BMJ Public Health

Comprehensive diagnostic testing identifies diverse aetiologies of acute febrile illness among hospitalised children and adults in Sri Lanka: a prospective cohort study

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To cite: Bodinayake CK, Nagahawatte A, Devasiri V, *et al*. Comprehensive diagnostic testing identifies diverse aetiologies of acute febrile illness among hospitalised children and adults in Sri Lanka: a prospective cohort study. *BMJ Public Health* 2023;**1**:e000073. doi:10.1136/bmjph-2023-000073

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjph-2023-000073).

Received 24 March 2023
Accepted 3 July 2023

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ABSTRACT

Introduction Acute febrile illness (AFI) is a common cause of hospital admissions in tropical settings. Identifying AFI aetiology is essential for guiding clinicians' diagnoses and developing diagnostic and management guidelines. We used rigorous, gold-standard testing for diverse viral and bacterial pathogens to confirm the aetiology of AFI in southern Sri Lanka.

Methods We prospectively enrolled children and adults with AFI admitted to Teaching Hospital Karapitiya, Galle, the largest tertiary care hospital in Southern Province, Sri Lanka from June 2012 to May 2013. We obtained sociodemographic and clinical data, an acute blood sample, a nasopharyngeal sample, and a urine sample at enrolment and a convalescent blood sample 2–4 weeks later. Laboratory testing was conducted for dengue, respiratory viruses, leptospirosis, scrub typhus, spotted fever group (SFG) and typhus group (TG) rickettsioses and Q fever.

Results A total of 976 patients were enrolled and a convalescent visit was completed in 878 (90.0%). Median age was 26.9 (14.2–41.4) years and the majority were male (628, 64.3%). A viral or bacterial aetiology was identified in 660 (67.6%). A viral aetiology was identified in 534 (54.7%), including 388 (39.8%) with dengue and 171 (17.5%) with respiratory viruses. Bacterial infection was found in 138 (14.1%) and included leptospirosis (79, 8.1%), SFG (17, 1.7%), TG (7, 0.7%), scrub typhus (53, 5.4%) and Q fever (5, 0.5%). Antibiotics were prescribed at enrolment for 45.5% with viral infections and 62.3% with bacterial infection. Overall, sensitivity of clinical diagnosis was low at approximately 50%.

Conclusion We identified an aetiology of AFI in two-thirds of patients in a setting where malaria is non-endemic. Sensitivity of clinical diagnosis was low, with overuse of antibiotics for viral infections and underuse of antibiotics for bacterial infections. Diagnostic algorithms for AFI may help improve clinical management in this and comparable settings with diverse AFI aetiologies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Acute febrile illness (AFI), or fever without localising symptoms and signs, is a common cause of hospital admissions in tropical and subtropical settings.
⇒ AFI is caused by a diverse array of pathogens that vary geographically and seasonally, and include viruses, bacteria, fungi and parasites.
⇒ The true aetiology of AFI is often unknown given similar clinical presentations and need for advanced laboratory testing for confirmation of diagnoses.

WHAT THIS STUDY ADDS

⇒ In a large cohort of children and adults hospitalised with AFI in Sri Lanka, we confirmed a specific aetiology in two-thirds using rigorous, gold-standard diagnostic testing.
⇒ Despite the study being conducted prior to the emergence SARS CoV-2, the majority of cases were due to viruses including dengue and respiratory viruses.
⇒ Sensitivity of clinical diagnosis was generally low, with overuse of antibiotics for viral infections and underuse of antibiotics for bacterial infections.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ There is an opportunity to improve the diagnosis of AFI for appropriate management.
⇒ We identified clinical and laboratory markers that were associated with specific aetiologies of AFI and confirmed using gold-standard laboratory testing.
⇒ We provide the clinical evidence base to develop diagnostic algorithms for management of AFI in this and comparable settings with diverse AFI aetiologies.

INTRODUCTION

Acute febrile illness (AFI) is a common cause of hospital admissions in many tropical and subtropical settings. The definition of AFI can vary greatly. AFI is defined as a recorded fever (>38°C) at presentation or within 48 hours

of hospital admission with no localising symptoms and signs.[1 2] AFI is caused by a diverse array of pathogens that vary geographically and seasonally, including viruses such as dengue and respiratory viruses (RSVs), bacteria such as *Leptospira* spp and *Salmonella typhi*, and parasites such as *Plasmodium* spp.[3] Sri Lanka has a high prevelance of dengue, leptospirosis and rickettsial diseases, which are reported islandwide, including in the Southern Province.[4–6] The accurate identification of pathogens causing AFI is challenging to the clinician due to overlapping clinical features, limited availability of accurate point-of-care (POC) diagnostic testing, and the emergence of novel pathogens.[7] Difficulties in identifying the pathogen often lead to therapeutic dilemmas, subsequent antimicrobial misuse and adverse patient outcomes including death. In this article, we use the term POC test for rapid detection tests which were done at the hospital laboratory complex, such as rapid influenza antigen, NS1 antigen for dengue, rapid antigen for SARS-CoV-2, rapid detection test for leptospirosis, urine dipsticks and urine antigen tests for pneumococcus and Legionella.

In most settings where AFI is common, POC testing is limited and gold-standard testing covering the full range of pathogens is not feasible due to the complexity and cost of testing. Given the potential for life-threatening illness, empiric treatment with antibacterials is often administered for AFI without laboratory confirmation.[8] However, many AFI agents are non-bacterial and do not warrant treatment with antibacterials. The frequent and unnecessary use of antibacterials leads to the emergence of multidrug-resistant pathogens, which are estimated to cause more than 1 million deaths annually.[9] On the other hand, undertreatment with antibacterials for bacterial aetiologies may also result in fatal outcomes.

