Etiologies of Acute Undifferentiated Febrile Illness in Bangkok, Thailand

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Abstract. Acute undifferentiated febrile illness (AUFI) has been a diagnostic dilemma in the tropics. Without accurate point-of-care tests, information on local pathogens and clinical parameters is essential for presumptive diagnosis. A prospective hospital-based study was conducted at the Bangkok Hospital for Tropical Diseases from 2013 to 2015 to determine common etiologies of AUFI. A total of 397 adult AUFI cases, excluding malaria by blood smear, were enrolled. Rapid diagnostic tests for tropical infections were performed on admission, and acute and convalescent samples were tested to confirm the diagnosis. Etiologies could be identified in 271 (68.3%) cases. Dengue was the most common cause, with 157 cases (39.6%), followed by murine typhus (20 cases; 5.0%), leptospirosis (16 cases; 4.0%), influenza (14 cases; 3.5%), and bacteremia (six cases; 1.5%). Concurrent infection by at least two pathogens was reported in 37 cases (9.3%). Furthermore, characteristics of dengue and bacterial infections (including leptospirosis and rickettsioses) were compared to facilitate dengue triage, initiate early antibiotic treatment, and minimize unnecessary use of antibiotics. In conclusion, dengue was the most common pathogen for AUFI in urban Thailand. However, murine typhus and leptospirosis were not uncommon. Empirical antibiotic treatment using doxycycline or azithromycin might be more appropriate, but cost-benefit studies are required. Physicians should recognize common causes of AUFI in their localities and use clinical and laboratory clues for provisional diagnosis to provide appropriate treatment while awaiting laboratory confirmation.

INTRODUCTION

The real burden of acute undifferentiated febrile illness (AUFI) has not been reported; data were available only for some important diseases.^{1,2} Among those is dengue, which was neglected in the past decades; however, its burden has been increasing worldwide.³ Although dengue infection does not need specific antiviral treatment, a previous study found that patients with dengue had a higher hospitalization rate than those with other febrile illnesses.⁴ Leptospirosis, a worldwide reemerging zoonosis, causing up to 40% mortality in its severe form, is still underreported.^{5,6} The incidence of rickettsioses, such as scrub typhus and murine typhus, as the under-recognized causes of acute fever in the past, has been increasing.^{7–9} Unfortunately, neither clinical signs nor basic laboratory investigations are specific enough in most AUFI cases, and standard investigations require time and are not available in all settings.^{2,10,11} Its diagnoses and treatment approach are difficult and still based on limited data from local epidemiological studies. With limited reliable tools for rapid diagnosis, empirical antibiotic treatment is still unavoidable, leading to unnecessary costs and, more importantly, antibiotic resistance.^{12,13}

Previous etiology studies in Thailand discovered that leptospirosis and scrub typhus were the common causative agents in AUFI.^{13–16} However, these studies were mainly conducted in rural and agricultural settings. Moreover, data on AUFI in urban and suburban areas are limited. This prospective epidemiological study was primarily performed in AUFI patients attending the fever clinic of the Hospital for Tropical Diseases (HTD) in Bangkok, Thailand, to determine the incidence of common infectious diseases endemic in an urban setting of Thailand.

Furthermore, clinical characteristics that can be used to differentiate dengue infection, which do not need antibiotic therapy, from bacterial and tropical diseases were determined to facilitate the triage of dengue patients, to minimize unnecessary antibiotic treatment, and to allow early treatment for diseases that require antibiotic treatment.

MATERIALS AND METHODS

Patients and study setting. The HTD, Bangkok, Thailand, is a 200-bed specialized university hospital in the capital city of Thailand. To facilitate and hasten the management of patients suffering from fever, the 24-hour fever clinic was established in October 2012. It has served as an urgent care center for all age febrile patients, and it has more than 3,000 patient visits per year. This prospective observational hospital-based study was conducted from July 2013 to April 2015. Adult (age ≥ 15 years) AUFI patients attending the fever clinic who fulfilled the eligibility criteria and agreed to participate in the study were enrolled. An AUFI case was defined as a patient who had acute fever (≤ 14 days) without obvious organ-specific symptoms and signs of infection, for example, abscess, pneumonia, and acute pyelonephritis. Patients with malaria diagnosed by positive blood smear and patients with obvious upper respiratory tract infections including influenza confirmed by rapid testing were excluded. Foreign travelers were also excluded to rule out the diseases harboring from their own countries. Thorough physical examination and tourniquet test (TT) were carried out. The World Health Organization criteria for TT were applied (a positive case was defined as ≥ 20 petechiae in 2.5 cm²).¹⁷ All patients were treated according to the attending doctors' decisions. Clinical outcomes were evaluated at the time of discharge in admitted cases and at the last visit in outpatient cases. The study was approved by the Ethic Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2013-023-1).

