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Febrile Phase Soluble Urokinase Plasminogen Activator Receptor and Olfactomedin 4 as Prognostic Biomarkers for Severe Dengue in Adults

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Background. Dengue cases continue to rise and can overwhelm healthcare systems during outbreaks. In dengue, neutrophil mediators, soluble urokinase plasminogen activator receptor (suPAR) and olfactomedin 4, and mast cell mediators, chymase and tryptase, have not been measured longitudinally across the dengue phases. The utility of these proteins as prognostic biomarkers for severe dengue has also not been assessed in an older adult population.

Methods. We prospectively enrolled 99 adults with dengue—40 dengue fever, 46 dengue with warning signs and 13 severe dengue, along with 30 controls. Plasma levels of suPAR, olfactomedin 4, chymase and tryptase were measured at the febrile, critical and recovery phases in dengue patients.

Results. The suPAR levels were significantly elevated in severe dengue compared to the other dengue severities and controls in the febrile ($P < .001$), critical ($P < .001$), and recovery ($P = .005$) phases. In the febrile phase, suPAR was a prognostic biomarker of severe dengue, with an AUROC of 0.82. Using a cutoff derived from Youden's index (5.4 ng/mL) and an estimated prevalence of severe dengue (16.5%) in our healthcare institution, the sensitivity was 71.4% with a specificity of 87.9% in the febrile phase, and the positive and negative predictive values were 54.7% and 95.8%, respectively. Olfactomedin 4 was elevated in dengue patients but not in proportion to disease severity in the febrile phase ($P = .04$) There were no significant differences in chymase and tryptase levels between dengue patients and controls.

Conclusions. In adult dengue, suPAR may be a reliable prognostic biomarker for severe dengue in the febrile phase.

Keywords. severe dengue; soluble urokinase plasminogen activator receptor; olfactomedin 4 biomarker; neutrophils; arbovirus.

Dengue is the most common arthropod-borne virus globally with an annual estimated 400 million infections and 96 million being symptomatic [1, 2]. Asia has 75% of the global dengue disease burden, with 19% of febrile dengue patients in the region requiring hospitalization. This represents a major ongoing burden on healthcare resources which may be further strained during periodic outbreaks [1].

Most individuals infected with dengue virus remain asymptomatic or present with a self-limiting febrile illness. However, a minority progress to severe dengue (SD), which typically occurs 4–6 days after symptom onset, and accurate early identification of these patients is important for clinical management and allocation of healthcare resources [1, 3]. Currently, the World Health Organization (WHO) recommends frequent monitoring and inpatient management for dengue patients who have warning signs (WS), a list of seven clinical or laboratory criteria [4]. Case management and triage of dengue patients rely mostly on clinical evaluation and laboratory parameters to assess for the presence or absence of WS [3]. However, studies in pediatric and adult cohorts have demonstrated WS to be limited in predicting SD in the febrile phase, including heterogeneity in interpretation, high sensitivity but poor specificity [3, 5–7]. Although several biomarkers including pro-inflammatory cytokines and vascular damage markers were predictive of SD, disparities have been observed in study populations who differ by age or geographical regions [8–10]. Thus, reliable and validated prognostic biomarkers in different study age groups and regions are urgently needed for the better management of dengue patients and to optimize healthcare usage [11].

We and others have shown that mediators released from host innate immune cells such as neutrophils and mast cells play a role in the pathogenesis of SD [8, 12, 13]. Urokinase plasminogen activator receptor (uPAR) is highly expressed on immune

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cells during infection, and its cleaved form soluble uPAR (suPAR) is involved in cell migration and chemotaxis [14]. Plasma suPAR concentrations are elevated in proportion to disease severity in infectious diseases such as malaria, bacterial sepsis, and COVID-19 [14–17]. Additionally, plasma suPAR was inversely correlated with neutrophil uPAR expression, suggesting neutrophils to be the main source of suPAR [16]. In pediatric dengue, levels of olfactomedin 4 (a neutrophil-specific mediator) and mast cell mediators, chymase and tryptase, are elevated in SD [8, 18, 19]. However, the prognostic potential of these mediators has not been examined in older adult dengue populations.

In a prospective longitudinal study, we measured plasma levels of the neutrophil mediators suPAR and olfactomedin 4, and mast cell mediators, chymase and tryptase, levels in adult dengue patients in the febrile, critical, and recovery phases and assessed their suitability as prognostic biomarkers of SD.

METHODS

Participants

Details of the dengue participants recruitment have been previously described [13, 20]. Briefly, in a prospective longitudinal study, patients (>16 years) were recruited (no exclusion criteria) and followed up from febrile, critical, early, and late recovery phases. Disease severities were assigned based on WHO 2009 classifications—dengue fever (DF), dengue fever with warning signs (DWS), and SD [4]. The critical phase was defined according to the day with the lowest platelet count concurrent with the highest hematocrit and defervescence. Controls were adults with no febrile episodes 2 weeks prior to recruitment and no previous dengue within the past six months.

