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A pilot study investigating severe community-acquired febrile illness through implementation of an innovative microbiological and nucleic acid amplification testing strategy in Timor-Leste (ISIN-MANAS-TL)

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ABSTRACT

Objectives: Acute febrile illness (AFI) causes significant health-seeking, morbidity, and mortality in Southeast Asia. This pilot study aimed to describe presentation, etiology, treatment, and outcomes of patients with AFI at one hospital in Timor-Leste and assessing the feasibility of conducting larger studies in this setting.
Methods: Patients attending Hospital Nacional Guido Valadares with tympanic or axillary temperature ≥37.5°C in whom a blood culture was taken as part of routine clinical care were eligible. Participants were followed up daily for 10 days and again after 30 days. Whole blood was analyzed using a real-time quantitative polymerase chain reaction assay detecting dengue virus serotypes 1-4 and other arthropod-borne infections.
Results: A total of 82 participants were recruited. Polymerase chain reaction testing was positive for dengue in 14 of 82 (17.1%) participants and blood culture identified a bacterial pathogen in three of 82 (3.7%) participants. Follow-up was completed by 75 of 82 (91.5%) participants. High rates of hospital admission (58 of 82, 70.7%), broad-spectrum antimicrobial treatment (34 of 82, 41.5%), and mortality (9 of 82, 11.0%) were observed.
Conclusions: Patients with AFI experience poor clinical outcomes. Prospective observational and interventional studies assessing interventions, such as enhanced diagnostic testing, clinical decision support tools, or antimicrobial stewardship interventions, are required and would be feasible to conduct in this setting.

Introduction

Acute febrile illness (AFI) is a significant cause of health-seeking behavior, morbidity, and mortality in adults and children in Southeast Asia. Infectious etiologies are wide-ranging and depend on the setting. However, arthropod-borne viral infections, including dengue viruses 1-4, systemic bacterial infections, and malaria, are all common causes in the region [1,2]. These require disparate treatments.

Targeted management of patients with acute febrile illness

Culture-based, molecular, and serological techniques can be used to analyze clinical samples from patients with AFI [3,4]. These "diagnostic tests" aim to determine the likely infecting organism and may indicate its antimicrobial susceptibilities. They include rapid diagnostic tests (RDTs), which can be operated at the point of care. Such diagnostic testing is important in the delivery of targeted care to patients with AFI, including antimicrobial treatments [5,6]. Diagnostic testing in AFI is also required in systems of passive disease surveillance [7,8]. Clinical decision support systems (CDSSs), which analyze various forms of clinically derived data to help health care providers make decisions, and antimicrobial stewardship interventions (AMSIs), which aim to improve antimicrobial prescribing, are other potentially useful interventions to improve the management of patients with AFI [9–12]. Although significant technological advances have been made across these areas, including the development of highly accurate tests for common causes of AFI, few

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studies have so far assessed the impact of such interventions on the clinical outcomes of AFI when they are implemented within patient pathways [13,14]. This is particularly true in low-middle–income countries. Studies of malaria RDTs and three recently published randomized controlled trials assessing the impact of implementing a package of diagnostic tools on antimicrobial prescribing are notable exceptions [15–19].

Timor-Leste as a potential study site

Timor-Leste is a half-island nation located between Indonesia and Australia with a population of 1.3 million. Despite significant recent improvements in diagnostic laboratory and infectious disease surveillance capacity, including the implementation of bacterial culture and molecular diagnostic platforms for SARS-CoV-2 and tuberculosis at multiple sites across the country, there have been no prospective studies of AFI in Timor-Leste [20,21]. In 2008-2010, a retrospective review of pediatric hospitalizations at the Hospital Nacional Guido Valadares (HNGV), Dili found that AFI was the most common reason for admission; however, only limited etiological, treatment-related and outcome data were available in this study [22]. Dengue is a major emerging public health concern in Timor-Leste, with widespread outbreaks occurring during rainy seasons [23].

Study aim

The primary aim of this pilot study was to describe the patient pathway for severe AFI at one site in Timor-Leste, gathering preliminary data on clinical features, etiology, antimicrobial treatment, and outcomes. The secondary aim was to assess the feasibility of conducting further prospective observational and/or interventional studies, which may assess the impact of novel diagnostic tests, CDSSs, and/or AMSIs when implemented in patient pathways in this setting.

Methods

Study design

This is a prospective, observational cohort study.

Setting

This study took place at the emergency department (ED) at the national referral hospital, HNGV, providing inpatient and outpatient care for adults and children who are either self-presenting or referred from municipal hospitals, community clinics, or health posts. Samples were analyzed at Laboratorio Nacional da Saúde (LNS), which is adjacent to HNGV and is the central diagnostic and surveillance laboratory in Timor-Leste, undertaking bacterial culture, molecular, and serological techniques.