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Sample collection, laboratory testing, and definitions.

On enrollment, blood samples were collected for basic laboratory investigations for the diagnosis of etiologic organisms. Participants were asked to come back at 2–3 weeks after enrollment for follow-up and convalescent blood tests. Several rapid diagnostic tests (RDTs) were performed at enrollment to guide treatments. Additional tests for less common diseases may be conducted depending on the clinical manifestations.

Basic laboratory testing and RDTs. Basic laboratory tests including complete blood count, blood chemistries, and urine analysis were performed in HTD. Malaria smears by Giemsa staining and chest radiography were performed in all cases on the day of enrollment at HTD. Rapid diagnostic tests included QuickProfile™ Dengue Duo Panel for dengue non-structural protein 1 (NS1) antigen (Ag) and immunoglobulin (Ig) G/IgM (LumiQuick Diagnostics, Inc., Santa Clara, CA), QuickView™ *Rickettsia* IgG/IgM Combo Test (LumiQuick Diagnostics, Inc.),¹⁸ ImmunoDOT™ test for *Rickettsia typhi* (GenBio, San Diego, CA),¹⁹ QuickProfile™ *Salmonella typhi/paratyphi* Antigen Duo Test (LumiQuick Diagnostics, Inc.), QuickView™ *S. Typhi* IgG/IgM Duo Test Card (LumiQuick Diagnostics, Inc.), and Leptorapide® for leptospirosis (Linnodee, Doagh, Northern Ireland).²⁰ All RDTs were performed according to manufacturers' instruction. Sequential laboratory tests and radiological examinations were performed based on the clinical evolution.

Blood cultures. Two aerobic blood cultures were taken at different sites using BACTEC™ 9050 system (Becton Dickinson, Franklin Lakes, NJ) at HTD.²¹ All cultures were incubated for 7 days. Positive specimens were subcultured in Columbia sheep blood, chocolate, and MacConkey's agars before identification with conventional routine biochemical methods (coagulase, indole, latex agglutination, and Lancefield typing).²²

Dengue infection. Laboratory confirmation of dengue infection was defined by positive NS1Ag and/or polymerase chain reaction (PCR) for dengue and/or positive enzyme-linked immunosorbent assay (ELISA) for dengue. Polymerase chain reaction and ELISA for dengue were performed at the Tropical Medicine Diagnostic Reference Laboratory, Faculty of Tropical Medicine, Mahidol University. Dengue RNA was extracted from plasma sample using the QIAamp RNA Viral kit (Qiagen GmbH, Heiden, Germany) according to the manufacturer's recommendations. Real-time reverse transcription PCR (TaqMan) assays were performed using serotype-specific dengue (DEN) virus primers and fluorogenic probes as previously described.²³ Fourplex reaction mixtures, 50 pmol (each) of DEN-1- and DEN-3-specific primers, 25 pmol (each) of DEN-2- and DEN-4-specific primers, and 9 pmol of each probe were combined in a 50-μL volume reaction mixture. Reverse transcription for 10 minutes at 50°C was followed by 45 cycles of amplification at 60°C annealing temperature in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA) according to iScript One-Step RT-PCR kit instructions. Confirmatory dengue virus IgM (capture ELISA) and IgG (indirect ELISA) assays were performed using the commercial ELISA test kit (Focus Diagnostics, Cypress, CA).²⁴ Assays were performed according to the manufacturer's instructions. A spectrophotometer at a wavelength of 450 nm was used to measure color density. Index values were calculated from the obtained optical density and the mean of the corrected cutoff calibrator absorbance values. An index value of higher than or equal to

one is presumptive for the presence of IgM or IgG antibodies to dengue virus. Positive IgM or 4-fold rising of IgG between the acute and convalescent sera was considered acute dengue infection (positive ELISA).²⁵ The IgM/IgG ratio ≥ 1.8 was interpreted as primary dengue infection.²⁶