Identifying the true aetiology of AFI is essential for guiding and refining clinicians' diagnoses and for developing diagnostic and management guidelines. In this study, we used rigorous, gold-standard diagnostic testing for a series of diverse viral and bacterial pathogens to confirm the aetiologies of AFI in southern Sri Lanka. We correlated physicians' clinical impression with laboratory-confirmed diagnoses and identified current practices in antimicrobial therapy for AFI. The overarching goal of this work is to provide the clinical evidence base to develop diagnostic algorithms for management of AFI in this and comparable settings with diverse AFI aetiologies.

METHODS

Febrile cohort

We prospectively enrolled patients with AFI admitted to the paediatric and adult wards at Teaching Hospital Karapitiya, Galle, the largest (1500 bed) tertiary care hospital in the Southern Province of Sri Lanka from June 2012 to May 2013. Consecutive patients 1 year of age and above with documented fever (>38°C) at presentation or within 48 hours of hospital admission were eligible for enrolment. We excluded patients who presented with focal bacterial infections such as pneumonia, urinary tract infection, or skin and soft tissue infection. We obtained sociodemographic and clinical data, an acute blood sample, a nasopharyngeal sample and a urine sample at enrolment and a second blood sample at a convalescent visit at 2–4 weeks follow-up. We recorded the treating physicians' clinical diagnosis at both admission and discharge based on the most likely differential diagnosis and extracted clinical and laboratory data during hospitalisation from the patients' medical records. Testing for research purposes was conducted retrospectively and results were not shared with the clinical team.

Laboratory testing

We performed laboratory testing for acute dengue in all patients, since dengue is the most common aetiology of AFI needing hospitalisation in Sri Lanka.[10] The blood samples of those who tested negative for dengue were tested for leptospirosis, rickettsiae, scrub typhus and Q fever, as described below. We also tested the upper respiratory samples of all patients for respiratory pathogens. Since we excluded focal bacterial infections, we did not obtain blood cultures in our study. In a prior study of hospitalised children and adults with AFI that we enrolled in 2007 using the same eligibility criteria and at the same hospital, the yield of positive blood cultures was low at 1%.[11] We chose not to perform HIV testing in our current study, since no patients tested positive for HIV in our prior AFI cohort and national HIV prevalence remains <0.1%.[12] Finally, we did not include testing for malaria in our study since Sri Lanka eliminated malaria in 2012.[13]

Confirmation of acute dengue

A combination of virological and serological testing methods was used to confirm acute dengue. We performed dengue IgG ELISA, dengue virus (DENV) isolation, real-time PCR (RT-PCR) testing for DENV and RT-PCR for flaviviruses, as previously reported.[14] We defined laboratory-confirmed acute dengue as (1) IgG seroconversion, (2) positive dengue PCR with either positive DENV isolation or positive flavivirus PCR or (3) positive convalescent IgG and either positive dengue PCR or positive DENV isolation. Acute primary and acute secondary dengue were distinguished by the absence or presence of IgG in acute-phase serum, respectively. Seroprevalence was defined as the presence of IgG in acute-phase serum.

Confirmation of upper respiratory pathogens

The nasopharyngeal samples of patients were selected for testing by reverse transcription RT-PCR using the Luminex Integrated System NxTAG Respiratory Pathogen Panel platform which detects 19 RSVs (A and B; non-specific influenza A; influenza A subtypes H1, H3 and 2009 H1N1; influenza B; parainfluenza 1–4; human metapneumovirus; adenovirus; human rhinovirus/human enterovirus (HRV/HEV); coronavirus types

