Lee et al, See et al did not find fever to be a reliable sign of possible coinfection.

We found that dengue patients with

coinfection have more severe clinical manifestations compared to those without coinfection. In our analysis, patients with coinfection have more fluid accumulation, spontaneous bleeding, and low blood pressure. All these results from excessive plasma leakage due to increased vascular permeability. This group of patients also exhibited lower albumin levels reflecting the severity of plasma leakage. These results share similar findings with a previous study done by Thein et al where patients with dengue and coinfection were more likely to be critically ill with lower albumin levels [6].

Similar to other studies, our results also showed that white blood cell and neutrophils counts were significantly higher in dengue patients with bacterial coinfections [25, 31]. Studies have shown that patients with dengue had significantly higher white blood cell and neutrophil counts [32] and an elevated count may indicate other etiology of febrile illnesses or a superimposed bacterial infection.

Interestingly, we included the use of acute phase reactants to see their usefulness and benefit in patients with dengue and bacterial coinfection. In a systematic review, CRP has an estimated 77% sensitivity and 79% specificity and diagnostic accuracy for bacterial infection in children with fever. However, its predictive value increases with the number of serial measurements, thus rendering it possibly useful to assess response to therapy [13]. The addition of CRP to the scoring system that we created is of novel use because this is the only risk score study in dengue patients with coinfection that included inflammatory markers.

Procalcitonin (PCT) is currently used as a novel biomarker for diagnostic and prognostic purposes [20, 33]. PCT has been assessed as a biomarker for local and systemic inflammatory responses, disease severity, and necrosis related to organ failure, particularly in patients with bacterial infection. To date, the greatest elevation of serum PCT are seen in bacterial infections. In previous studies, the level of PCT in viral diseases is <0.5 ng/mL [34, 35]. In our study, CRP and PCT was found to be significantly elevated in dengue patients with coinfection compared to dengue without coinfection. A CRP value > or = 12 mg/l was observed in 95 out of 99 patients (95%) with coinfection. Procalcitonin, on the other hand, has an optimal cutoff value of > 2.5 ng/mL in predicting coinfection among dengue patients, with sensitivity of 67.68% and specificity of 83.33%. A study done by Chen et al in adult patients admitted in the ICU showed that procalcitonin has a sensitivity and specificity of 81.5% and 59.5% respectively for diagnosing bacteremia using 1.14 ng/mL as a cutoff [20]. It should be noted that our study included localized and systemic bacterial infections, hence, further studies need to be done to determine the discriminative power of CRP and PCT in detecting bacterial infections between local and systemic infections in patients with dengue. Nevertheless, the addition of CRP and PCT as adjunct tests in diagnosing bacterial coinfection in dengue patients may be of value.

PCT has also been associated with dengue shock and/or multiple organ failure [33, 36, 37]. Thanachartwet, et al. showed that PCT > 0.7 ng/mL was independently associated with dengue shock and/or organ failure. It is probable that the increased levels of PCT during dengue virus infection is due to widespread inflammation in multiple organs [33]. Another study explained that organ failure may be due to the broader tropism of dengue virus leading to drastic lesions and damage in several organs [38].

Physicians are often faced with a dilemma whether or not to initiate antimicrobial treatment in dengue patients, especially in those with severe manifestations. In our study, majority of patients were given antibiotics. The most common indication for initiating antibiotics is pneumonia and a consideration of sepsis due to recurrent shock. Similar to other studies, Syue, et. al. [39] and Hadinegoro, et. al. [40] have noted that hypotension/shock or recurrent shock might be a clue to the occurrence of bloodstream infections

and suggested that cultures be obtained and antibiotics be administered in these settings. In our study, Cephalosporins, specifically cefotaxime, is the most common antibiotic used empirically for dengue patients suspected with coinfection. Since majority of bacteremic pathogens are gram-negative enteric bacteria, the use of 3rd generation cephalosporin maybe appropriate pending the results of cultures. In other studies, although done in adults, empiric antibiotic regimen recommended is levofloxacin, cefepime, or piperacillin/tazobactam [39].