Plasma suPAR, Olfactomedin 4, Chymase and Tryptase Measurements

Plasma suPAR (suPARnostic E001, Virogates) and human olfactomedin 4 (ab267805, Abcam), and human mast cell chymase (E01M0368, BlueGene Biotech) and tryptase (SEB070, Cloud-Clone Corp) concentrations were assayed by enzyme-linked immunosorbent assays based on manufacturers' protocols. All available samples, in duplicate, were read at 450 nm with biotech Synergy H1 and protein concentrations were extrapolated from respective standard curves (4-PL) with Prism (GraphPad V9).

Dengue Virus Serotyping Assay

Dengue virus serotyping and viral threshold cycle were obtained using the Food and Drug Administration (FDA)-approved CDC DENV1-4 reverse transcription

Table 1. Baseline Characteristics and Disease Outcomes

	Controls (n = 30)	DF (n = 40)	DWS (n = 46)	SD (n = 13)	*P* Value[a]
Male (%)	15 (50.0)	28 (70.0)	27 (58.7)	8 (61.5)	.32
Median age (IQR) [range], y	44 (32–59) [23–75]	44 (29–55) [18–73]	48 (35–58) [21–80]	63 (45–68) [24–83]	.123
Median body mass index (IQR), kg/m^2	24.3 (21.9–26.4)	25.2 (22.7–28.1)	24.0 (21.2–27.8)	26.0 (24.5–31.0)	.39
Median CCI (IQR) [range]	0 (0–0) [0–2]	0 (0–0) [0–3]	0 (0–0) [0–3]	0 (0–1) [0–5]	.072
Acute myocardial infarction, n (%)	0	0	1 (2)	2 (15.4)	**.020**
Diabetes Mellitus, n (%)	3 (10)	3 (7.5)	8 (17.4)	3 (23)	.42
Hypertension, n (%)	4 (13.3)	9 (22.5)	13 (28.3)	9 (69.0)	**.001**
Previous Dengue, n (%)	3 (10)	1 (2.3)	2 (3.9)	2 (16.7)	.13
Days after first reported symptoms					
Median day of illness for febrile phase (IQR)	NA	4 (3–5)	4 (3–5)	5 (3–6)	.87
Median day of illness for critical phase (IQR)	NA	6 (5–7)	6 (5–7)	6 (5.5–7)	.28
Median day of illness for early recovery phase (IQR)	NA	8.5 (7–10.5)	8 (7–8)	8 (7–10)	.19
Median day of illness for late recovery phase (IQR)	NA	20.5 (16–24.5)	17.5 (15–21.5)	18 (15–26)	.10
Hospitalization outcomes					
Length of hospital stay (IQR), d	NA	4 (3–5.5)[b]	5 (4–6)	7 (5–8)	**.010**
ICU admission (%)	.	0	0	2 (15.4)	.14
Inotrope use (%)	NA	0	0	2 (15.4)	**.018**
Transfusion of blood and blood products (%)	NA	0	0	1 (7.6)	.14

Data are presented in median (interquartile range) or no. (%) of patients, unless otherwise indicated. *P* values of <.05 were considered significant (in bold). Abbreviations: CCI, Charlson comorbidity index; DF, dengue fever; DWS, dengue with warning signs; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; SD, severe dengue.

[a] *P* values tabulated by ANOVA/Kruskal-Wallis or χ^2 test/Fishe exact for comparisons across groups.

[b] Includes 24 participants with dengue fever who were admitted during dengue illness.

polymerase chain reaction (RT-PCR) assay. Briefly, viral RNA was extracted from the first plasma sample obtained, and the RT-PCR assay was performed in singleplex reactions following manufacturer's instructions in 25 μL of reaction mixture with 2 μL of RNA template.

Statistical Methods

Analysis of variance or the Kruskal-Wallis test was performed to determine intergroup differences for parametric or nonparametric continuous variables, respectively. Categorical variables were assessed using the χ^2 test. Post hoc pairwise comparisons were used to compare differences between DF, DWS, and SD. Logistic regression was done to determine the association between SD and the respective mediator levels. Laboratory variables (platelet count, albumin, aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were reported to be associated with SD, and these variables were included in a multiple logistic regression model if $P < .05$ in the univariate analysis [3]. Longitudinal mixed-effects models were used to analyze repeated measures per dengue participant with restricted maximum likelihood estimations. The area under the receiver operating curve (AUROC) and corresponding 95% confidence interval (CI) was calculated to evaluate the suitability of suPAR, olfactomedin 4, tryptase and chymase as potential biomarkers for SD. For mediators with an AUROC $\geq$ 0.7, a Youden index was calculated to determine an optimal cutoff value for SD. The negative predictive value (NPV, proportion of true negatives), positive predictive value (PPV, proportion of true positives), sensitivity, and specificity were also determined. Additionally, PPVs were calculated based on determined cutoff values together with the expected prevalence of SD in patients admitted for inpatient management, in our institution, and well as affiliated primary care settings [6, 21]. Statistical analysis was conducted with Stata version 16 (Stata Corp., College Station, Texas, USA) and graphical representations by Prism. A 2-sided value of $P < .05$ was considered significant.

