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High Yield of HIV Testing in Dengue-Like Febrile Illness in Singapore

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Where dengue virus infections are endemic, acute febrile illness is often managed as dengue fever (DF) without diagnostic testing. In a prospective study of 140 patients with clinical features of DF, 3 (2.1%) had acute HIV infection (AHI). We recommend testing for AHI in dengue-like febrile illness.

Keywords. fourth-generation enzyme immunoassays; acute febrile illness; acute HIV; dengue; diagnostics.

People who receive antiretroviral treatment during acute HIV infection benefit from reduced viral load and arrested CD4 cell decline [1]. Programs that increase the diagnosis of acute HIV infection (AHI) may reduce HIV transmission [2], as viral load and infectivity are highest during seroconversion. HIV testing is now more sensitive to primary HIV infection as recommended testing algorithms include fourth-generation enzyme-linked immunoassays (EIAs) and confirmatory nucleic acid amplification tests (NAATs) [3]. To realize the benefits of these advances, we need to identify clinical populations in whom HIV testing yields a high number of cases.

AHI causes a febrile illness in up to 89% of cases [4]. However, the presentation is nonspecific and is often confused with other infections. In Asia, an estimated 350 000 new HIV infections each year [5] must be distinguished from other causes of fever, including more than 66.8 million symptomatic episodes of dengue fever (DF) [6]. Singapore promotes

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outpatient management of DF in hospital-based fever clinics, with inpatient admission offered to those with warning signs of severe DF [7]. Confirmatory testing is not routine, as is the case in many dengue-endemic countries [8]. We sought to estimate the yield of testing people with acute febrile illnesses consistent with DF for AHI using fourth-generation EIA followed by confirmatory NAAT.

METHODS

In this cross-sectional study, we prospectively identified febrile patients with 2 or more symptoms of DF and offered testing for AHI. The study recruited from fever clinics and acute medicine wards based at National University Hospital, Tan Tock Seng Hospital, and Alexandra Hospital between April 2012 and March 2015.

Participants were eligible if they were aged 21 years or older, had a fever >37.5°C, and had 2 symptoms of DF—defined as anorexia, nausea or vomiting, rash, aches and pains, leukopenia, a positive tourniquet test, or any warning sign (abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy, restlessness, liver enlargement >2 cm, or an increasing hematocrit). Cases were excluded if they were pregnant, non-Singapore citizens, or permanent residents. Those with a laboratory diagnosis for their illness or a known dengue test result at the time of recruitment were also excluded.

Trained research assistants administered a structured questionnaire on risks for HIV exposure in the last 3 months and collected clinical and laboratory data as they became available. The 3-month interval comfortably includes the period from exposure to symptoms of AHI [9]. HIV testing was by HIV fourth-generation Ag-Ab EIA (ARCHITECT HIV Ag/Ab Combo assay) and NAAT (Roche COBAS AmpliScreen HIV-1 Test, version 1.5). Positive results on either test were further evaluated with a quantitative viral load (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0) and immunoblot performed at Singapore's National Reference Laboratory. A positive immunoblot was defined as any 2 of p24, gp41, or gp120/160, or 2 of 3 env bands with or without gag and/or pol bands.

The proportion of febrile patients with AHI was calculated overall and for those reporting particular risk factors. Ninety-five percent confidence intervals were calculated for each estimate. Statistical analysis was performed using Stata, version 13.

Participants gave informed consent to participate in the study, which was approved by the National Healthcare Group Domain Specific Review Board.

RESULTS

In total, 4559 patients with febrile illness were screened at the 3 sites; 4240 were not eligible, mostly because DF rapid testing

was introduced during the study period, and the results were known at the time of recruitment. Of the 263 eligible participants, 140 consented. Their median age (range) was 34.6 (21–77) years, and 110 (65.9%) were male; 47.9% had fever <5 days, and a further 37.7% had fever lasting 5–7 days. Aside from fever, the most common symptoms were lethargy (70.1%), aches and pains (61.7%), anorexia (51.5%), nausea and vomiting (41.9%), and rash (35.9%). Characteristics of the participants are shown in Table 1. Three of 140 participants had AHI, 2.1% (95% confidence interval [CI], 0.6%–6.5%) of the study participants.

Case 1 was a 59-year-old man with fever for 3 days, nausea and vomiting, anorexia, and petechial rash. He had had vaginal intercourse with an anonymous female partner from another country within the last 3 months. He had a quantitative viral load of 506 000 copies/mL and a positive immunoblot without p31 band.

Case 2 was a 21-year-old man who presented after 12 days of intermittent fever, nausea and vomiting, rash, myalgias, and lethargy. He had had 3 male partners in the last 3 months, including receptive anal intercourse without a condom. He had a quantitative viral load of 406 000 copies/mL and a positive immunoblot without p31 band.