Rickettsioses and leptospirosis. The diagnosis of leptospirosis, scrub, and murine typhus was based on either real-time PCR or serological tests. Real-time PCR assays for murine typhus and scrub typhus were performed using primers specific to the rickettsial citrate synthase gene²⁷ and partial 16S rRNA gene of scrub typhus,²⁸ respectively. In brief, the DNA template was extracted from 200 μL of blood using Blood Genomic DNA Extraction kit, FavorPrep™ (Ping-Tung, Taiwan). The DNA template was then mixed with LightCycler® 480 Probes Master (Rotkreuz, Switzerland) reaction mixture as recommended by the company. For leptospirosis, the primers and probe sequences specific to partial 16S rRNA gene were Lepto-F (5'-CCC GCG TCC GAT TAG-3'), Lepto-R (5'-TCC ATT GTG GCC GRA CAC-3'), and Lepto-P probe (5'-FAM-TCA CCA AGG CGA CGA TCG GTA GC-BHQ-3').²⁹ For murine typhus detection, primers and probe sequences were CS-F (5'-TCG CAA ATG TTC ACG GTA CTT T-3'), CS-R (5'-TCG TGC ATT TCT TTC CAT TGT G-3') and the probe was CS-P (5'-CY5-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-BHQ-3'). For scrub typhus detection, primers and probe sequences were OT3-F (5'-CCC ATC AGT ACG GAA TAA CA-3'), OT1-R (5'-CTC TCA GAC CAG CTA CAG ATC ACA-3'), and the probe was OT-P (5'-HEX-TAA GTG CTA ATA CCG TAT GCC CTC TA-BHQ-3'). Real-time PCR was performed and analyzed using the LightCycler 480. Moreover, serological tests for IgM and IgG for scrub typhus and murine typhus were performed in both acute and convalescent sera using immunofluorescent assay (IFA) tests prepared by the Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand, as described elsewhere.^{14,30} A positive PCR result and/or a seroconversion (≥ 4 -fold increase in either IgM or IgG titer) was considered a confirmed case.^{31,32} Microscopic agglutination test (MAT) for leptospirosis was performed using a set of 24 serovars at the Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, as described elsewhere.³³ The seroconversion was used to determine the positive result.

Influenza. Serological test for influenza was performed in acute and convalescent sera using Anti-Influenza A Virus ELISA IgG (EUROIMMUN, Lübeck, Germany). Sera were diluted 1:101 in a sample buffer. Quantitative analysis was performed as per manufacturer's protocol. The color intensity was measured by a spectrophotometer at a wavelength of 450 nm. The standard curve was obtained by point-to-point plotting of the extinction values measured for the three calibration sera. The results were calculated and reported in relative units (RU)/ml according to the manufacturer's instruction. The $\geq 50\%$ increase in the RU/ml value of the convalescent sera compared with acute sera was found to correlate with a 4-fold increase in titer.³⁴ Thus, it was used as the diagnostic criteria of a current influenza infection.

Case definitions. Diagnosis was made using clinical pictures compatible with diseases plus confirmatory laboratory results as stated previously. Medical record and laboratory investigations of each case were reviewed for diagnosis by infectious diseases physician and tropical medicine clinicians.

Statistical analysis. Data were extracted and checked. Double data entries were applied, and data were validated, cleaned, and analyzed using SAS software version 9.4 (SAS Institute, Inc., Cary, NC). Chi-square test and *t*-test were used for comparison of categorical data and continuous data between two groups, respectively. Log-binomial regression (PROC GENMOD) was used to calculate relative risk (RR) and 95% confidence interval (CI). Multiple regression model analysis was performed to adjust for occupation, underlying diseases, and age of patients.

RESULTS

A total of 4,621 adult patients had attended the fever clinic from July 2013 to April 2015. Of those cases, 2,859 were excluded, and the most common exclusion criterion was organ-specific infection followed by malaria. Among patients who were eligible for inclusion, only 397 patients consented to participate in the study (Figure 1). Complete history taking, physical examinations, and first blood collection were performed in all cases, but the convalescent sera could be obtained in 315 cases (79.3%).