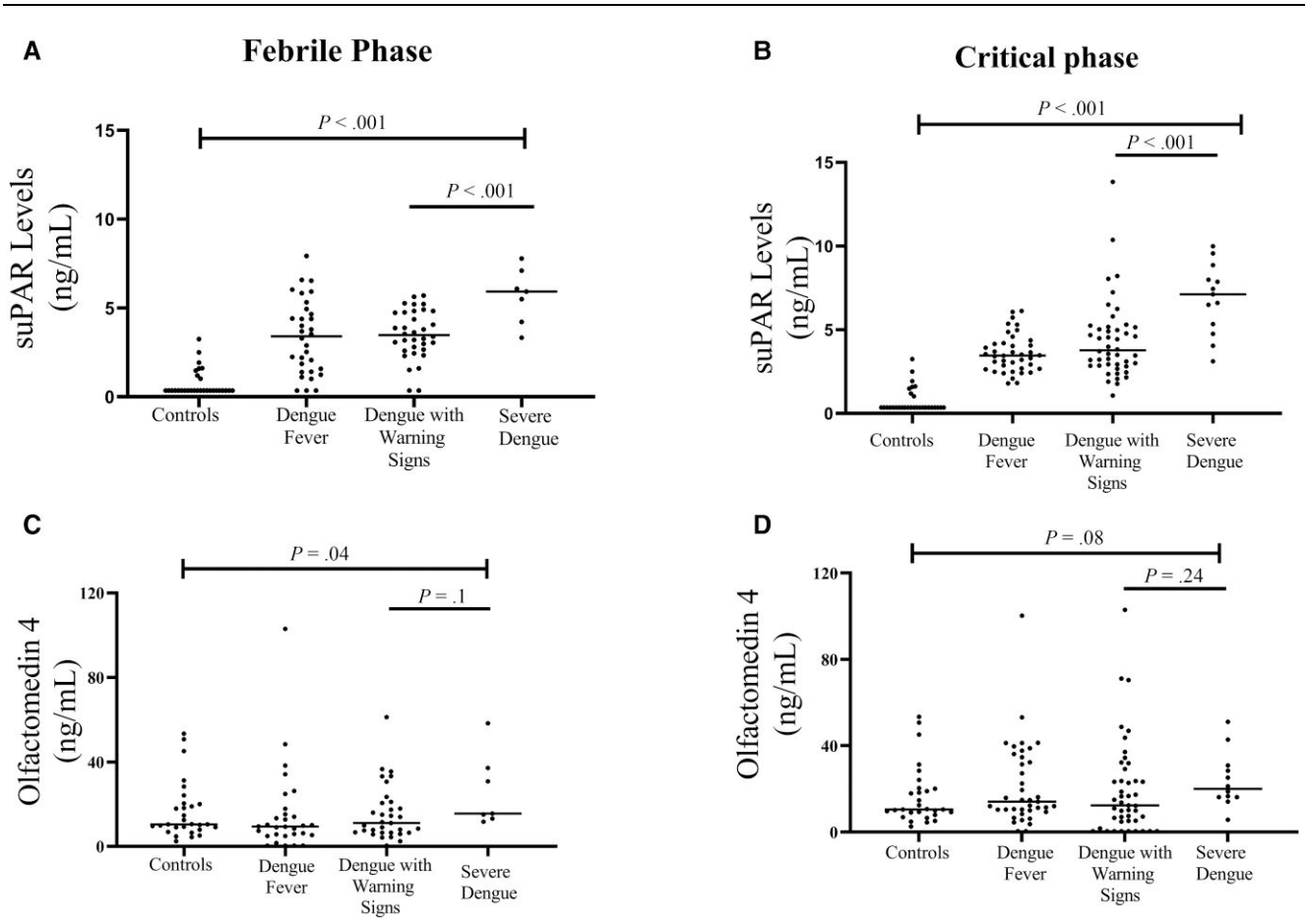

Figure 1. Plasma soluble urokinase plasminogen activator receptor (suPAR) and olfactomedin 4 concentrations by dengue severity at febrile and critical phases. Dengue subjects (n = 99) classified based on WHO2009 dengue classifications: dengue fever (n = 40), dengue with warning signs (n = 46), and severe dengue (n = 13); controls (n = 30). *A*, Plasma suPAR concentrations at febrile phase stratified by disease severity. *B*, Plasma suPAR concentrations at critical phase stratified by disease severity. *C*, Plasma olfactomedin 4 concentrations at febrile phase stratified by disease severity. *D*, Plasma olfactomedin 4 concentrations at critical phase. *A–D*, Data presented as dot plot and horizontal dash lines represent median; *P* values by Kruskal-Wallis tests.