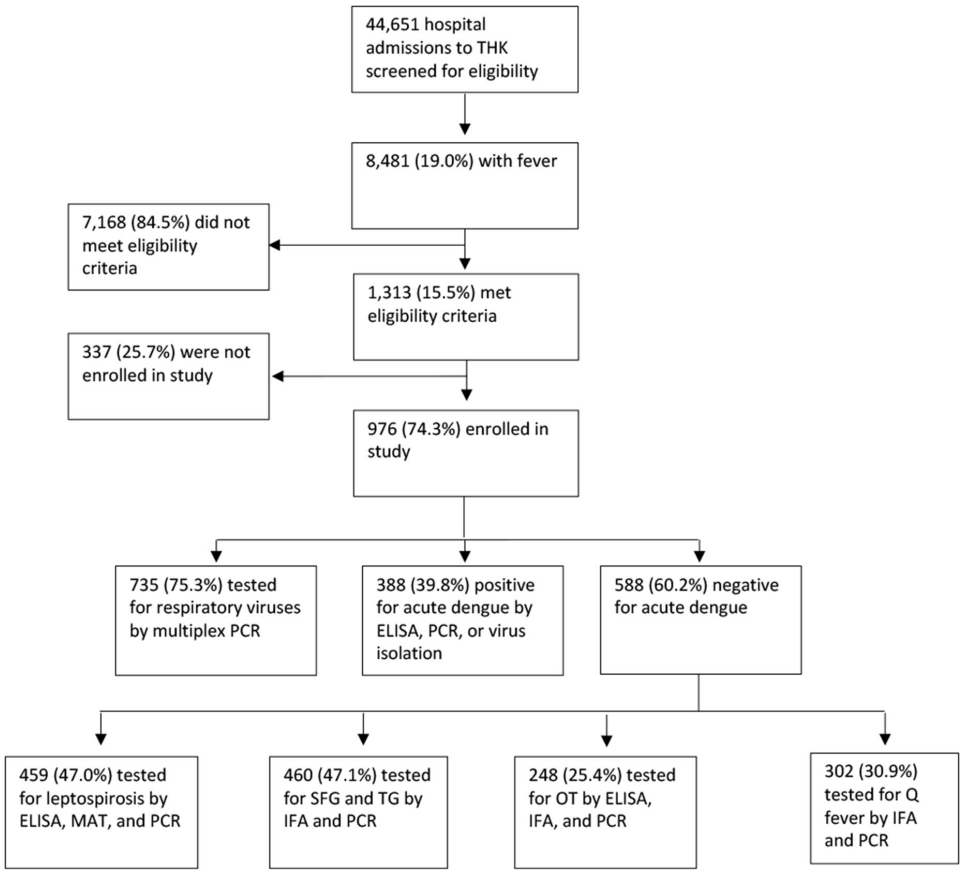

Figure 1 Flow chart of enrolment and aetiological confirmatory testing among patients with acute febrile illness in southern Sri Lanka, 2012–2013. IFA, immunofluorescent antibody; MAT, microscopic agglutination testing; OT, *Orientia tsutsugamushi*; SFG, spotted-fever rickettsioses; TG, typhus group; THK, Teaching Hospital Karapitiy.

NL63, HKU1, 229E and OC43; and human bocavirus) and three bacteria (*Chlamydophila pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae*).[15]

Confirmation of leptospirosis

We screened convalescent sera by IgG ELISA, and if the convalescent sample was IgG ELISA-positive, we tested acute-phase sera by separate IgG and IgM ELISA kits. All patients with positive convalescent IgG and positive acute IgM were tested by microscopic agglutination testing (MAT) to confirm or exclude acute leptospirosis. We also tested a subset (n=50) of convalescent IgG-negative patients by MAT as a gold-standard comparator.

We defined a confirmed case of acute leptospirosis according to PCR and MAT criteria as proposed by the Centers for Disease Control and Prevention in 2013 (http://wwwn.cdc.gov/NNDSS/). For MAT, we used twenty reference strains representing 17 serogroups. For RT-PCR amplification, we targeted the *lipL32* gene found in pathogenic *Leptospira*. Samples with fluorescence before 40 cycles were considered positive. Specimens that were positive for pathogenic *Leptospira* using the LipL32 real time PCR assay were also tested using species-specific primers targeting four species (*Leptospira interrogans*, *Leptospira kirschneri*, *Leptospira borgpetersenii* and *Leptospira noguchi*).[16]

Confirmation of rickettsioses

We screened convalescent sera for spotted fever group (SFG) and typhus group rickettsioses (TGR) IgG antibodies using immunofluorescent antibody (IFA). For those who were convalescent IgG screen positive (positive at 1:80), paired sera were titered to endpoint. Two pan-rickettsial PCR assays were run. We defined acute rickettsioses as (1) a fourfold rise in IgG titre with convalescent titer >160, (2) PCR positive with consistent IFA or (3) amplicon confirmed by gel electrophoresis. We assigned SFGR versus TGR group based on a higher convalescent titre and/or alternative target PCR.

Confirmation of scrub typhus

We screened convalescent sera for IgG antibodies by in-house ELISA. Those inhouse IgG ELISA-positive and a subset of those IgG ELISA-negative were tested by IFA. Among those positive by IgG ELISA, convalescent sera were screened by IFA at 1:80 for IgG and at 1:40 for IgM antibodies. For those convalescent IgG and/or IgM positive by IFA, acute sera were tested. A multiplex RT-PCR assay was run for *Orientia tsutsugamushi*. We defined acute scrub typhus as (1) fourfold rise in IgG±IgM titre by IFA with convalescent titer >160 or (2) PCR positivity with >1 PCR assay with product confirmed by gel and sequencing.

Confirmation of Q fever

We screened convalescent sera at 1:80 for IgG phase II antibodies by IFA. For those who were convalescent IgG screen-positive, acute sera were screened. PCR testing was conducted for Q fever. We defined acute Q fever as those confirmed by (1) fourfold rise in phase II IgG by IFA with convalescent titer >160 or (2) PCR positivity with product confirmed by gel electrophoresis.

Statistical analysis

Sample size calculation: We did not conduct a formal sample size analysis a priori, as we sought to describe varying aetiologies of AFI over season and time. We enrolled all consecutive patients meeting eligbility criteria who consented to participate in the study, in order to increase the precision of our estimates. At a confidence level of 95%, with 3% margin of error and estimated proportion of 0.67 with a specific identified aetiology, a sample size of 944 would be required.

We determined the prevalence of individual pathogens and coinfections with 95% CIs. We identified demographic features, exposures, and clinical symptoms and signs associated with individual infections using the Fisher' exact test for categorical variables and Kruskall-Wallis test for continuous variables. All analyses were conducted using R (V.3.6.3).

Written informed consent was obtained from all patients 18 years of age, parental informed consent was obtained from patients 1–17 years of age and additionally written assent was obtained from all those aged 12–17 years to participate in the study. Study doctors (MBBS) with paediatric experience obtained informed consent in a predesigned consent form.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

RESULTS

Demographic findings

Of the 1313 patients who met eligibility criteria during the study period, 976 (74.3%) were enrolled (figure 1). A convalescent visit was completed in 878 (90.0%) of the cohort and median follow-up time was 3.3 (IQR 2.3–5.6) weeks. Most patients (669, 68.5%) were adults ≥18 years and median age was 26.9 (14.2–41.4) years. The majority of patients were male (628, 64.3%). Overall, 298 (30.5%) self-reported that they lived in rural settings and 211 (21.6%) reported a history of travel within the country or internationally in the prior 30 days. A total of 248 (25.4%) reported using antibiotics for the current illness prior to admission.