Demographic and clinical data of all enrolled patients are shown in Table 1. There were more female than male patients, with an approximate ratio of 3:2. The mean (SD) age of patients was 33.9 (14.1) years. Most patients (69%) did not have underlying medical conditions. The mean (standard deviation [SD]) days of fever before enrollment were 4.2 (2.3) days. Other than fever, the most common symptom was myalgia (86.4%), followed by chills (76.3%) and headache (70.5%). Mucosal bleeding was reported in 11.1% of patients. However, there were only four cases of active bleeding during hospitalization, that is, two cases of gastrointestinal tract bleeding and two cases of hematoma. Although 96.4% of patients lived in Bangkok Metropolitan area, almost 60% of patients reported a history of recent travel (within 3 months before the onset of fever) and half of them traveled to a forested area where malaria and scrub typhus are endemic. Approximately 12% of patients received antibiotic treatments for this fever episode

before enrollment either by doctor-prescribed or over-the-counter medicine. On physical examination, rash was reported in 18.6%. Only one-third of patients had positive TT (112 of 323 cases, 34.7%).

Causes of fever could be identified in 271 cases (68.3%). Dengue infection was the most common cause, with 157 cases (39.6%), followed by 20 cases of murine typhus (5.0%) and 16 cases of leptospirosis (4.0%) (Figure 1). Coinfection identified by confirmatory tests was reported in 37 cases (9.3%) (Table 2).

Hemocultures were positive in 11 of 397 (2.8%) cases. Among these, three coagulase-negative *Staphylococci* without signs and symptoms of skin infection were regarded as contamination. The identified pathogens were two cases of *Escherichia coli*, two cases of *Streptococcus agalactiae*, and one case each of *S. typhi*, *S. paratyphi A*, *Salmonella* group C, and *Streptococcus viridans*. Two cases were diagnosed as coinfection, *S. typhi*/dengue and *S. agalactiae*/influenza. Human immunodeficiency virus infection was found in one case of *Salmonella* septicemia. Interestingly, community-acquired extended-spectrum beta-lactamase strains were identified in two cases (*E. coli* and *Salmonella* group C).

Diagnostic laboratory tests for dengue were performed in all cases and were positive in 188 patients (47.4% of all participants), including those coinfecting with other diseases. The PCR for dengue, NS1 Ag, and ELISA for dengue were positive in 144 (36.6%), 105 (26.4%), and 135 (34.0%) cases, respectively. Around one-third of the cases had positive results in all investigations (Table 3). The most common identified serotype was serotype 3 (70 cases, 48.6%), followed by serotype 4 (33 cases, 22.9%), serotype 1 (24 cases, 16.7%), and serotype 2 (11 cases, 7.6%). Interestingly, six cases were infected with more than one serotype at the same time. There were 10 (5.3%) cases of primary infection and 178 (94.7%) cases of secondary infection.

Among 31 cases of murine typhus (20 cases of murine typhus alone and 11 cases of coinfection with other diseases), diagnoses were confirmed by both PCR and IFA in seven cases, PCR alone in 11 cases, and IFA alone in 13 cases.

FIGURE 1. Study flow.

TABLE 1

Demographic and clinical data at the time of enrollment, 397 cases

Demographic and clinical data	n	%
Gender: Male	171	43.1
Age (years), mean (SD)	33.86 (14.08)	—
Age group		
15–20	85	21.4
21–30	110	27.7
31–40	79	19.9
41–50	57	14.3
51–60	50	12.6
61–70	11	2.8
> 70	5	1.3
Race		
Thai	351	88.4
Burmese	25	6.3
Cambodian	3	0.8
Others	18	4.5
Occupation		
Labor	33	8.6
Sedentary worker	179	46.7
Student	85	22.2
Others	86	22.5
Preexisting medical illnesses	122	30.7
Diabetes mellitus	22	5.6
Hypertension	31	7.9
Others	69	17.6
Pattern of care seeking		
First medical attention	291	73.3
Referred from other hospitals	106	26.7
Clinical manifestations*		
Days of fever (days), mean (SD)	4.2 (2.3)	—
Body temperature (°C), mean (SD)	38.3 (0.9)	—
Chills	302	76.3
Headache	279	70.5
Myalgia	343	86.4
Joint pain	47	13.0
Cough	126	31.7
Nausea	211	53.2
Vomiting	127	32.0
Diarrhea	89	22.4
Bleeding	44	11.1
Anemia	7	1.8
Jaundice	9	2.3
Presentation of petechiae	37	9.5
Rash	73	18.6
Hepatomegaly	45	11.4
Positive tourniquet test†	112	34.7
Recent travel history	230	58.1
Recent travel history to forested area	101	25.4
Dengue infection in neighborhood	59	15.1
Antibiotic before enrolment	49	12.3
Patient treatment		
Outpatient care and follow-up	96	24.2
Inpatient care	301	75.8
Length of stay‡ (days), mean (SD)	4.87 (3.77)	—
Outcome		
Recovered	378	95.2
Transferred out	10	2.5
Died	2	0.5
Loss to follow-up	7	1.8

* Missing responses in some parameters.