Table 2. Plasma Soluble Urokinase Plasminogen Activator Receptor, Olfactomedin 4, Chymase and Tryptase Levels in Febrile, Critical, Early Recovery and Late Recovery, and in Controls

	Controls (n = 30)	DF (n = 40)	DWS (n = 46)	SD (n = 13)	*P* Value[a]
Soluble urokinase plasminogen activator receptor levels, median (IQR) (ng/mL)					
Febrile phase	0.35 (0.35–1.02)	3.40 (1.49–4.80)	3.47 (2.66–4.75)	5.92 (3.33–7.11)	**<.001**
Critical phase		3.46 (2.68–4.17)	3.77 (2.86–5.15)	7.11 (5.33–7.99)	**<.001**
Early recovery phase		3.14 (2.08–4.12)	3.09 (2.22–3.84)	5.90 (4.10–6.62)	**.005**
Late recovery phase		1.81 (1.13–2.50)	2.00 (1.59–2.75)	4.18 (2.03–4.70)	**.03**
Olfactomedin 4, median (IQR) (ng/mL)					
Febrile phase	10.46 (8.47–22.28)	9.67 (5.55–21.35)	11.35 (6.57–21.1)	15.61 (13.16–37.13)	**.04**
Critical phase		14.13 (10.01–34.67)	13.02 (5.18–29.18)	19.99 (16.16–29.61)	.08
Early recovery phase		11.63 (0.41–23.77)	11.18 (0.44–19.20)	16.92 (14.55–29.66)	.10
Late recovery phase		10.80 (0.42–32.88)	17.93 (12.89–28.12)	22.28 (4.46–41.32)	.06
Chymase levels, median (IQR) (ng/mL)					
Febrile phase	1.28 (1.07–1.77)	1.82 (0.95–3.40)	1.32 (0.79–2.71)	1.12 (0.77–2.77)	.41
Critical phase		1.41 (1.30–1.78)	1.36 (1.27–1.49)	1.49 (1.29–2.00)	.21
Early recovery phase		1.50 (1.42–1.98)	1.41 (1.28–1.49)	1.34 (1.25–1.51)	.08
Late recovery phase		1.48 (1.41–1.55)	1.48 (1.46–1.56)	1.52 (1.48–1.6a4)	.43
Tryptase levels, median (IQR) (ng/mL)					
Febrile phase	20.08 (12.59–56.12)	14.02 (7.97–30.35)	10.604 (2.85–23.79)	23.85 (8.92–38.79)	.1
Critical phase		12.53 (5.57–21.96)	11.86 (6.38–19.28)	19.54 (6.65–44.15)	.23
Early recovery phase		14.52 (11.99–1829)	9.47 (5.66–15.75)	19.18 (8.04–19.18)	.15
Late recovery phase		13.42 (10.89–15.86)	12.97 (11.73–15.86)	10.03 (6.18–15.47)	.35

Data are presented in median (interquartile range). *P* values of <.05 were considered significant (in bold).

Abbreviations: DF, dengue fever; DWS, dengue with warning signs; IQR, interquartile range; SD, severe dengue.

[a]*P* values tabulated by Kruskal-Wallis for comparisons across groups.

Ethical Review

This study was approved by the National Healthcare Group Domain Specific Review Board (E/2016/00982). Written informed consent was obtained from all patients prior to enrolment.

RESULTS

Baseline Characteristics

Detailed baseline and clinical characteristics have previously been published [13, 20]. Briefly, we recruited 129 patients for the study: 40 DF, 46 DWS, 13 SD, and 30 controls. For the DF, DWS, and SD groups, 32, 34, and 7 patients, respectively, were enrolled in the febrile phase. Generally, DF and DWS patients were examined on median day 4 (range 3–5) and SD patients on median day 5 (3–6) after symptoms onset. There were no significant differences across disease severities except in the SD group, a higher proportion had hypertension (69.0%), previous myocardial infarction (15.4%) and lengthier hospitalization stay compared with DF and DWS groups. Table 1. For dengue subjects, 53 had detectable serotypes—30 (56.6%) serotype 2, 21 (39.6%) serotype 3, and 1 (1.8%) each with serotype 1 and 4. Of note, serotypes 2 and 3 were the predominant serotypes circulating in Singapore during the study period [13].

Plasma suPAR and Olfactomedin 4 Levels Were Increased in Dengue Patients Versus Controls

Plasma suPAR levels were significantly increased in SD (febrile: median [interquartile range {IQR}], 5.92 [3.33–7.11] ng/mL; critical: 7.11 [5.33–7.99] ng/mL) compared to DWS (febrile: 3.47 [2.66–4.75] ng/mL; critical: 3.77 [2.86–5.15] ng/mL), DF (febrile: 3.40 [1.49–4.80] ng/mL; critical: 3.46 [2.86–5.15] ng/mL) and controls (0.35 [0.35–1.02] ng/mL) in the febrile and

Table 3. Differences in Plasma Soluble Urokinase Plasminogen Activator Receptor, Olfactomedin 4, Chymase, and Tryptase Levels Between Dengue Serotype 2 and 3 in Febrile and Critical Phases

	Serotype 2	Serotype 3	*P* Value[a]
Soluble urokinase plasminogen activator receptor levels, median (IQR) (ng/mL)			
Febrile phase	3.33 (2.30–5.32)	3.50 (1.51–4.83)	.62
Critical phase	3.55 (2.62–4.68)	3.44 (2.86–4.43)	.72
Olfactomedin 4, median (IQR) (ng/mL)			
Febrile phase	11.12 (6.57–17.82)	10.31 (5.75–17.41)	.58
Critical phase	14.86 (6.54–25.16)	14.98 (9.86–23.43)	.66
Chymase levels, median (IQR) (ng/mL)			
Febrile phase	1.88 (0.84–2.98)	1.30 (0.78–2.10)	.40
Critical phase	1.45 (1.30–1.54)	1.37 (1.28–1.41)	.26
Tryptase levels, median (IQR) (ng/mL)			
Febrile phase	26.25 (10.60–33.04)	13.62 (8.25–27.11)	.27
Critical phase	11.58 (7.21–25.73)	13.50 (7.83–20.20)	.32

Data are presented in median (interquartile range).