Participants

All patients presenting to the department and undergoing triage by an ED nurse between 09:00 and 16:00 on weekdays between March 22, 2023 and June 23, 2023 were assessed for inclusion. Those aged over 1 year with a tympanic or axillary temperature ≥37.5°C in whom a blood culture was taken as part of routine clinical care were eligible. These pragmatic inclusion criteria were required owing to the limited resources available to conduct this pilot study and to minimize its impact on care delivery in a busy ED.

Initially, only patients presenting directly to the ED from the community were included. However, during the 6th week of recruitment, eligibility was changed to additionally include participants who had spent up to three nights as an inpatient in another hospital and were then transferred to HNGV and assessed at the ED. These individuals were still considered most likely to have a community-acquired cause of AFI, despite their 1-3–night stay in another hospital. This was in response to early data that showed that the majority of HNGV ED attendees presented elsewhere first, which resulted in low recruitment.

Data and sample collection

Baseline data including demographic, clinical, and treatment-related details were collected by ED nurses or doctors by interviewing the participant and their family and by reviewing their medical records. This occurred after the participant's initial assessment by a clinician and included provisional diagnoses made based on history-taking, clinical examination, and vital signs. Data were collected onto a paper questionnaire and later transcribed into electronic format on a mobile application with the REDCap application installed. Adult participants then provided 4 ml of whole blood and child participants provided 1 ml of whole blood by phlebotomy into an ethylenediamine tetraacetic acid sample collection tube. This was transported within 8 hours to the laboratory, where it was refrigerated at 4°C until it was analyzed.

Follow-up data were collected by research nurses who were interviewing participants on days 1-10 and day 30 after their recruitment. This was done face-to-face if the participant was an inpatient at HNGV or by telephone if otherwise. If a participant was not contactable (and when follow-up was scheduled for a weekend) the interview was marked as unsuccessful and the research nurse moved to the next scheduled interview (usually the next day). This pragmatic follow-up strategy was required owing to the limited human resources available to conduct this pilot study. If the 30-day interview was unsuccessful, then up to two further attempts were made on days 31 and 32. Each follow-up was focused on ascertaining whether the participant was still experiencing fever (or fever symptoms), whether they were being treated with antimicrobial medications, and whether they had experienced any adverse outcomes since the last follow-up. For adult participants, disability was assessed at the 30-day interview only, using the World Health Organization Disability Assessment Schedule 12-item functional questionnaire. Follow-up data were input directly into electronic format on a mobile application with the REDCap application installed.

Sample analysis

Diagnostic testing was conducted using the Australia Diagnostics Mosquito Panel, which is a research-only multiplexed quantitative polymerase chain reaction (PCR) assay with dengue virus 1-4, chikungunya virus, West Nile virus, Barma Forest virus, zika virus, Japanese encephalitis virus, Murray Valley encephalitis virus, Ross River virus, malaria, and artemisinin resistance targets (PCR test). Nucleic acid extraction was done using MT-Prep Viral/Pathogen Nucleic Acids Extraction kits on the MT-Prep Extraction platform. This assay was implemented at LNS as part of this study. Before the start of the study, detection of dengue viruses using this assay was verified by analyzing 22 samples which were positive and two samples which were negative for dengue nonstructural protein 1 by lateral-flow RDT, with 100% concordance. Detection of non-dengue targets could not be verified because samples determined positive by other laboratory methods were not available. Results from blood culture (undertaken as part of each participant's standard clinical care) were also collected from LNS records.

Data analysis

For adults, baseline shock index was calculated by dividing their pulse rate (beats per minute) by their systolic blood pressure (mm Hg) and their SpO_2/FiO_2 ratio was calculated by dividing their oxygen saturation by the fraction of inspired oxygen. These were chosen as pragmatic measures of cardiovascular and respiratory compromise, respectively, which could be derived from vital signs being measured routinely in ED (i.e. without the need for arterial blood gas and lactate

measurement). For all participants, the duration of antimicrobial therapy in whole days was calculated from available data at each follow-up. When a participant went from receiving antimicrobials to not receiving antimicrobials (or *vice versa*) during a period when they missed ≥1 day of follow-up, the change was assumed to have taken place on the middle day (rounded up). The duration of inpatient hospital admission was calculated in a similar way.

Descriptive analysis was undertaken with categorical and continuous demographic, clinical, treatment-related, diagnostic testing, and outcome variables being presented as proportions and median values (with interquartile ranges [IQRs]), respectively. Participants in whom PCR testing was positive for dengue were compared with other participants in the univariable analyses with associations with categorical and continuous variables tested using chi-square and Mann–Whitney U tests, respectively. This *a posteriori* decision was made because dengue was the most commonly identified cause of AFI and there was significant interest among researchers in understanding patient pathways and outcomes in this group of participants. R (version 4.2.2) in RStudio version 2023.06.2+561 was used for all data manipulation, analysis, and visualization.