Results of aetiological testing

A viral or bacterial aetiology was identified in 660 (67.6%) of patients, table 1 and figure 2. A viral aetiology was identified in 534 (54.7%), including 388 (39.8%) with dengue and 171 (17.5%) with RSVs. The most common RSVs were influenza A (74, 10.0%), influenza B (52, 7.1%), parainfluenza virus (9, 1.2%), HRV/HEV (7, 1.0%) and RSV (9, 1.2%). Seasonal coronaviruses were found in 1 (0.1%). Bacterial infection was found in 138 (14.1%) of patients and included leptospirosis (79, 8.1%), rickettsiae (21, 2.2%) including SFG (17, 1.7%) and TG (7, 0.7%), scrub typhus (53, 5.4%), and Q fever (5, 0.5%). Coinfections were present in 52 (5.3%), with the most common coinfections including 10 with dengue and influenza A, 8 with dengue and influenza B, and eight with leptospirosis and scrub typhus. We were not able to identify an aetiology in 317 (32.5%) patients, with children being more likely to have no aetiology identified (43.8%). Adults were more likely than children to have a bacterial infection (18.5% vs 4.6%, p<0.001).

Demographic features and exposures associated with AFI aetiologies

Respiratory viral illness was identified in younger patients (median age 14.3 years), while leptospirosis and rickettsial infections (including SFG/TG, scrub typhus and Q fever) were found in much older patients (median age for leptospirosis 37.6 years and median age for rickettisal infections 43.7 years (table 2, online supplemental tables 1 and 2). All aetiologies were found more commonly in males, with the exception of rickettsial infections. Patients with dengue were more likely to report travel within the country within the prior 30 days than patients with other identified infections. Rickettsial infections were more common among those who were housebound (housewives/unemployed/retired). Leptospirosis was more common among those who had contact with standing water/mud.

Clinical and laboratory features associated with AFI aetiologies

Patients with rickettsial infections were more likely to present later in illness compared with other aetiologies (median 6 vs 4 days). Headache, anorexia and fatigue were prominent symptoms among patients with all identified aetiologies in the cohort, with over 65% reporting these symptoms. Rhinitis/congestion and cough were significantly more common among patients with RSVs compared with other aetiologies. Patients with dengue and leptospirosis were more likely to report both joint pain and muscle pain, while patients with respiratory infection were least likely to report these symptoms. On examination, patients with dengue had a higher temperature (median 100.2F) at enrolment compared with patients with other aetiologies. Among patients with leptospirosis, conjunctival injection and jaundice were significantly more common, whereas patients with respiratory infection were more likely to have lung crackles. Patients with dengue and rickettsial infections were more likely to have rash on exam, but less than 25% had this finding. Flushing was significantly more common in patients with dengue. Eschar was found in only 5 (10.2%) of patients

Table 1 Laboratory-confirmed aetiologies among patients with acute febrile illness in southern Sri Lanka, 2012–2013

Aetiology	Children n=306		Adults n=669		All n=976	
	n confirmed cases	(%)	n confirmed cases	(%)	n confirmed cases	(%)
Viral infections	**161**	**52.6**	**373**	**55.8**	**534**	**54.7**
Dengue	83	27.1	305	45.6	388	39.8
Influenza A	27	8.8	47	7.0	74	7.6
Influenza B	27	8.8	25	3.7	52	5.3
Parainfluenza virus	7	2.3	2	0.3	9	0.9
Human rhinovirus/enterovirus	3	1.0	4	0.6	7	0.7
Respiratory syncytial virus	9	2.9	0	0	9	0.9
Coronavirus 229E	1	0.3	0	0	1	0.1
Other respiratory viruses	25	8.2	6	0.9	31	3.2
Bacterial infections	**16**	**5.2**	**142**	**21.1**	**158**	**16.2**
Leptospirosis	9	2.9	70	10.5	79	8.1
SFG/TG	3	1.0	18	2.7	21	2.2
Scrub typhus	4	1.3	49	7.3	53	5.4
Q fever	0	0	5	0.7	5	0.5
Coinfections*	**17**	**5.6**	**35**	**5.2**	**52**	**5.3**
Aetiology not identified	**134**	**43.8**	**182**	**27.2**	**317**	**32.5**

*The individual aetiologies found in coinfections are also listed separately under bacterial and/or viral infections, thus proportions do not add up to 100%. The most common coinfections included dengue and influenza A (10, 17.5%), dengue and influenza B (8, 12.7%), leptospirosis and scrub typhus (8, 12.7%), and parainfluenza and other respiratory viruses (5, 7.9%).
SFG/TG, spotted fever group/typhus group.

with rickettsial infection. Patients with dengue were significantly more likely to have leucopenia, thrombocytopaenia, and elevated transaminases at both enrolment and during hospitalisation. Patients with all other aetiologies had a median white cell count over 7.0 cells/μL at enrolment, while patients with dengue had a median white cell count of 4.1 cells/μL.