It is important to correctly identify patients with dengue who are likely to have bacterial coinfections. To help us identify patients who are likely in need of empirical antibiotics and distinguish patients who will benefit most from early intervention and initiation of antimicrobial therapy, we created a scoring system to help us diagnose coinfection in dengue patients, especially in patients with no obvious focus of bacterial infection. This is the first study done in the pediatric population to determine a scoring system to diagnose coinfection in dengue patients. Previous scoring systems have been done but studied exclusively the adult population. See et. al., [19] created and validated a Dengue Dual Infection Score (DDIS) for early identification of dengue patients in need of empirical antibiotic treatment. The DDIS was a summation of five variables (each scored as 0 or 1) which were pulse rate ≥90 bpm, total leukocyte count ≥6, 000/μL, hematocrit <40%, sodium <135 mmol/L, and urea ≥5 mmol/L). A DDIS score of ≥4 was associated with coinfection in 94.4% of cases. In our study, the scoring system created had an AUC of 0.7884 in the derivation set. Only about 6% of patients with a score of 0-1 had bacterial coinfection, whereas 73.7% of patients with score of ≥ 3 had bacterial coinfections. Given this data, and using this simple scoring system, it is possible to identify patients who are likely to need close monitoring and early empiric antimicrobial therapy. We neither recommend the indiscrimate use and administration of empiric antibiotic nor the overutilization of cultures on every dengue infected patient meeting the cutoff point without carefully considering other relevant clinical and laboratory parameters. On the contrary, in cases where antibiotics are not given, patients may deteriorate and die. We should carefully use this scoring system on every dengue patient we encounter, especially those admitted in healthcare settings where all the diagnostic tests are available.

CONCLUSION

In conclusion, we have found a significant portion of dengue cases with various bacterial coinfections (64.29%). Patients with dengue and bacterial coinfections were younger with comorbidities. They presented with significantly abnormal vital signs, physical examinations findings, and elevated acute phase reactants. Using age ≤ 9 years, fever >5 days, dengue severe, and CRP >12mg/l, a scoring system was developed. A score of ≥3 can help diagnose patients with dengue and bacterial coinfection who will most likely need early empiric antimicrobial therapy.

LIMITATIONS

There are some limitations in this present study. First, the sample size is limited to 154 children and this may not be representative of all Filipino children with dengue. Second, it was conducted at a single tertiary medical center, and the patient population and clinical characteristics may not be generalizable to other settings such as in primary, or secondary hospitals and community hospitals. Lastly, we did not determine if mortalities can be prevented through early antimicrobial therapy of patients with bacterial coinfection.

RECOMMENDATIONS

We recommend further prospective studies to obtain information on clinical characteristics of dual infections in the pediatric population, especially since this is the first study of its kind.

Prospective validation of the risk scoring is also recommended to investigate its usefulness and effectiveness so that we can be more confident of its wider application in our setting.

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REFERENCES

1. Bhatt, S. et al. The global distribution and burden of dengue. Nature. 2013
2. Edillo, Frances, et al. Economic Cost and Burden of Dengue in the Philippines. Am J Trop Med Hyg. 2015
3. World Health Organization. www.who.int/denguecontrol/en/
4. Van de Weg CAM, Pannuti CS, de Araùjo ESA, van den Ham HJ, Andeweg AC, Boas LS, et al. Microbial translocation is asso- ciated with extensive immune activation in dengue virus infected patients with severe disease. PLoS Negl Trop Dis. 2013
5. Singh, RK et al. Comparison between three rare cases of co-infection with Dengue, Leptospira, and Hepatitis E: Is Early Endothelial Involvement the Culprit in Mortality? Ann Med Health Sci Res. 2014
6. Thein, Tun-Linn, Ng, Ee-Ling, et al. Risk factors for concurrent bacteremia in adult patients with dengue. Taiwan Society of Microbiology. 2015.
7. Nunez- Garhin A. et al. Coinfection of dengue and leptospirosis in a girl from the Peruvian amazon. Rev peru med exp salud publica. 2015
8. Srinivasaraghavan, Rangan. Et al. Culture proven Salmonella typhi co-infection in a child with Dengue fever: a case report. J Infect Dev Ctries 2015
9. Green AM, Beatty PR, Hadjilaou A, Harris E. Innate immunity to dengue virus infection and subversion of antiviral responses. J Mol Biol. 2014
10. Pierson TC, Diamond MS. Flaviviruses. In: Knipe DM, Howley PM. FieldsVirology, 6th ed. 2013
11. Chen C, Chan K, et al. Diagnostic performance of procalcitonin for bacteremia in patients with severe dengue infection in the intensive care unit. Journal of Infection. 2016.
12. Chen, CC., Lee, IK et al. Utility of C-Reactive Protein Levels for Early Prediction of Dengue Severity in Adults. Biomed Research International. Vol 2015.
13. Sanders S, Barnett A et al. Systemic review of the diagnostic accuracy of C-reactive protein to detect bacterial infection in nonhospitalized infants and children with fever. J Pediatr. 2008;153(4): 570-574
14. Cherry, et al. Feigin and Cherry's Textbook of Pediatric Infectious Diseases. Elsevier. Eight edition. 2019
15. 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
16. Tai BC, Machin D. 2014. Regression methods for medical research. Chichester: Wiley; 161-164.
17. 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.
18. Syue, L. et al, Bloodstream infections in hospitalized adults with dengue fever: Clinical characteristics and recommended empirical therapy. Journal of Microbiology, Immunology and Infection (2019) 52, 225e232
19. 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
20. Chen C, Chan K, et al. Diagnostic performance of procalcitonin for bacteremia in patients with severe dengue infection in the intensive care unit. Journal of Infection. 2016.
21. Chai, Louis YA, et al. Cluster of Staphylococcus aureus and Dengue Co-infection in Singapore. Ann Acad Med Singapore 2007
22. Van de Weg CAM, Pannuti CS, de Araùjo ESA, van den Ham HJ, Andeweg AC, Boas LS, et al. Microbial translocation is asso- ciated with extensive immune activation in dengue virus infected patients with severe disease. PLoS Negl Trop Dis. 2013
23. Lin CF, Lei HY, Shiau AL, Liu CC, Liu HS, Yeh TM, et al. Anti- bodies from dengue patient sera cross-react with endothelial cells and induce damage. J Med Virol. 2003
24. Premaratna R, Dissanayake D, Silva FHDS, Dassanayake M, de Silva HJ. Secondary