Ethical considerations

Participants aged ≥16 years provided written informed consent. Those aged <16 years provided verbal assent, with their parent or guardian providing written consent. For those assessed by the HNGV ED nurses or doctors as lacking the capacity to make decisions about participation in this study, the next of kin were asked to provide a declaration that they support their relative's participation. This study received ethical approval from the Instituto Nacional da Saúde Research Ethics Committee, Timor-Leste (Reference 157-MS-INS/GDE/I/2023) and the Northern Territory Research Ethics Committee, Australia (Reference 2023-4505).

Results

Recruitment and baseline characteristics

A total of 5141 patients presented to HNGV ED between 09:00 and 16:00 on weekdays between March 22, 2023 and June 23, 2023 and were screened for inclusion. A total of 93 of these were identified by ED staff to have community-acquired AFI and were referred to the study team for formal assessment against inclusion criteria. A total of 85 of these fulfilled the criteria, of whom 82 agreed to participate. Figure 1 shows the flow of individuals through study recruitment and participation.

A total of 38 (46.3%) participants were male. A total of 52 (63.4%) participants were adults (aged ≥16 years, median [IQR] age 33 [25-52.8] years). The adults' median (IQR) duration of fever symptoms was 2 (1-4) days. A total of 30 (36.6%) participants were children whose median (IQR) age was 4.5 (3-7) years. The children's median (IQR) duration of fever was 2 (1.3-3) days. All participants reported clinical features, which localized the infection to a possible anatomic site, including 70 (85.4%) with one or more gastro-intestinal feature and 32 (39.0%) with one or more respiratory feature. A total of 35 (42.7%) participants had received antimicrobials before recruitment and 19 (23.2%) had been an inpatient for 1-3 days at another health care facility before recruitment. There was a presumptive diagnosis of dengue in 30 (33.3%) cases, of which eight of 30 (26.7%) either had "warning signs" or were "severe."

Results from diagnostic testing

PCR testing was positive for dengue in 14 (15.6%) participants. This included 10 participants with serotype III infection and one participant each with serotypes I, II, and IV infections. One participant tested positive for serotypes I and III. One participant with a clinical diagnosis of acute appendicitis (and not dengue) was PCR-positive for dengue. In 17 of 30 (18.9%) cases, PCR testing was negative, despite a clinical diagnosis of dengue having been made. Blood culture was positive in four (4.4%) cases, with *Klebsiella pneumoniae* in two participants, *Staphylococcus aureus* in one participant, and *Pseudomonas stutzeri* (not considered to be a clinically significant result but rather a blood culture contamination) in one participant. Table 1 summarizes the results from diagnostic testing.

Participant follow-up and outcomes

Of the 82 included participants, 75 (91.5%) were followed up for the full 30 days or until they died. The median (IQR) follow-up duration for the seven (8.5%) individuals who were lost to follow-up was 9 (8-10) days. The median (IQR) antimicrobial treatment duration of all 47 treated participants was 7 (4.5-9) days. The most commonly used antimicrobials were ceftriaxone (received by 72.3% of treated participants), ampicillin/amoxicillin (40.4%), metronidazole (42.6%), cloxacillin (19.1%), and meropenem (4.2%). The median (IQR) duration of hospital admission was 5 (3.3-9) days and there were no re-admissions within the 30-day follow-up period to HNGV or other health care facilities. At 30 days, the median (IQR) World Health Organization Disability Assessment Schedule 12-item functional outcome (only collected for adults) was 60 (58-60). Nine (11.0%) participants died within 30 days of recruitment. The causes of death included hypoxic encephalopathy, meningoencephalitis, bronchopneumonia and cardiomyopathy, severe hypokalemia, tuberculosis, severe dengue with hemorrhagic complications, and aplastic anemia, with one participant for each. The cause of death was "unknown, reported by the family" for two participants. Table 2 summarizes the demographic, clinical, treatment-related, and outcome data. Figure 2 summarizes each participant's journey, including whether they were an inpatient and whether they were receiving antimicrobials over time during their follow-up. Figure 3 is a Kaplan–Meier plot showing the survival of participants with and without dengue.

In the univariable analyses, participants with dengue were more likely to be children ($P = 0.005$), had a higher SpO_2/FiO_2 ratio (assessed in adults only, $P = 0.031$), and received shorter durations of antimicrobial treatment (assessed in treated individuals only, $P < 0.001$) than those without dengue.

Discussion

To the best of our knowledge, this is the first prospective observational study enrolling participants with severe community-acquired AFI in Timor-Leste, describing the patient pathway of 82 individuals presenting to the national referral hospital in Dili, including their clinical features, etiology, antimicrobial treatment, and outcomes.

In addition to fever, many participants presented with clinical feature(s) which potentially localized the infection to the gastro-intestinal (85.4%) or respiratory (39.0%) tracts. Many had been an inpatient at another health care facility before enrollment (23.2%) or were already receiving antimicrobial treatments (42.7%). This suggests that a significant number of participants were recruited late in their patient pathway and that future studies of community-acquired AFI should additionally be conducted at smaller hospitals and health posts in the region.