Abbreviation: IQR, interquartile range.

[a]*P* values tabulated by Kruskal-Wallis for comparisons across groups.

critical phases, both $P < .001$] (Figure 1*A* and 1*B*). In the recovery phases, suPAR levels, in SD patients, remained elevated compared to DWS, DF, and control groups (see Table 2.) By pairwise comparisons, suPAR concentrations were significantly elevated in the SD group versus either DWS or DF in all the disease phases ($P < .001$), but no significant difference between the DWS and DF groups was observed ($P > .05$) (Table 2). Longitudinally, in all dengue patients, suPAR kinetics increased from febrile to critical phase and decreased thereafter (Supplementary Figure 1*A*). Olfactomedin 4 levels were significantly increased in all dengue patients in the febrile phase compared to controls, but not in the critical and recovery phases (febrile—DF: 9.67 [5.55–21.35] ng/mL; DWS: 11.35 [6.57–21.1] ng/mL; SD: 15.61 [13.16–37.13] ng/mL, $P = .04$) (Figure 1*C* and 1*D*). By pairwise comparison, there were no significant differences between SD versus DWS or DF groups in any of the disease phases. Longitudinally, there was also no significant change in olfactomedin 4 levels from the febrile phase to late recovery (Supplementary Figure 1*B*).

There were no significant differences in suPAR and olfactomedin 4 levels between subjects with serotypes 2 and 3 in the febrile and critical phases (Table 3, all $P > .5$). There was also no association between suPAR or olfactomedin 4 concentrations with dengue virus cycle threshold values in the acute phase.

Plasma Chymase and Tryptase Levels Were Not Different in Dengue Patients and Controls

There were no significant differences in plasma chymase and tryptase levels between dengue patients and controls in each dengue phases (Figure 2*A*–*D*, both $P > .1$). There were also no differences in the levels of both mediators among the different severities in each dengue phase (Table 2). Longitudinally, no significant change in either chymase or tryptase levels was observed (Supplementary Figure 1*C* and *D*).

There were no significant differences in mast cells mediators, chymase and tryptase, levels between subjects with serotypes 2 and 3 in the febrile and critical phases (Table 3, all $P > .2$). There was also no association between chymase or tryptase concentrations with dengue virus cycle threshold values in the acute phase.

Increased suPAR Levels Were Associated With an Increased Risk of Severe Dengue

By logistic regression models, a unit increase in suPAR level was associated with increased risks of SD in the febrile phase (odds ratio [OR]: 2.1, 95% CI [1.2–3.7], $P = .009$) and critical phase (OR: 1.70, 95% CI [1.27–2.28], $P < .001$). In the critical but not febrile phase, albumin, AST, and ALT were associated with higher risk of SD. After adjusting for these variables and a previous history of cardiac failure and coronary artery disease, suPAR and albumin levels remained as risk factors for SD (AOR: 1.64, 95% CI [1.27–2.10], $P < .001$), in the critical phase. In contrast, olfactomedin 4, chymase and tryptase were not associated with increased odds of SD.

Febrile Phase suPAR and Olfactomedin 4 are Potential Prognostic Biomarkers of Severe Dengue

In the febrile and critical phases, the AUROC for suPAR to predict SD was 0.82 (95% CI [.63–.99]) and 0.86 (95% CI [.75–.97]), respectively (Figure 3*A* and 3*B*). Olfactomedin 4 showed modest ability in predicting SD, the AUROC was 0.73 (95% CI [.59–.87]) and 0.64 (95% CI [.51–.77]) in the febrile and critical phases, respectively (Figure 3*C* and 3*D*). The corresponding AUROCs of chymase were 0.41 (95% CI [.11–.70]) and 0.59 (95% CI [.39–.78]) in the febrile and critical phases, and for tryptase it was 0.63 (95% CI [.30–.96]) and 0.64 (95% CI [.45–.81]), respectively.

The Youden index for suPAR to predict SD was 5.4 ng/mL and based on a retrospective study we assessed that 16.5% of all dengue cases admitted to our institution progressed to SD [6]. We demonstrated that at this cutoff and prevalence, it was 71.4% sensitive and 87.9% specific in the febrile phase, and in the critical phase, it was 69.2% sensitive and 87.2% specific to predict SD. The PPV for SD were 54.7.% (febrile) and 52.7% (critical), and the corresponding NPV were 95.8% (febrile) and 93.5% (critical). In affiliated primary care facilities, the overall prevalence of patients who progressed to SD was 8.8% (readout from the discovery cohort) [21]. In this setting, in the febrile phase, the sensitivity was 71.4% and specificity was 87.9%, whereas in the critical phase, it was 69.2% sensitive and 87.3% specific. The PPV for febrile and critical phases were 36.2% and 34.3%, respectively, and the corresponding NPVs were 97.0% and 96.7%.