Clinical management of AFI

Over half of the cohort (489, 50.1%) received antibiotic therapy at enrolment. Antibiotics were prescribed for 45.5% of patients with viral infections at enrolment, including 39.5% with dengue and 58.6% with RSVs. Among patients with bacterial infections, 62.3% received antibiotics at enrolment, including 61.9% for leptospirosis and 55.1% for rickettsial infections. Median duration of hospitalisation was 3 (IQR 2–5) days, and the overall fatality was very low (9 deaths, 0.9%). Overall, the sensitivity of clinical diagnosis was low (approximately 50%), with the sensitivity of clinical diagnosis for rickettsial infections being very low at less than 15% (table 3). The sensitivity of clinical diagnosis did not improve during hospitalisation.

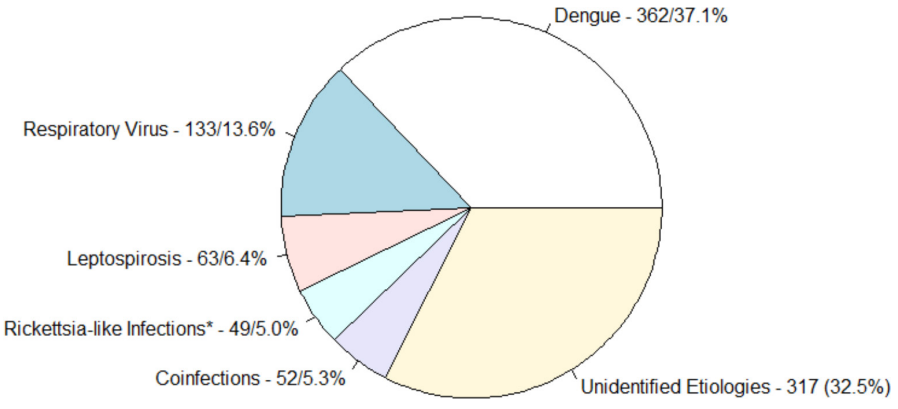

Figure 2 Laboratory-confirmed aetiologies of acute febrile illness in Sri Lanka, 2012–2013. The total number of patients enrolled was used as the denominator in calculating proportions. *Rickettsia-like infections include Spotted Fever Group and Typhus Group Rickettsiae, scrub typhus, and Q fever.

Table 2 Demographic and clinical features associated with specific aetiologies of acute febrile illness in southern Sri Lanka, 2012–2013

Characteristic	Dengue only n=362	Respiratory viruses only n=133	Leptospirosis only n=63	Rickettsial infections, scrub typhus and Q fever n=49	P value	Unidentified aetiology n=317	Overall n=976
Demographic features and exposures							
Age	28.72 (20.3–42.2)	14.28 (4.19–34.21)	37.55 (26.67–45.64)	43.71 (32.08–57.50)	<0.001	23.05 (5.77–37.10)	26.91 (14.15–41.45)
Male sex	233 (64.4)	82 (61.6)	47 (74.6)	22 (44.9)	0.012	210 (66.2)	628 (64.3)
Rural residence	140 (38.7)	36 (27.1)	22 (34.9)	12 (24.5)	0.042	70 (22.1)	298 (30.5)
Travel in past 30 days	106 (29.3)	20 (15.0)	10 (15.9)	12 (24.5)	0.003	54 (17.0)	211 (21.6)
Education							
<Ordinary level (O/L)	148 (40.9)	92 (69.2)	31 (49.2)	30 (61.2)	<0.001	186 (58.7)	516 (52.9)
≥O/L	212 (58.6)	39 (29.3)	32 (50.8)	17 (34.7)		127 (40.1)	450 (46.1)
Occupation							
Housewife/unemployed/retired	67 (18.5)	17 (12.8)	13 (20.6)	25 (51.0)		42 (13.2)	171 (17.5)
School/student	31 (8.6)	8 (6.0)	5 (7.9)	2 (4.1)	0.009	12 (3.8)	61 (6.2)
Indoor workers (labourer/factory/ merchant/office)	114 (31.5)	17 (12.8)	27 (42.8)	8 (16.3)		80 (25.2)	266 (27.2)
Outdoor workers (farmer/labourer/ agriculture/construction)	46 (12.7)	10 (7.5)	10 (15.9)	6 (12.2)		27 (8.5)	104 (10.6)
Military/police/security	12 (3.3)	4 (3.0)	2 (3.2)	2 (4.1)		10 (3.2)	30 (3.1)
Other	18 (5.0)	2 (1.5)	0	2 (4.1)		12 (3.8)	34 (3.5)
Exposures to fresh water							
Standing water/mud	35 (9.7)	11 (8.3)	22 (34.9)	3 (6.1)	<0.001	22 (6.9)	100 (10.2)
River/pond/lake	37 (10.2)	6 (4.5)	6 (9.5)	1 (2.0)	0.073	21 (6.6)	77 (7.9)
Clinical symptoms at enrolment							
Days of fever at enrolment	4 (3–6)	4 (3–5)	4 (3–5.5)	6 (4–10)	0.001	5 (3–7)	4 (3–6)
Headache	293 (80.9)	87 (65.4)	57 (90.5)	39 (79.6)	0.001	190 (59.9)	705 (72.2)
Rhinitis/congestion	36 (9.9)	68 (51.1)	1 (1.6)	2 (4.1)	<0.001	88 (27.8)	215 (22.0)
Sore throat	64 (17.7)	25 (18.8)	14 (22.2)	2 (4.1)	0.057	51 (16.1)	178 (18.2)
Cough	121 (33.4)	109 (82.0)	15 (23.8)	18 (36.7)	<0.001	159 (50.2)	454 (46.5)
Joint pain	255 (70.4)	48 (36.1)	46 (73.0)	25 (51.0)	<0.001	127 (40.1)	532 (54.5)
Muscle pain	247 (68.2)	55 (41.4)	48 (76.2)	27 (55.1)	<0.001	132 (41.6)	542 (55.5)