† Tourniquet test was performed in 323 cases.

‡ In-patients only.

Scrub typhus was identified positive in both PCR and IFA in one case, positive PCR alone in one case, and positive IFA alone in six cases. Leptospirosis was diagnosed in 28 cases (16 cases of leptospirosis alone and 12 cases of coinfection with other diseases). Of the 315 cases with paired sera, MAT for leptospirosis showed seroconversion in four cases, and the identified serovar were *Leptospira hebdomadis*, *Leptospira pomona*, *Leptospira*

TABLE 2

Coinfection identified in the study

Coinfections	n
Dengue + leptospirosis	9
Dengue + influenza	9
Dengue + murine typhus	8
Dengue + scrub typhus	2
Dengue + leptospirosis + murine typhus	1
Dengue + hepatitis A	1
Dengue + bacteremia	1
Murine typhus + influenza	2
Leptospirosis + scrub typhus	1
Leptospirosis + influenza	1
Scrub typhus + other	1
Influenza + bacteremia	1

sejroe, and *Leptospira pyrogenes*. Among 26 cases with positive PCR for leptospirosis, MAT was positive in two cases, negative in 20 cases, and no convalescent sera in four cases.

The empirical antibiotic treatment was prescribed in 208 cases. Among 65 (16.4%) confirmed cases of leptospirosis and rickettsioses, both with and without concurrent infections, antibiotics were prescribed in 53 (81.5%) cases. The most common regimen was third-generation cephalosporin plus doxycycline (19 cases), whereas doxycycline or azithromycin alone was given in 17 and four cases, respectively. Moreover, 21.0% (33 of 157) of cases diagnosed with dengue infection alone received antibiotics.

Although dengue-specific treatment is not available, dengue-infected patients need close monitoring. By contrast, antibiotics should be prescribed in other diseases such as leptospirosis, rickettsioses, and bacteremia as soon as possible. Further analysis was conducted to determine whether clinical parameters at presentation can be used to distinguish dengue from other etiologies that require antibiotics. Patients with influenza (14 cases), unidentified etiology (126 cases), and coinfection of dengue and other diseases (31 cases) were excluded from this analysis. The demographic data, clinical information, and laboratory results of 157 dengue cases were compared with those of 69 non-dengue cases with probable benefit from antibiotic treatments. The multivariate analysis was performed by adjusting age groups (Table 4). Significantly independent variables associated with laboratory-confirmed dengue infections were hematocrit $\geq 40\%$ (RR: 1.21, 95% CI: 1.01–1.44), white blood count (WBC) $\leq 4,500/\text{mm}^3$ (RR: 2.31, 95% CI: 1.79–2.99), lymphocyte $\geq 30\%$ (RR: 1.26, 95% CI: 1.07–1.49), atypical lymphocyte $\geq 8\%$ (RR: 1.44, 95% CI: 1.23–1.69), platelet count $\leq 80,000/\text{mm}^3$ (RR: 1.52, 95% CI: 1.32–1.75), and an aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio ≥ 1.3 (RR: 1.29, 95% CI: 1.10–1.51). Neither history (history of dengue in neighborhood, nausea, and vomiting) nor physical

TABLE 3

Investigations of dengue infection, 188 cases

Positive results of investigations	n (N = 188)	%
All: PCR + ELISA + NS1 antigen	67	35.6
PCR + ELISA	29	15.4
PCR + NS1 antigen	24	12.8
ELISA + NS1 antigen	9	4.8
PCR alone	24	12.8
ELISA alone	30	15.9
NS1 antigen alone	5	2.7

ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; NS1 = non-structural protein 1.