For olfactomedin 4, the Youden index was 14.1 ng/mL to distinguish SD cases. In our institution (16.5% SD prevalence), in the

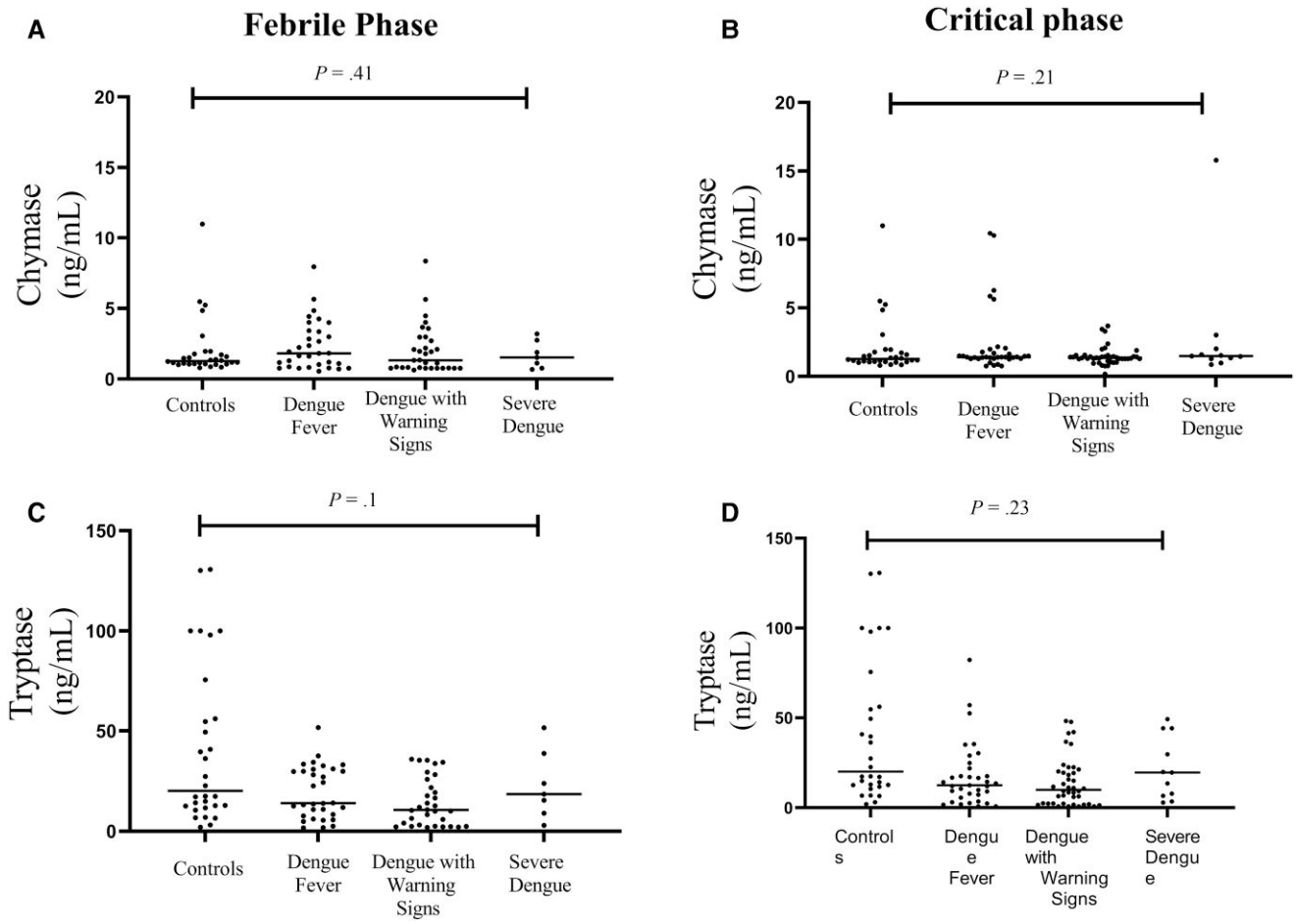

Figure 2. Plasma chymase and tryptase concentrations by dengue severity at febrile and critical phases. Dengue subjects (n = 129) classified based on WHO2009 dengue classifications—dengue fever (n = 40) dengue with warning signs (n = 46) and severe dengue (n = 13); controls (n = 30). *A*, Plasma chymase concentrations at febrile phase stratified by disease severity. *B*, Plasma chymase concentrations at critical phase stratified by disease severity. *C*, Plasma tryptase concentrations at febrile phase stratified by disease severity. *D*, Plasma tryptase concentrations at critical phase. *A* and *B*, Data presented as dot plot and horizontal dash lines represent median; *P* values by Kruska-Wallis test.

febrile phase, the sensitivity and specificity were 71.4% and 62.1%, respectively. Additionally, the PPV was 27.1% and NPV was 91.7%. Applying this to the primary care settings, the sensitivity and specificity in was 71.4% and 62.1% and the respective PPV and NPV were 16.7% and 95.8% in the febrile phase.