Dengue (15.6%) and systemic bacterial infections (3.3%) were the only identified etiologies, despite all participants undergoing enhanced diagnostic testing with a newly implemented PCR assay for other potentially relevant arthropod-borne infections, including chikungunya virus, West Nile virus, Japanese encephalitis virus, zika virus, and malaria. Therefore, the majority of participants did not receive a confirmed diagnosis. It is possible that the positive detection rate in cases of dengue and/or other arthropod-borne infections would have been higher had participants who presented earlier in their patient pathway (i.e. during the viremic phase of illness). Additional retrospective serological and/or molecular testing of samples from the present study may be useful. Other

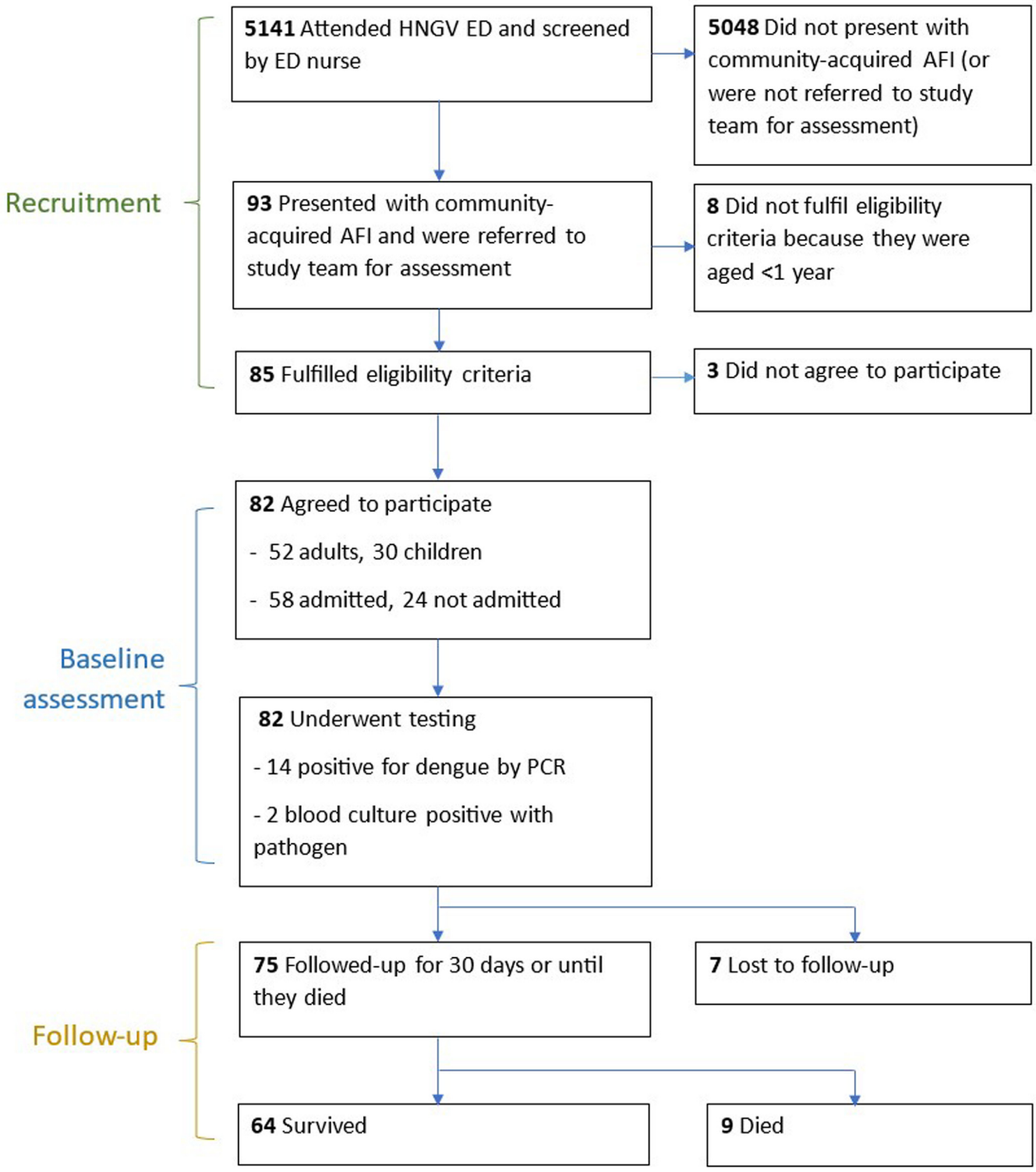

Figure 1. Flow of individuals through recruitment and study participation. AFI, acute febrile illness; ED, emergency department; HNGV, Hospital Nacional Guido Valadares; PCR, polymerase chain reaction.

observational studies of severe AFI in Southeast Asian countries have found high incidence of dengue infection [1,2].