TABLE 4
Predicting factors for distinguishing dengue infection from other acute undifferentiated febrile illness: multivariate analysis

Factor	Non-dengue <i>n</i> (%), <i>N</i> = 69	Dengue <i>n</i> (%), <i>N</i> = 157	Age-adjusted	
			Relative risk	95% confidence interval
Gender				
Female	24 (26)	70 (74)	1.04	0.90–1.21
Male	45 (34)	87 (66)	1	–
History of dengue in the neighborhood				
Yes	7 (16)	37 (84)	1.10	0.97–1.25
No	61 (34)	116 (66)	1	–
Nausea				
Yes	33 (25)	99 (75)	1.11	0.94–1.32
No	36 (38)	58 (62)	1	–
Vomiting				
Yes	17 (21)	64 (79)	1.08	0.93–1.26
No	52 (36)	93 (145)	1	–
Presentation of petechiae				
Yes	2 (7)	25 (93)	1.12	0.77–1.63
No	67 (34)	130 (66)	1	–
Tourniquet test				
Positive	13 (18)	59 (82)	1.15	1.00–1.33
Negative	43 (41)	61 (59)	1	–
Hematocrit (%)				
≥ 40%	28 (24)	88 (76)	1.21*	1.01–1.44
< 40	41 (37)	69 (63)	1	–
White blood count (/mm ³)				
≤ 4,500	12 (9)	120 (91)	2.31*	1.79–2.99
> 4,500	57 (61)	37 (39)	1	–
Lymphocyte (%)				
≥ 30%	8 (17)	39 (83)	1.26*	1.07–1.49
< 30%	61 (34)	118 (66)	1	–
Atypical lymphocyte (%)				
≥ 8%	6 (11)	50 (89)	1.44*	1.23–1.69
< 8	55 (38)	89 (62)	1	–
Platelet (/mm ³)				
≤ 80,000	4 (7)	54 (93)	1.52*	1.32–1.75
> 80,000	65 (39)	103 (61)	1	–
Aspartate aminotransferase/alanine transaminase ratio				
≥ 1.3	18 (15)	104 (85)	1.29*	1.10–1.51
< 1.3	51 (49)	53 (51)	1	–

* Relative risk adjusted for age.

signs (petechiae and positive TT) were significant in the multivariate analysis.

DISCUSSION

As expected, the most common identifiable cause of AUI in this study was dengue infection, followed by murine typhus. This might be explained mainly by the urban setting of our hospital and the global increase in the incidence of dengue infection.³ Notably, there was a huge outbreak of dengue infection throughout Thailand in 2013. This result was different from that reported in previous AUI etiology studies in Thailand. The study conducted at the Thai–Myanmar border found malaria as the most common pathogen, followed by leptospirosis.^{8,16} In Thailand, malaria occurs mainly in border areas where the vector is available; thus, we excluded malaria from our AUI study. However, two studies which took place in an agricultural/rural setting found leptospirosis and scrub typhus as the common causative agents.^{13,15} Study sites, timing, and laboratory confirmation investigation methods might mainly contribute to these differences.¹

Coinfection is not uncommon in AUI, with varying proportions depending on study methodology.^{1,35} Furthermore, concurrent infection with two serotypes of dengue found in six cases in our report is occasionally reported in

hyperendemic areas, especially during outbreaks.^{36,37} However, there were limited data about the characteristic of cases and pathogenesis. It would be interesting to explore further on the study in both epidemiological data and pathogenesis.

The high unidentified etiology rate in this study may be explained by unobtainable convalescent sera in 82 cases (20.7%). In addition, 12.4% of participants received antibiotic treatment before enrollment, both over-the-counter medicine and from previous health care. By contrast, among 315 cases that completed vigorous investigations, we could not identify the causative pathogens in 95 (30.2%) cases. This finding emphasized the impact and challenges of AUI.

Nevertheless, this result provides information to guide the clinical practice and prevention policy. Doxycycline was proved to have cost–benefit as the empirical approach in rural setting in Thailand and was suggested as useful treatment in a study from Egypt because it is an effective treatment of leptospirosis and rickettsioses.^{35,38} Azithromycin has been recommended in the case of doxycycline allergy or pregnancy.^{8,39} According to our result, the advantage of using doxycycline or azithromycin to empirically treat leptospirosis, rickettsioses, and typhoid should be weighed against risks of adverse events and drug resistance. In addition, the low incidence of bacteremia in AUI cases from our study reflected the limited advantage of blood culture and empirical cephalosporin

treatment as a routine in all AUI cases. The nationwide surveillance in both urban and rural settings should be conducted to determine common pathogens in different settings together with the cost-benefit analysis of diagnostic and therapeutic approaches.