Case 3 was a 64-year-old man with fever for 11 days, anorexia, rash, and abdominal pain. He reported vaginal intercourse without a condom with a female sex worker from overseas within the last 3 months. His viral load was 89 000 copies/mL, and he had an indeterminate immunoblot.

All 3 cases had a reactive fourth-generation EIA and positive NAAT. The laboratory findings are shown in Table 1. Case 2 had a negative HIV test 2 months before enrollment. Rapid testing for dengue, performed after recruitment, was negative in cases 1 and 3; however, case 2 had dengue NS1 and IgM detected but not IgG or DENV RNA. All cases of AHI had a consultation with an HIV specialist within 17 days of their inclusion in the study.

Sexual activity in the last 3 months was reported by 56.4% of our cohort of suspect DF patients, including all 3 cases of AHI. In our cohort, a carefully collected sexual history had a sensitivity (95% CI) of 100% (29.2%–100%) and negative predictive value of 100%, but this could have been lower (94.1%–100.0%). The prevalence of AHI in patients with symptoms of DF who reported sex in the last 3 months (95% CI) was 2.7% (2.2%–11.2%). Notably, 48.6% of the cohort reported never or only sometimes using condoms. This group had an AHI prevalence (95% CI) of 4.4% (1.4%–13.1%); however, the sensitivity and negative predictive value of this risk factor for AHI was lower: 66.7% (9.4%–99.2%) and 98.4% (91.3%–100.0%), respectively.

DISCUSSION

A prevalence of AHI of 2.1% is high and warrants a systematic approach to diagnosing AHI as a serious cause of acute dengue-like febrile illness. This finding, in a prospective cohort recruited at 3 sites, is consistent with our findings from a retrospective review, that 2.4% of suspected DF admissions to our university hospital had AHI [10]. The rate of HIV in this population is higher than that detected by any other local surveillance program [11].

We included participants with dengue-like febrile illness as this is a well-recognized clinical syndrome for health care workers in our setting. Although this syndrome could reflect other infectious etiologies, including other flaviviruses, most do not require specific treatment, with the notable exception of HIV. Targeting febrile illness presentations to test for AHI has been tried in other clinical settings but found to occur in a lower prevalence than in our study. In a United States hospital, 1% of those tested for acute mononucleosis had AHI [12], and in a Ugandan study, 1% of those presenting with malaria symptoms had AHI [13]. As increasing use of fourth-generation EIAs and NAATs further narrows the "window period," the yield of testing acute febrile patients for HIV may increase.

This strategy was evaluated during a dengue outbreak in Brazil, where investigators analyzed pooled samples of seronegative dengue cases for HIV RNA [14]. An HIV-1 prevalence of 0.73% was found. Though lower than our study, this prevalence was noted to be almost twice that expected for the general population. In the 3 cases identified, 1 sample exhibited the K103N resistance-associated mutation, highlighting another potential cost of such missed opportunities for AHI diagnosis.

Our results suggest that restricting testing to those reporting HIV risk factors could marginally increase the yield of testing, but this needs to be balanced against the risk that the requirement of taking a sexual history may discourage clinicians from offering testing outside of the study setting. A universal offer of AHI testing for acute febrile illness presentations is encouraged in our setting.

Case 2 in our study had antidengue IgM and NS1 detected by rapid test. These results could be consistent with primary dengue, but convalescent serology would be required to confirm the diagnosis. Alternatively, this may be a false-positive NS1 result, a finding recently observed in 2 patients with hematological malignancies, although concurrent AHI and DF is also a possibility [15]. The similar clinical and hematological findings in DF and AHI necessitate either sensitive tests to rule out AHI or specific tests to confirm DF. As the lower limit of estimates for the specificity of NS1 dengue rapid tests are as low as 87.3%, these tests are not useful for ruling in DF when the probability of a serious condition like AHI is high [16]. Although polymerase chain reaction (PCR) for DENV RNA early in the illness and convalescent serology are specific "confirmatory" tests for DF, the former is only useful in the first 5 days, which would only capture 47.9% of participants in this series. Convalescent serology requires recalling the patient. In contrast, AHI testing with fourth-generation EIA and/or NAAT identifies a high prevalence of AHI and would not miss cases of co-infection in settings where both viruses circulate.