Most participants (57.3%) received antimicrobial treatment at enrollment or during their follow-up, with broad-spectrum antibiotics (primarily ceftriaxone) being commonly prescribed. The treatments were largely empirical because only four participants had a positive blood culture. Improved access to diagnostic testing, CDSSs, and AMSIs may be beneficial to target therapies and reduce the inappropriate use of antimicrobials in this population.

A high proportion of participants in this study completed follow-up (91.5%), with successful data collection from face-to-face and telephone interviews. Highly variable hospital admission duration and antimicrobial treatment durations were observed and, overall, the participant mortality was high (11.0%).

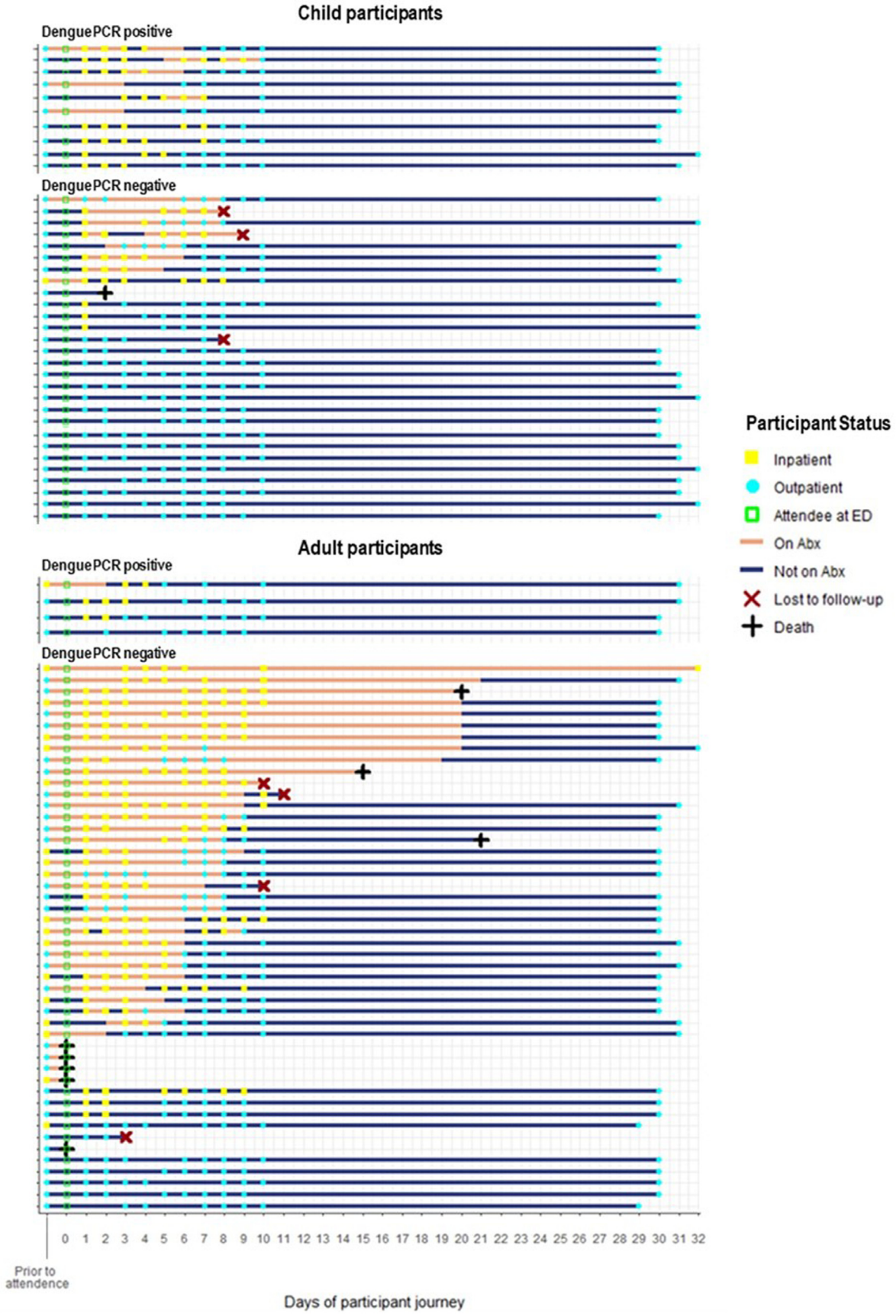

Figure 2. Patient pathway during the study. ED, emergency department; PCR, polymerase chain reaction.

Table 1
Summary of results from RT-qPCR and blood culture testing.

	Adults	Children	Total
Positive RT-qPCR (AusDiagnostics Mosquito Panel) result:			
- Dengue 1 detected	1	0	1
- Dengue 2 detected	1	0	1
- Dengue 3 detected	2	8	10
- Dengue 4 detected	0	1	1
- Dengue 1 & 3 detected	0	1	1
No target detected	48	20	68
Significant blood culture result:			
- *Klebsiella pneumoniae* isolated	1	0	1
- *Staphylococcus aureus* isolated	1	0	1
No significant blood culture result:			
- *Pseudomonas stutzeri* isolated	0	1	1
- No growth	49	22	71
- Blood culture not successfully undertaken	1	7	8
Total	52	30	82

RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

Table 2
Demographic data, clinical features, and outcome of participants with acute febrile illness who were diagnosed with dengue virus infection compared with those who were not.