Continued

Table 2 Continued

Characteristic	Dengue only n=362	Respiratory viruses only n=133	Leptospirosis only n=63	Rickettsial infections, scrub typhus and Q fever n=49	P value	Unidentified aetiology n=317	Overall n=976
Anorexia	329 (90.9)	110 (82.7)	58 (92.1)	40 (81.6)	0.019	250 (78.9)	834 (85.4)
Abdominal pain	99 (27.3)	28 (21.0)	11 (17.5)	13 (26.5)	0.246	65 (20.5)	235 (24.1)
Vomiting	191 (52.8)	59 (44.4)	30 (47.6)	21 (42.8)	0.134	142 (44.8)	467 (47.8)
Diarrhoea	43 (11.9)	10 (7.5)	12 (19.0)	8 (16.3)	0.101	47 (14.8)	123 (12.6)
Dysuria	39 (10.8)	4 (3.0)	12 (19.0)	6 (12.2)	0.004	22 (6.9)	87 (8.9)
Oliguria	48 (13.2)	11 (8.3)	11 (17.5)	9 (18.4)	0.172	21 (6.6)	108 (11.1)
Fatigue	288 (79.6)	86 (64.7)	51 (81.0)	36 (73.5)	0.013	209 (65.9)	707 (72.4)
Clinical signs at enrolment							
Temperature (F)	100.2 (98.9–100.9)	99.4 (98.4–100.4)	99.3 (98.4–100.4)	99.6 (98.6–101.0)	0.001	99.2 (98.4–100.2)	99.5 (98.6–100.6)
Heart rate	82.0 (74.0–100.0)	92.0 (84.0–100.0)	80.0 (72.0–96.0)	84.0 (80.0–100.0)	<0.001	90.0 (80.0–100.0)	88.0 (80.0–100.0)
Systolic blood pressure	110.0 (100.0–110.0)	110.0 (100.0–110.0)	110.0 (100.0–110.0)	110.0 (100.0–120.0)	0.009	110.0 (100.0–120.0)	110.0 (100.0–120.0)
Diastolic blood pressure	70.0 (70.0–80.0)	70.0 (60.0–70.0)	70.0 (70.0–80.0)	70.0 (60.0–80.0)	0.073	70.0 (60.0–80.0)	70.0 (60.0–80.0)
Conjunctival injection	57 (15.7)	13 (9.8)	16 (25.4)	6 (12.2)	0.036	39 (12.3)	139 (14.2)
Pharyngeal erythema/exudate	30 (8.3)	8 (6.0)	2 (3.2)	1 (2.0)	0.214	18 (5.7)	63 (6.4)
Lymphadenopathy	40 (11.0)	22 (16.5)	5 (7.9)	6 (12.2)	0.274	50 (15.8)	133 (13.6)
Jaundice	3 (0.8)	0	4 (6.3)	1 (2.0)	0.002	5 (1.6)	13 (1.3)
Lung crackles	10 (2.8)	27 (20.3)	5 (7.9)	7 (14.3)	<0.001	44 (13.9)	102 (10.4)
Right upper abdominal tenderness	99 (27.3)	28 (21.0)	11 (17.5)	13 (26.5)	0.246	65 (20.5)	235 (24.1)
Hepatomegaly	28 (7.7)	5 (3.8)	3 (4.8)	7 (14.3)	0.079	24 (7.6)	70 (7.2)
Rash	73 (20.2)	9 (6.8)	4 (6.3)	9 (18.4)	0.001	38 (12.0)	136 (13.9)
Flushing	49 (13.5)	4 (3.0)	0	4 (8.2)	<0.001	9 (2.8)	68 (7.0)
Laboratory parameters at enrolment							
WCC (white cell count) $\times 10^9$/L	4.10 (2.70–6.10)	7.20 (6.00–8.65)	7.35 (5.50–11.32)	7.30 (4.88–11.90)	<0.001	7.55 (4.40–11.28)	5.90 (3.70–9.25)
Leucopenia (WCC<4.0)	153 (42.3)	5 (3.8)	5 (7.9)	2 (4.1)	<0.001	50 (15.8)	221 (22.6)
Leucopenia during hospitalisation	260 (71.8)	15 (11.3)	10 (15.9)	8 (16.3)	<0.001	65 (20.5)	370 (37.9)
Absolute neutrophil count $\times 10^9$/L	2.76 (1.68–4.39)	5.05 (3.88–6.34)	5.89 (3.88–9.61)	5.00 (3.55–9.15)	<0.001	5.19 (2.79–8.64)	4.13 (2.36–6.70)