bacteraemia in adult patients with pro- longed dengue fever. Ceylon Med J. 2015
25. Nagassar, R. et al. Staphylococcus aureus pneumonia and dengue virus coinfection and review of implications of coinfection. BMJ Case Reports. 2012.
26. van de Weg, Cornelia, et al. Evaluation of the 2009 WHO Dengue Case Classification in an Indonesion Pediatric Cohort. Am J Trop Med Hyg. 2012
27. Guzmán MG, Kouri G, Bravo J, et al. Effect of age on outcome of secondary dengue 2 infections. Int J Infect Dis. 2002;6:118–124.
28. Lovera, D. et al. Clinical Characteristics and Risk Factors of Dengue Shock Syndrome in Children. Pediatr Infect Dis J 2016;35:1294–1299
29. Amancio, F. et al. Fatal Outcome of Infection by Dengue 4 in a Patient with Thrombocytopenic Purpura as a Comorbid condition in Brazil. 2014. Rev Inst Med Trop Sao Paulo. 2014 May-Jun; 56(3): 267–270.
30. S. A. M. Kularatne et al. Series of 10 dengue fever cases with unusual presentations and complications in Sri Lanka: a single centre experience in 2016. BMC Infectious Diseasesvolume 18, Article number: 674 (2018)
31. Triunfo, Mattia, et al. Bacterial coinfections in dengue virus disease: what we know and what is still obscure about an emerging concern. 2016
32. Potts, et al. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Trop Med Int Health. Nov; 13(11): 1328–1340. 2008
33. Thanachartwet V, Desakorn V, et al. Serum Procalcitonin and Peripehral Venous Lactate for predicting dengue shock and/or organ failure: A prospective observational study. Negl Trop Dis 10(8). 2016.
34. Branche AR, Walsh EE, Vargas R, Hulbert B, Formica MA, Baran A, et al. Serum procalcitonin mea- surement and viral testing to guide antibiotic use for respiratory infections in hospitalized adults: a ran- domized controlled trial. J Infect Dis. 2015; 212: 1692–1700. doi: 10.1093/infdis/jiv252 PMID: 25910632
35. Gendrel D, Raymond J, Coste J, Moulin F, Lorrot M, Guérin S, et al. Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentiation of bacterial vs. viral infections. Pediatr Infect Dis J. 1999; 18: 875–881. PMID: 10530583
36. Anand, D. et al. Interrelationship Between Procalcitonin and Organ Failure in Sepsis. Indian J Clin Biochem. 2014 Jan; 29(1): 93–96.
37. Dewi, R. et al. Procalcitonin, C-Reactive Protein and its Correlation with Severity Based on Pediatric Logistic Organ Dysfunction-2 (PELOD-2) Score in Pediatric Sepsis. American Journal of Epidemiology and Infectious Disease. Vol. 4, No. 3, 2016, pp 64-67. doi: 10.12691/ajeid-4-3
38. Povoa, T. et al. The Pathology of Severe Dengue in Multiple Organs of Human Fatal Cases: Histopathology, Ultrastructure and Virus Replication. PLoS One. 2014; 9(4): e83386.
39. Syue, L. et al, Bloodstream infections in hospitalized adults with dengue fever: Clinical characteristics and recommended empirical therapy. Journal of Microbiology, Immunology and Infection (2019) 52, 225e232
40. Hadinegoro S, Moedjito I, Chairulfatah A (2014) Guidelines of Diagnosis and Treatment of Dengue Virus Infection in Children. Jakarta: Working Group on Infectious and Tropical Pediatric-Indonesian Pediatric Society.