	Dengue virus detected	Dengue virus not detected	*P* value	All participants
Demographic information	9 (64.3)	29 (42.6)	0.155	38 (46.3)
- Male gender (%)	9.5 (7.3-17.3)10 (71.4)1	28.5 (6.75-47.5)20 (29.4)18	0.010	24.5 (7-40.8)30 (36.6)19
- Median (IQR) age in years	(7.1)	(26.5)	**0.005**	(23.2)
- Child (<16 years) (%)			0.170	
- Inpatient for 1-3 days before recruitment (%)				
Clinical features at presentation	3 (2-3)3 (21.4)14 (100.0)14	2 (1-4)20 (29.4)68 (100.0)56	0.371	2 (1-4)23 (28.0)82 (100.0)70
- Median (IQR) duration of fever in days	(100.0)2 (14.3)3 (21.4)0	(82.4)30 (44.1)11 (16.2)8	0.747-	(85.4)32 (39.0)14 (17.0)8
- Fever for >3 days (%)	(0.0)0 (0.0)3 (21.4)	(11.8)8 (11.8)32 (47.0)	0.115	(9.8)8 (9.8)35 (42.7)
- Clinical features localizing a potential infection (%)	0.724 (0.696-0.864)	0.953 (0.802-1.184)	0.068	0.947 (0.791-1.184)
- Gastro-intestinal tract (%)	0.214(0.214-0.214)	0.216(0.216-0.219)	0.698	0.216(0.214-0.219)
- Respiratory tract (%)			0.339	
- Urinary tract (%)			0.339	
- Skin/soft tissue (%)			0.136	
- Central nervous system (%)			0.236	
- Antimicrobials received before recruitment (%)			**0.031**	
- Median (IQR) shock index				
- Median (IQR) SpO2/FiO2 ratio				
Clinical diagnosis at presentation	13 (92.9)6 (24.9)1 (7.1)	17 (22.4)2 (2.6)59 (77.6)	**<0.001**	30 (33.3)8 (8.9)60 (66.7)
- Dengue (%)				
- Warning signs/severe dengue (%)				
- Other (not including dengue) (%)				
Outcomes				
- Dengue polymerase chain reaction positive (%)	14 (100.0)	0 (0.0%)	**<0.001**	14 (17.1)
- Blood culture with significant organism (%)	0 (0.0)	3 (3.94)	1.000	3 (3.33)
- Completed 30-day follow-up (or died during follow-up) (%)	14 (100.0)	61 (89.7)	0.596	75 (91.5)
- Antimicrobial treatment given (%)	6 (42.9)	41 (60.3)7 (6-10)	0.365	47 (57.3)7 (4.5-9)
- Median (IQR) antimicrobial treatment duration in days	3 (2.3-3.8)	45 (66.2)	**<0.001**	58 (70.7)
- Admitted to hospital (%)	13 (92.9)	6 (3-14)	0.055	5 (3.3-9)
- Median (IQR) admission duration in days	5 (4-7)0 (0.0)	0 (0.0)	0.375	0 (0.0)
- Need for readmission after discharge (%)	60 (60-60)	60 (58 - 60)	-	60 (58-60)
- Median (IQR) WHODAS functional score at 30 days (/60)	0 (0.0)	9 (13.2)	0.264	9 (11.0)
- 30-day mortality (%)			0.345	
Total	14	68		82

IQR, interquartile range; WHODAS, World Health Organization Disability Assessment Schedule.

The limitations of this study include its small sample size and occurrence at only one site in Timor-Leste during a 3-month period. Recruitment was between 09:00 and 16:00 on weekdays only and was restricted to patients in whom a blood culture was taken as part of their routine clinical care. Participants are, therefore, unlikely to accurately represent the broader group of patients with AFI in Timor-Leste. Larger multicenter studies conducted throughout the year would yield more representative data on the clinical features, antimicrobial treatment, and outcomes of AFI in Timor-Leste. The Australia Diagnostics Mosquito Panel used in this study is a research-only multiplexed quantitative PCR assay, which has not yet undergone diagnostic accuracy studies. Low sensitivity (or specificity) in the detection of one or more target could potentially have contributed to the spuriously low (or high) pathogen detection rate. Conducting additional relevant molecular and/or serological testing on samples (and potentially collecting convalescent serum samples on day 10 or day 30 so for comparison of antibody titers and assessing for seroconversion) may have given a more complete picture of AFI etiology. The majority of participants completed follow-up (i.e. were contactable at 30 days after recruitment, 91.5%). However, many of these participants missed one or more follow-up interviews between day 0 and day 10. In part, this was because interviews were not attempted on weekends. When this occurred, some antimicrobial stop or start dates and hospital discharge dates were estimated to have occurred at the midpoint between successful follow-ups. Outcome data were lacking for those who