Continued

Table 2 Continued

Characteristic	Dengue only n=362	Respiratory viruses only n=133	Leptospirosis only n=63	Rickettsial infections, scrub typhus and Q fever n=49	P value	Unidentified aetiology n=317	Overall n=976
Absolute lymphocyte count $\times 10^9$/L	0.81 (0.57–1.26)	1.57 (1.00–2.15)	0.88 (0.55–1.09)	1.84 (1.24–2.63)	<0.001	1.40 (0.86–2.12)	1.09 (0.68–1.77)
Haemoglobin	13.10 (12.00–14.30)	12.74 (11.90–13.85)	12.70 (11.70–13.80)	12.00 (11.20–13.20)	0.002	12.70 (11.47–14.10)	12.90 (11.70–14.10)
Haematocrit	39.20 (36.20–42.40)	38.60 (36.26–41.45)	37.40 (34.60–41.60)	34.85 (33.17–39.30)	0.003	38.20 (34.90–42.00)	38.60 (35.40–42.00)
Platelets$\times 10^9$/L	127.0 (86.0–183.0)	220.0 (175.0–277.5)	161.8 (119.2–200.0)	148.0 (101.9–219.0)	<0.001	205.0 (143.0–261.0)	165.0 (112.0–227.0)
Thrombocytopaenia at enrolment	210 (58.0)	4 (3.0)	12 (19.0)	11 (22.4)	<0.001	41 (12.9)	288 (29.5)
Thrombocytopaenia during hospitalisation	293 (80.9)	24 (18.0)	38 (60.3)	26 (53.1)	<0.001	104 (32.8)	505 (51.7)
Elevated transaminases	64 (17.7)	1 (0.8)	6 (9.5)	6 (12.2)	<0.001	22 (6.9)	104 (10.6)
Transaminases during hospitalisation	65 (18.0)	1 (0.8)	6 (9.5)	6 (12.2)	<0.001	25 (7.9)	108 (11.1)
Clinical management and outcomes							
Antibiotics at enrolment	143 (39.5)	78 (58.6)	39 (61.9)	27 (55.1)	<0.001	171 (53.9)	489 (50.1)
Hospitalisation duration (days)	5 (4–7)	4 (3–5)	5 (4–6)	5 (4–7)	<0.001	4 (3–6)	5 (4–6)
Death during hospitalisation	0	0	0	0	---	0	0
Follow-up rate	327 (90.3)	117 (88.0)	63 (100)	49 (100)	---	270 (85.2)	877 (89.8)
Median time to follow-up (days)	25 (18–42)	20 (15–34)	21 (16–38)	24 (18–48)	0.016	21 (16–40)	23 (16–39)
Death at follow-up	0	1 (0.8)	0	1 (2.0)	0.108	7 (2.2)	9 (0.9)

The association of demographic and clinical features with dengue, respiratory viruses, leptospirosis and rickettsial infections was determined using the χ^2 test, and the p value is depicted in the table below. Unidentified aetiologies are also depicted in the table, but were not included in calculating the p value.

Table 3 Sensitivity, specificity, positive predictive value and negative predictive value of clinical diagnosis relative to laboratory-confirmed testing for specific aetiologies of acute febrile illness, Sri Lanka, 2012–2013

Clinical diagnosis at discharge	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Dengue	0.599	0.922	0.831	0.781
Respiratory viruses	0.504	0.678	0.208	0.890
Leptospirosis	0.444	0.935	0.333	0.958
Rickettsial infection*	0.102	0.984	0.263	0.951

*Rickettsial infection includes spotted fever group and typhus group rickettsioses, scrub typhus, and Q fever. Coinfections were excluded when calculating sensitivity, specificity, positive predictive value and negative predictive value.

DISCUSSION

Our study is the first comprehensive research study detailing the aetiologies of AFI in a cohort of hospitalised patients in Sri Lanka. We used gold-standard diagnostic testing for dengue, RSVs, leptospirosis, rickettsiae, scrub typhus and Q fever to ascertain the aetiology of AFI. We were able to identify a specific aetiology for AFI in two-thirds of patients in a setting where malaria is non-endemic. Despite the study being conducted prior to the emergence SARS-CoV-2, the majority of cases were due to viruses including dengue and RSVs. Bacterial infections, including leptospirosis and rickettsial infections, were identified in a smaller but important proportion of the cohort (16%). We found that several clinical and basic laboratory features were associated with dengue and leptospirosis, but in the majority of cases, clinical findings were indistinguishable between aetiologies. Sensitivity of clinical diagnosis for dengue, respiratory viral infection and leptospirosis was close to 50%, while that for rickettsial infections was very low (10%). Our results show that the sensitivity of clinical diagnosis was low for diseases of low prevalence in our setting, as would be expected. According to our findings, there is an opportunity to improve the clinical diagnosis of AFI for appropriate management. We identified clinical and laboratory markers that were associated with specific aetiologies of AFI and confirmed using gold-standard laboratory testing. These disease-specific identifiers, in conjunction with real-time regional disease surveillance data and low-cost POC tests, could be incorporated into clinical decision-making algorithms for improved diagnosis and management of AFI. These algorithms could be digitised and applied to comparable settings where multiple pathogens causing AFI are present.

We identified an aetiology in two-thirds of our AFI cohort, with more than 50% of identified illnesses being viral. The two most commonly identified viruses were DENV and influenza virus. The most common bacterial infections were leptospirosis and scrub typhus. Coinfections were identified in 5% of our cohort. Overall, one-third of our cohort did not have an identifiable aetiology for AFI. Very few other studies globally have conducted exhaustive, gold-standard testing to identify aetiologies of AFI, and many studies have not collected convalescent sera, which is necessary for confirming aetiologies serologically. Compared with many other studies of AFI worldwide, our study identified a higher proportion of aetiological agents of AFI. In Northern Tanzania where malaria was uncommon (1.6%), bacterial zoonoses and arboviruses were prevalent among children and adults admitted with AFI and 50% of the cohort remained undiagnosed despite comprehensive testing for bacteraemia, brucella, leptospirosis, Q fever, arboviruses, and SFGR and TGR.[17] In an Indonesian cohort of children and adults hospitalised with AFI, an aetiology was identified in 67.5% of patients and the most common aetiological agents were dengue, *Salmonella* spp and *Rickettsia* spp.[18] In a prospective study of 8996 Puerto Rican children and adults presenting to an emergency room with AFI, chikungunya, influenza A/B and dengue were the most common aetiologies and 54.8% had a pathogen identified.[19] We were able to confirm an aetiology in a majority of our cohort with the deployment of rigorous laboratory methods, including the use of multiple tests to confirm a single aetiology, gold-standard testing at reference laboratories, and paired serologic testing given our high rate of convalescent follow-up. We found that viral infections were the most likely to lead to hospitalisation for AFI, even prior to the global pandemic due to COVID-19.