DISCUSSION

In a cohort of Singaporean adult dengue patients, levels of the neutrophil mediators, suPAR and olfactomedin 4, were increased in dengue patients compared to controls in the febrile phase. In SD, suPAR but not olfactomedin 4 levels were elevated compared to DF and DWS patients across the different time points. Using a defined cutoff for suPAR (5.4 ng/mL) and with SD prevalence of 16.5% the PPV for SD was 54.7% and the NPV was 95.8% in the febrile phase. For olfactomedin 4 (14.1 ng/mL) in the same setting, the PPV was 27.1% and NPV was 91.7%. In contrast, levels of mast cells mediators, chymase and tryptase, did not differ between dengue patients, and controls, importantly, were not predictive of SD.

Increased circulating suPAR and olfactomedin 4 levels are associated with increased inflammation, which is a hallmark of SD [22]. Our observation of elevated suPAR concentrations in SD agrees with recent observations of positive associations and poorer outcomes in other infectious diseases [14–16]. Similarly, an earlier observation, in Singapore, also reported higher suPAR concentrations in hospitalized dengue patients [21]. Importantly, increased suPAR and olfactomedin 4 levels, together with observations of higher myeloperoxidase levels and increased neutrophil activation in SD, suggests a pathogenic role for neutrophils in dengue [12, 13]. Therefore, the attenuation of neutrophil activation should be explored for the management of clinical dengue.

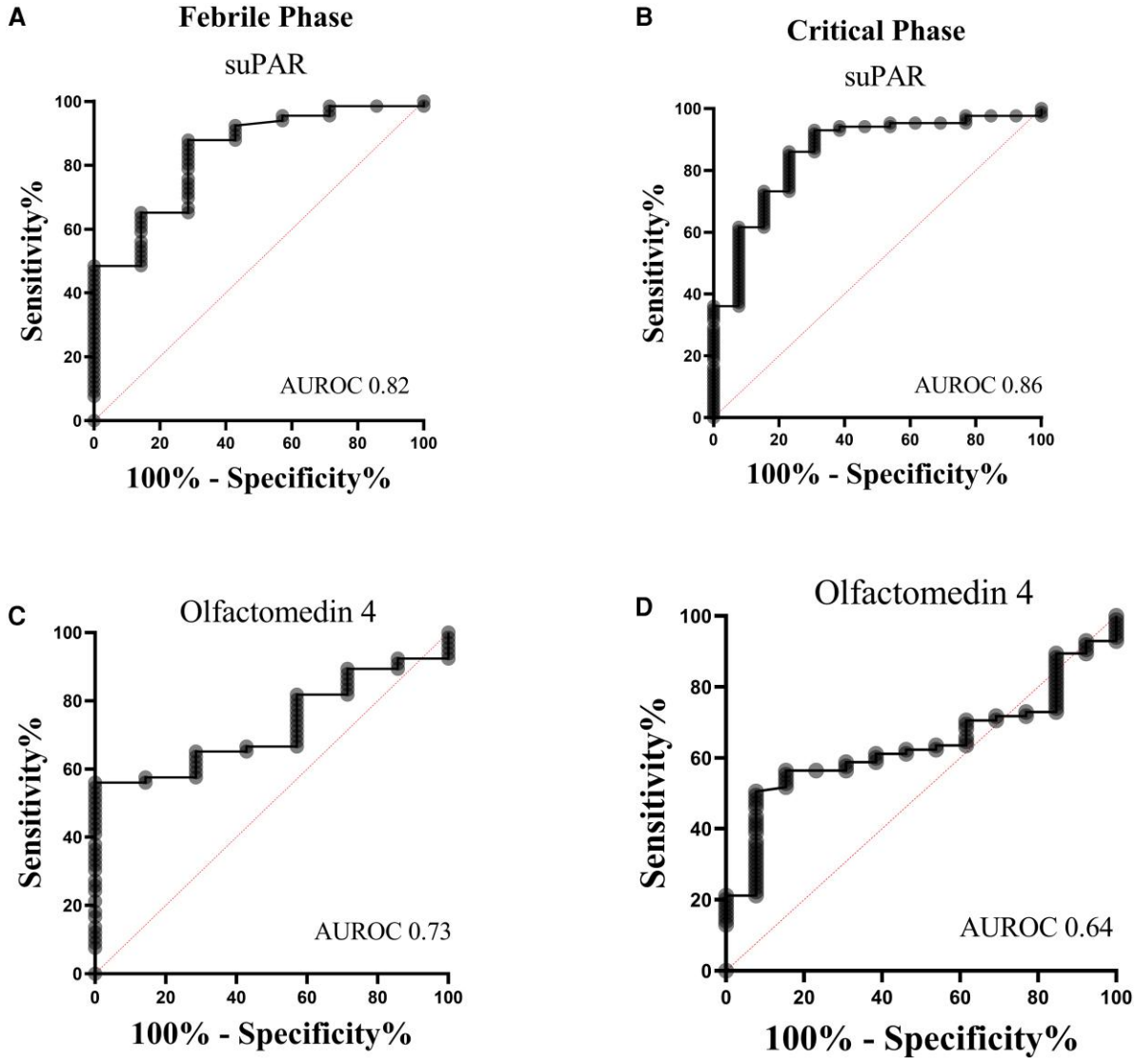

Figure 3. Area under the receiver operating curve of plasma soluble urokinase plasminogen activator receptor (suPAR) and olfactomedin 4 in predicting severe dengue at febrile and critical phases. *A*, The area under the receiver operating curve (AUROC) for plasma suPAR in predicting severe dengue assayed at febrile phase. *B*, AUROC for plasma suPAR in predicting severe dengue assayed at critical phase. *C*, AUROC for plasma olfactomedin 4 in predicting severe dengue assayed at febrile phase. *D*, AUROC for olfactomedin 4 in predicting severe -dengue assayed at critical phase. Nonparametric AUROC with 95% confidence interval are presented.