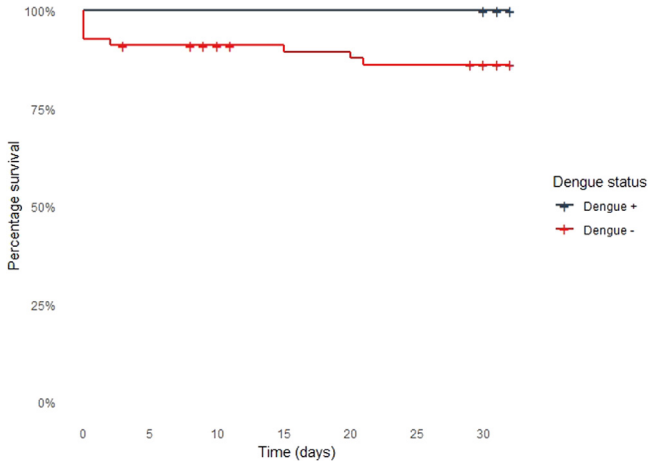

Figure 3. Kaplan–Meier chart showing survival of participants with or without dengue during the study.

did not complete the follow-up (8.5%). It is possible that these individuals experienced adverse outcomes (for example, readmission to hospital or death). Apart from attempting telephone follow-up on three occasions, no further efforts were made to record these participants' whereabouts. Future studies must include recruitment and follow-up activities on weekends and weekdays and should include home visits for discharged participants who are not contactable by telephone.

Overall, this study shows that prospective studies that enroll patients with severe AFI in Timor-Leste and collect their baseline, treatment-related, and clinical outcome data are feasible. Future studies could assess the impact of novel diagnostic tests, CDSSs, and/or AMSIs when implemented in patient pathways in this setting.

Declarations of competing interest

The authors have no competing interest to declare.

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Ethical approval

This study received ethical approval from the *Instituto Nacional de Saúde* Research Ethics and Technical Committee, Timor-Leste (Reference Number 157/MS-INS/GDE/I/2023) and Human Research Ethics Committee of the Northern Territory, Department of Health and Menzies School of Health Research, Australia (HREC Reference Number: 2023-4505).

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Author contributions

PA, GJ, LA, HG, JY, JRF, NM, SA, and DX conceived the study and designed the study protocol. FB, CS, LCA, JADC, ASCFCS, NS, and TO assisted in sample collection and laboratory testing. DX and PA performed data analysis. PA and DX wrote the manuscript. PA, JY, JRF, and NM provided overall guidance to the study. The manuscript was reviewed and approved by all the authors. PA, JRF, and JY are joint senior authors of the study.

References

[1] Shrestha P, Dahal P, Ogbonnaa-Njoku C, Das D, Stepniewska K, Thomas NV, et al. Non-malarial febrile illness: a systematic review of published aetiological studies and case reports from southern Asia and South-Eastern Asia, 1980–2015. *BMC Med* 2020;**18**:299. doi:10.1186/s12916-020-01745-0.

[2] Wangdi K, Kasturiaratchi K, Nery SV, Lau CL, Gray DJ, Clements ACA. Diversity of infectious aetiologies of acute undifferentiated febrile illnesses in south and Southeast Asia: a systematic review. *BMC Infect Dis* 2019;**19**:577. doi:10.1186/s12879-019-4185-y.

[3] Robinson ML, Manabe YC. Reducing uncertainty for acute febrile illness in resource-limited settings: the current diagnostic landscape. *Am J Trop Med Hyg* 2017;**96**:1285–95. doi:10.4269/ajtmh.16-0667.

[4] Bhaskaran D, Chadha SS, Sarin S, Sen R, Arafah S, Dittrich S. Diagnostic tools used in the evaluation of acute febrile illness in South India: a scoping review. *BMC Infect Dis* 2019;**19**:970. doi:10.1186/s12879-019-4589-8.

[5] Grundy BS, Houpt ER. Opportunities and challenges to accurate diagnosis and management of acute febrile illness in adults and adolescents: a review. *Acta Trop* 2022;**227**:106286. doi:10.1016/j.actatropica.2021.106286.

[6] Iroh Tam PY, Obaro SK, Storch G. Challenges in the etiology and diagnosis of acute febrile illness in children in low- and middle-income countries. *J Pediatric Infect Dis Soc* 2016;**5**:190–205. doi:10.1093/jpids/piw016.

[7] Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. *Nat Microbiol* 2019;**4**:46–54. doi:10.1038/s41564-018-0295-3.

[8] Beatty ME, Stone A, Fitzsimons DW, Hanna JN, Lam SK, Vong S, et al. Best practices in dengue surveillance: a report from the asia-pacific and Americas dengue prevention boards. *PLoS Negl Trop Dis* 2010;**4**:e890. doi:10.1371/journal.pntd.0000890.