Certain epidemiological and clinical features were associated with specific aetiologies of AFI. We found that history of travel, higher temperature, flushing on examination, leucopenia, thrombocytopaenia and elevated transaminases were associated with dengue compared with other aetiologies. Such features have been commonly reported in the literature.[20–22] Exposure to standing water or mud, conjunctival injection and jaundice were associated with a diagnosis of leptospirosis, as previously reported in the literature.[23–25] As expected, patients with respiratory viral infection were more likely to report rhinitis/congestion, cough and lung crackles compared with patients with other aetiologies. Patients with rickettsial infection were more likely to be female and to present later in illness compared with other aetiologies. Patients with rickettsial infection commonly had headache, anorexia and fatigue (>70%), consistent with the literature, but these symptoms were also identified in the majority of other patients with AFI.[26 27] We expected patients with rickettsial infections to have eschar, but this was not frequently documented in the medical record

and may be due to unrecognised lesions given the painless nature of lesions and their propensity for warm, covered areas such as under the breasts, axillae or groin. Others have also shown that eschar may be found in only a minority of patients with rickettsial infections such as scrub typhus and SFG rickettsioses.[26 28] Our findings indicate that eschar may not be a defining feature in identifying rickettsial infection, and may be the reason for poor clinical sensitivity for rickettsial infection.

Overall, our study demonstrated that the sensitivity of physicians' clinical diagnosis was low for all AFI aetiologies. This fact could be related to the non-discriminatory and overlapping clinical features of AFI diagnoses. We found a high prevalence of inappropriate antibiotic use in our cohort, with both overuse of antibiotics for viral infections and underuse of antibiotics for bacterial infections. Clinicians in this and comparable settings may feel compelled to treat patients presenting with acute fever with antibiotics until a specific diagnosis is arrived at with investigations. Our study highlights the need for data-driven, diagnostic algorithms incorporating multiple elements such as real-time regional surveillance data, patients' epidemiological and clinical features, basic laboratory test results such as white cell count and low-cost POC tests such as dengue NS1 and rapid influenza antigen tests for optimising AFI management. Others have initiated work developing diagnostic algorithms in settings where AFI is prevalent. In Africa and Asia, Salami *et al* proposed a clinical trial for treating AFI using an algorithm of POC pathogen and biomarker testing.[29] For low-resource settings, Brintz *et al* proposed a modular approach integrating epidemiological data, clinical information and a POC test to predict bacterial versus viral diarrhoea.[30] In Tanzania, Keitel *et al* developed a novel electronic algorithm (e-POCT) with POC pathogen and biomarker tests for children with AFI, and found that it was safe and reduced antibacterial use.[31]

The strengths of our study include unbiased, consecutive recruitment of both children and adults admitted to a tertiary care hospital. In addition, we used rigorous, gold-standard testing and were able to complete convalescent sampling and testing in a very high proportion of our cohort. Limitations of our study include the lack of blood cultures based on our prior experience and exclusion of focal bacterial infections. Furthermore, we only studied hospitalised patients with AFI, thus our results are not generalisable to ambulatory patients. In addition, we were not able to conduct advanced molecular testing to confirm aetiologies in the remainder of our cohort. Finally, we were not able to conduct testing for all aetiologies in all patients given limited resources and availability of serum. Our estimates regarding prevalence of aetiologies would therefore be underestimates. Minority of viral infections can coinfect with bacteria. In this study, we did not gather any information to assess subsequent bacterial infections.

In conclusion, a definitive of aetiology can be identified in a majority of patients with AFI when using comprehensive, gold-standard testing which is not feasible in a clinical setting. Availability of a few reliable, rapid POC tests will help distinguishing between AFI which present acutely with overlapping clinical features. Given the poor sensitivity of clinical diagnosis, new tools need to be explored to bridge the gap. Diagnostic and management algorithms for AFI that are data driven are currently in development, and this study provides a decisive evidence base for comparable settings.

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Acknowledgements The authors would like to acknowledge the patients, research staff, and laboratory technicians who were involved in this study.

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Funding This study was funded by a grant from the Office of Naval Research to the Emerging Infectious Diseases Programme, Duke-NUS Graduate Medical School, Singapore; NIH Research Training Grant #R25 TW009337 funded by the Fogarty International Center and the National Institute of Mental Health (LGT); NIH/National Institute of Allergy and Infectious Diseases (NIAID) K23AI083931 (MR); a Johns Hopkins Center for Global Health Junior Faculty Grant (MR) and the Hubert-Yeargan Center for Global Health. LGT was supported by a grant from the National Institutes of Allergy and Infectious Diseases (1K23AI125677).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and the Ethical Review Committee, Faculty of Medicine, University of Ruhuna (Sri Lanka) (ERC approval received from the letter dated 21 May 2012), and the Institutional Review Boards of Duke University (USA) (IRB No: 00014461), Duke-National University Singapore (Singapore), and Johns Hopkins University (USA). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article. If individual-level data are desired, please contact the authors such that we can contact the appropriate ethical review bodies to determine possibility of sharing data.

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