Table 1. Characteristics of Participants With and Without Acute HIV Infection

	Acute HIV Cases			HIV Negative[a]
	Case 1	Case 2	Case 3	
Demographic characteristics				
Age, y	59	21	64	35 (21–77)
Male sex	Male	Male	Male	89 (65)
Chinese ethnicity	Other	Chinese	Chinese	107 (78)
Symptoms/signs of DF				
Fever duration, d	3	29	11	6 (1–20)
Nausea or vomiting	Yes	Yes	No	61 (45)
Anorexia	Yes	No	Yes	77 (56)
Rash	Yes	Yes	Yes	52 (38)
Petechial rash	Yes	No	No	12 (9)
Aches and pains	No	Yes	No	91 (66)
Abdominal pain or tenderness	No	No	Yes	24 (18)
Clinical fluid accumulation	No	No	No	3 (2)
Mucosal bleeding	No	No	No	10 (7)
Lethargy	No	Yes	No	103 (75)
Restlessness	No	No	No	39 (28)
Increasing hematocrit	No	No	No	6 (4)
Liver ≥2 cm below costal margin	No	No	No	0 (0)
Leukopenia	No	No	No	27 (20)
Tourniquet test positive	No	No	No	1 (1)
Any warning sign	No	Yes	Yes	113 (82)
AHI risk factors				
Previous HIV test	No	Yes	No	51 (37)
No. of sex partners in last 3 mo	1	3	1	1 (0–5)
Men who have sex with men	No	Yes	No	1 (1)
STIs in last 3 mo	No	No	No	4 (3)
IV drug use in the last 3 mo	No	No	No	6 (4)
Sex with a person known to have HIV	No	No	No	1 (1)
Sex with IVDU	No	No	No	1 (1)
Sex with MSM	No	Yes	No	1 (1)
Sex without a condom	No	Yes	Yes	31 (23)
Sex while intoxicated	No	Yes	No	3 (2)
With a commercial sex worker	No	No	No	1 (1)
Sex in exchange for something	No	No	Yes	5 (4)
Sex with an anonymous partner	Yes	No	Yes	4 (3)
Sex with a foreigner	Yes	No	Yes	8 (6)
Laboratory findings (for other participants)				
Hemoglobin, g/dL	7.3	14.4	16.8	14.1 (5.4–17.7)
Hematocrit, %	45	42.1	46.5	42.2 (30.0–53.2)
Platelets, ×10^9/L	147	169	112	133 (16–455)
Leucocytes, ×10^9/L	7.3	5.37	8.79	4.2 (1.2–19.7)
Neutrophils, ×10^9/L	3.21	2.09	6.32	2.0 (0.3–74.8)
Lymphocytes, ×10^9/L	2.92	2.68	1.22	1.1 (0.3–8.0)
Creatinine, μmol/L	97	74	87	63 (46–112)
Albumin, g/L	34	41	33	38 (15–46)
Alanine aminotransferase, U/L	170	277	34	60 (2–395)
Aspartate aminotransferase, U/L	190	92	49	58 (2–641)

Abbreviations: AHI, acute HIV infection; DF, dengue fever; IVDU, intravenous drug user; MSM, men who have sex with men; STI, sexually transmitted infection.

[a]No. (%) with symptom/risk factor or median (range).

This study has important limitations. First, an acceptance rate of 53%, while considerably higher than local screening programs, means that AHI prevalence may be underestimated if high-risk individuals chose not to participate in the study. Nonetheless, our estimate reflects the real yield of offering screening with informed consent in our setting and may be higher in settings where HIV testing is more widely accepted. Second, introduction of rapid tests for DF during

the study period, when Singapore experienced a dengue outbreak, meant many patients were ineligible because their rapid test results were known at recruitment. This limited the size of the study and the precision of the estimated prevalence of AHI but prevented a biased estimate. As case 2 illustrates, HIV testing irrespective of dengue test results may detect additional cases. Third, for ethical reasons, we could not include temporary or long-term visitors to Singapore, who may have different risks for both AHI and dengue. Last, the precise proportion of patients with AHI among those with dengue-like illness will vary across time and place, in line with changes in the incidence of both diseases. However, the coexistence of HIV and dengue is not unique to Singapore. More than 2 billion people live in regions at risk of dengue, many in countries where HIV is highly prevalent. These settings need approaches to investigating acute febrile illness to identify cases of acute HIV.

The clinical and hematological features of AHI overlap with those of DF. A systematic approach to testing febrile patients with dengue-like presentations for AHI with fourth-generation EIA is justified by the high yield of AHI cases. DF rapid tests lack the specificity to exclude a serious differential diagnosis like AHI, and DENV PCR, if available, would only be suitable for half of this cohort. Testing of patients with dengue-like presentations for AHI could be expanded as physicians in DF-endemic countries readily recognize the syndrome and testing is acceptable to the majority of patients. It also enables prompt HIV care for those who test positive and creates opportunities for preventive interventions during the most infective period.

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