[9] Sim I, Gorman P, Greenes RA, Haynes RB, Kaplan B, Lehmann H, et al. Clinical decision support systems for the practice of evidence-based medicine. *J Am Med Inform Assoc* 2001;**8**:527–34. doi:10.1136/jamia.2001.0080527.

[10] Ming DK, Hernandez B, Sangkaew S, Vuong NL, Lam PK, Nguyet NM, et al. Applied machine learning for the risk-stratification and clinical decision support of hospitalised patients with dengue in Vietnam. *PLoS Digit Health* 2022;**1**:e0000005. doi:10.1371/journal.pdig.0000005.

[11] Siribhadra A, Ngamprasertchai T, Rattanaumpawan P, Lawpoolsri S, Luvira V, Pitisuttithum P. Antimicrobial stewardship in tropical infectious diseases: focusing on dengue and malaria. *Trop Med Infect Dis* 2022;**7**:159. doi:10.3390/tropicalmed7080159.

[12] Godman B, Egwuenu A, Haque M, Malande OO, Schellack N, Kumar S, et al. Strategies to improve antimicrobial utilization with a special focus on developing countries. *Life (Basel)* 2021;**11**:528. doi:10.3390/life11060528.

[13] Ferrante di Ruffano LF, Harris IM, Zhelev Z, Davenport C, Mallett S, Peters J, et al. Health technology assessment of diagnostic tests: a state of the art review of methods guidance from international organizations. *Int J Technol Assess Health Care* 2023;**39**:e14. doi:10.1017/S0266462323000065.

[14] Ferrante di Ruffano L, Davenport C, Eisinga A, Hyde C, Deeks JJ. A capture-recapture analysis demonstrated that randomized controlled trials evaluating the impact of diagnostic tests on patient outcomes are rare. *J Clin Epidemiol* 2012;**65**:282–7. doi:10.1016/j.jclinepi.2011.07.003.

[15] Odaga J, Sinclair D, Lokong JA, Donegan S, Hopkins H, Garner P. Rapid diagnostic tests versus clinical diagnosis for managing people with fever in malaria endemic settings. *Cochrane Database Syst Rev* 2014;**2014**:CD008998. doi:10.1002/14651858.CD008998.pub2.

[16] Hopkins H, Bruxvoort KJ, Cairns ME, Chandler CIR, Leurent B, Ansah EK, et al. Impact of introduction of rapid diagnostic tests for malaria on antibiotic prescribing: analysis of observational and randomised studies in public and private healthcare settings. *BMJ* 2017;**356**:j1054. doi:10.1136/bmj.j1054.

[17] Kapisi J, Sserwanga A, Kitutu FE, Rutebemberwa E, Awor P, Weber S, et al. Impact of the introduction of a package of diagnostic tools, diagnostic algorithm, and training and communication on outpatient acute fever case management at 3 diverse sites in Uganda: results of a randomized controlled trial. *Clin Infect Dis* 2023;**77**:S156–70. doi:10.1093/cid/ciad341.

[18] Kiemde F, Valia D, Kabore B, Rouamba T, Kone AN, Sawadogo S, et al. A randomized trial to assess the impact of a package of diagnostic tools and diagnostic algorithm on antibiotic prescriptions for the management of febrile illnesses among children and adolescents in primary health facilities in Burkina Faso. *Clin Infect Dis* 2023;**77**:S134–44. doi:10.1093/cid/ciad331.

[19] Adjei A, Kukula V, Narh CT, Odopey S, Arthur E, Odonkor G, et al. Impact of point-of-care rapid diagnostic tests on antibiotic prescription among patients aged <18 years in primary healthcare settings in 2 peri-urban districts in Ghana: randomized controlled trial results. *Clin Infect Dis* 2023;**77**:S145–55. doi:10.1093/cid/ciad328.
[20] Sarmento N, Oakley T, Soares da Silva ES da, Tilman A, Monteiro M, Alves L, et al. Strong relationships between the Northern Territory of Australia and Timor-Leste. *Microbiol Aust* 2022;**43**:125–9. doi:10.1071/MA22039.
[21] Sarmento N, Soares da Silva E, Barreto I, Ximenes JC, Angelina JM, Correia DM, et al. The COVID-19 laboratory response in Timor-Leste; a story of collaboration. *Lancet Reg Health Southeast Asia* 2023;**11**:100150. doi:10.1016/j.lansea.2023.100150.
[22] Bucens IK, Reid A, Barreto AC, Dwivedi V, Counahan M. Three years of paediatric morbidity and mortality at the national hospital in Dili, East Timor. *J Paediatr Child Health* 2013;**49**:1004–9. doi:10.1111/jpc.12305.
[23] Wangdi K, Clements ACA, Du T, Nery SV. Spatial and temporal patterns of dengue infections in Timor-Leste, 2005–2013. *Parasit Vectors* 2018;**11**:9. doi:10.1186/s13071-017-2588-4.