For the management of acute febrile illness in low-and-middle income settings, the optimal use of limited resources and clinical management of patients will require both reliable diagnostic and prognostic tests that can be utilized in a variety of healthcare settings [11]. A major priority in dengue research is the development of prognostic biomarkers that can accurately predict the development of SD in the febrile phase. This would potentially reduce the healthcare burden and allow resources to be focused on high-risk patients. Currently, practitioners in dengue endemic countries rely extensively on warning signs as defined by WHO to triage and manage dengue patients [4]. A recent systematic review reported vomiting, abdominal pain and tenderness, spontaneous or mucosal bleeding, and detection of clinical fluid accumulation to be associated with higher risk of progression to severe disease in the febrile phase [3]. The study supported WHO's guidelines using WS; however, a major limitation, despite high sensitivity, is the poor specificity [5, 7]. In corroboration, a retrospective Singapore cohort evaluated the utility of WS as predictors of SD and the presence of any single WS had a sensitivity of 96.0% with a specificity of 18.0% and a PPV of 15% and NPV of 96% [6]. In another prospective study involving Vietnamese children, a prognostic model consisting of a history of vomiting, platelet count, AST and a positive rapid NS1 test, were predictive of SD in the early febrile phase [23]. In the same study, where 6% of the study population developed complications, the model had a PPV of 10% and an NPV of 99% for SD.

In our adult cohort, with known SD prevalence, we showed that suPAR levels > 5.4 ng/mL had higher PPVs (36.2%–54.7%) and NPVs (95.8%–97.0%) in discriminating for SD in the febrile phase. The high NPV observed suggests that at a concentration below the cutoff, the probability developing SD (~3 to 4 in every 100 cases) is low, thus safe for home recovery. Of clinical significance, a point-of-care quantification for suPAR is available and was demonstrated to reliably prognosticate coronavirus disease 2019 (COVID-19) patients for treatments [17]. Together, these findings suggests that point-of-care quantification of suPAR may potentially be implemented in various clinical settings during dengue outbreaks. Although multiple inflammatory and vascular markers have been demonstrated to be predictive of SD, these tests required sophisticated equipment as well as trained staffs, limiting their usability in low- and middle-income settings [9, 24].

Similarly, increased olfactomedin 4 has been reported to be a marker of disease severity in several infectious diseases [25]. Olfactomedin 4 levels, measured in samples collected between symptoms onset and defervescence, have been demonstrated to be increased in Cambodian children, and there was a trend of increased concentrations in young Colombian adults with SD [19]. In the same study, using only olfactomedin 4 levels determined in Cambodian children, the AUROC was 0.95 in discriminating SD. It is worth noting that the tabulated AUROC was based on readouts from the febrile and critical phases; therefore, it remains unclear whether olfactomedin 4 could be a reliable prognostic biomarker of SD in the febrile phase. In our study, olfactomedin 4 was elevated in all dengue patients compared to controls but was not higher in SD patients. The AUROC, PPV, and NPV results suggest olfactomedin 4 to be only a modest prognostic biomarker in the febrile phase.

Mast cell mediators chymase and tryptase have been shown to elicit vascular leakage in vitro and shock in dengue animal models [18, 26]. In a Sri Lankan dengue cohort, chymase was shown to predict dengue hemorrhagic fever (DHF) [8]. Additionally, at a cutoff of >1.5 ng/mL, chymase was reported to have a sensitivity of 96.0% and a specificity of 79.0% to predict DHF, with a PPV of 76.0% and a NPV of 97.0% for children [8]. However, chymase appears to be a weak predictor of SD in adults over 45 years old, and our cohort of older adults mirrors the earlier observations.

Strengths of this study include the longitudinal follow-up throughout the disease phases. Limitations are the small number of SD patients, the lack of pediatric patients, and most of the patients were also enrolled later in the febrile phase.

In conclusion, plasma suPAR levels were elevated in adult dengue patients in proportion to disease severity and was a more reliable predictor of SD at both the febrile and critical phases, whereas olfactomedin 4 also modestly predicted SD in the febrile phase. Human mast cell mediators chymase and tryptase were not elevated in our adult cohort and did not predict severe disease. Critically, larger studies in both pediatric and adult patients recruited earlier in the febrile phase are urgently needed to validate our results.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. Conceptualization of study: A. T. Investigation and data curation: A. T., C. T. T. L., T. T., P. Y. C. Formal analysis: T. W. Y. Writing original draft: T. W. Y. Edits and revisions: A. T., C. T. T. L., TT, P. Y. C., T. W. Y. All authors contributed to data interpretation, critically reviewed the manuscript, and approved the final manuscript for submission.

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