Differentiating dengue from other tropical diseases is necessary toward close medical observation for dengue and appropriate antibiotic treatment. However, it is difficult to distinguish dengue from leptospirosis and rickettsioses, especially in the early stages because of the common geographic distribution and similar clinical presentations.^{6,10,40} The sensitivity and specificity of RDTs for dengue depend on the day after onset and serotypes. NS1-based RDTs have decreased sensitivity in secondary infections, which is most of the adult cases.^{41,42} The mean duration between fever onset to enrollment for patients in this study was 4.2 days, which is the “window period” where dengue NS1 Ag sensitivity begins to decline and IgM is usually undetectable. Although PCR is a method of choice, availability and cost limit its usage in the tropics. Predictive factors, multivariable model, or decision tree algorithms were used to distinguish dengue from other AUI.^{4,43–47} However, inconsistencies in study findings may have resulted from differences in study parameters, such as population, timing, and febrile illness comparators.⁴³

In this study, symptoms and signs were not useful to predict dengue infection. Although previous studies from HTD found the usefulness of TT in dengue diagnosis⁴⁸ (Putri et al., unpublished data), TT did not show a significant predictive value for dengue in this study. Among basic laboratory parameters, leucopenia and thrombocytopenia were commonly proposed as clinical predictors with different definitions, for instance, WBC of $< 5,000/\text{mm}^3$ in Gregory et al.'s study and WBC $\leq 6,000/\text{mm}^3$ Tanner et al.'s study.^{43,44,49} Furthermore, the diagnostic value of leucopenia was found to have increased with age in a previous study.⁴⁵ We proposed that the cutoff point of platelets would be $\leq 80,000/\text{mm}^3$, not the classic definition of $150,000/\text{mm}^3$, as leptospirosis and murine typhus frequently have mild thrombocytopenia.^{6,9,50} The proportions of lymphocyte and atypical lymphocyte were less mentioned in studies compared with leucopenia. The atypical lymphocyte increase by time from onset to defervescence and the cutoff point of 10% was proposed as indicator in a previous study.⁵¹ Higher AST than ALT level is one of the characteristics of dengue.^{47,52,53} Although the retrospective study from our hospital found an average AST/ALT ratio of 1.8:1 in dengue cases, no previous study mentioned the cutoff point of AST/ALT ratio to distinguish dengue from other AUI.⁵³ This study demonstrated that then AST/ALT ratio ≥ 1.3 has a predictive value.

There was no perfect single parameter in clinical practice.⁴³ The diagnosis model of dengue for acute febrile illness patients, using Bayesian network model, was developed from the data of this study and could demonstrate similar sensitivity and specificity as experienced physicians.⁵⁴ Multiple parameters should be applied to guide clinical approaches, and prospective diagnostic validation should be conducted.

A number of limitations are worth mentioning. The single-center study design might limit the interpretation and application of results. As HTD is one of the referral hospitals for fever patients, there might be a selection bias resulting in the high admission rate of this cohort. Given the limited funding, we focused our workup for common pathogens reported in Thailand,

and investigations of some diseases such as Zika, chikungunya, and cytomegalovirus were performed only in suspected cases. Spotted fever group rickettsioses, Q fever, and other emerging pathogens that existed in Thailand were not investigated.^{15,16} However, we are still attempting to identify new emerging pathogens such as Zika virus in our specimens. Finally, the use of in-house PCR for leptospirosis, scrub typhus, and murine typhus may limit the generalization of the results.

In conclusion, dengue was the common etiology of AUI in this study, followed by murine typhus and leptospirosis. The empiric antibiotic treatment with third-generation cephalosporin in mild cases might have limited advantages. The advantage of empirical treatment using doxycycline or azithromycin in AUI in urban setting still needs further evaluation. The proposed predicting factors to distinguish dengue from other tropical diseases that required antibiotic treatment were hematocrit $\geq 40\%$, leucopenia, atypical lymphocyte $\geq 8\%$, platelets $\leq 80,000/\text{mm}^3$, and the AST/ALT ratio ≥ 1.3 . Further improvement for point-of-care diagnostic test would be valuable.

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