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Early circulating biomarkers to predict plasma leakage in dengue fever

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SUMMARY

Background: Dengue, a mosquito-borne viral infection, poses a rapidly growing burden, particularly in low- and middle-income countries. Without early identification of patients at risk of severe outcomes (dengue haemorrhagic fever, severe dengue, and plasma leakage- the latter typically occurring on days 5–7 of illness), untriaged admissions lead to hospital overcrowding and suboptimal care.
Methods: This nested case-control study compared early-stage plasma samples (within the first 96 hours of fever) from dengue patients with and without plasma leakage. Thirty-four potential biomarkers, selected through systematic review, were tested on a multiplex bead-based immunoassay platform. Subgroup analysis stratified patients by primary or secondary dengue infection.
Findings: A total of 228 patient samples (114 had plasma leakage) were tested. Elevated Vascular cell adhesion molecule-1 (OR:3.289, 95% CI: 1.090–9.926, $p < 0.05$), and Interleukin 33 receptor levels (OR: 2.677, 95% CI: 1.244–5.856, $p < 0.05$) were associated with an increased risk of plasma leakage while eotaxin-1 was associated with a decreased risk (OR: 0.166, 95% CI: 0.057–0.483, $p < 0.05$). When adjusted for prior dengue exposure, additional biomarkers (C-X-C motif chemokine 11, serum amyloid A) were also associated with plasma leakage.
Interpretation: Plasma leakage in dengue, being more objectively measurable than other severe outcomes, offers a reliable endpoint for biomarker studies. Identifying biomarkers that predict plasma leakage strengthens the evidence base in dengue research. These biomarkers could improve clinical assessment and patient care in dengue cases.

Research in Context

Evidence before this study

Plasma leakage is a key pathophysiological feature in dengue fever which characterises a subgroup of patients that can progress to life-threatening severe disease. Thus, identifying biomarkers that can predict the likelihood of plasma leakage can help to triage patients for hospital admission in early infection. A previously published systematic review by us collated evidence on early predictive biomarkers for adverse outcomes in dengue fever (severe dengue, dengue hemorrhagic fever and plasma leakage) from published literature between 1997 and 2022. It failed to find high-certainty evidence for biomarkers that are predictive of plasma leakage. This was because individual studies had often considered the outcomes of dengue haemorrhagic fever and severe dengue to correlate with predictive biomarkers instead of plasma leakage. The former outcomes are defined by more than one criterion (hence subject to more inter-observer bias) while plasma leakage is an objectively demonstratable single, stand-alone criterion that can still identify at-risk patients needing close monitoring.

Added value of this study

This study identified biomarkers in early dengue infection that can differentiate patients at risk of plasma leakage. We found that elevated levels of VCAM-1 (OR: 3.289, 95% CI: 1.090–9.926, $p < 0.05$) and IL-33R (ST2) (OR: 2.677, 95% CI: 1.244–5.856, $p < 0.05$) were independently associated with an increased risk of plasma leakage, while elevated CCL11 (OR: 0.166, 95% CI: 0.057–0.483, $p < 0.05$) was associated with a

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decreased risk. Notably, the protective role of CCL11 is a novel finding. When the patients were stratified as having primary or secondary dengue infections, elevated CXCL11 (OR: 0.130, 95% CI: 0.038–0.447, $p < 0.05$) was associated with a decreased risk of plasma leakage in primary dengue patients. For secondary dengue infections, higher VCAM-1 concentrations (OR: 32.979, 95% CI: 2.429–447.708, $p < 0.05$) were associated with an increased risk of plasma leakage, while higher SAA concentrations were associated with a reduced risk (OR: 0.031, 95% CI: 0.002–0.433, $p < 0.05$).

Implications of all the available evidence

This work fills a critical knowledge gap in dengue research by identifying early infection biomarkers that are predictive of plasma leakage.

Introduction

The clinical manifestations of symptomatic dengue infection range from simple undifferentiated fever to potentially life-threatening dengue shock syndrome or severe dengue.[1] Two widely used clinical classification systems published by the World Health Organization in 1997 and 2009 classify patients at risk of life-threatening complications within the subgroups of dengue haemorrhagic fever (DHF) and severe dengue (SD) respectively.[2,3] The typical course of symptomatic dengue infection begins with a febrile phase lasting approximately 1–4 days. In a subset of the patients, the illness advances to the critical phase around defervescence (days 4–7), marking a time of elevated risk for serious complications.[2] Currently, it is not possible to predict which dengue patients are at risk of life-threatening complications, early in the course of illness (within the first 96 hours of fever). Consequently, during seasonal outbreaks, hospital admissions overburden the limited healthcare resources in many endemic countries, compromising the safety of both dengue and non-dengue patients.[4,5] Accurate triage of patients during the early febrile phase could allow for safe discharge of low-risk patients, potentially reducing unnecessary hospitalizations and optimizing resource allocation.

The incidence of dengue fever has increased more than tenfold from 2000 to 2023, and the numbers reported to the World Health Organization (WHO) reached an all-time high of 6.5 million cases in 2023, with over 7300 dengue-related deaths across 80 countries.[6] The reported cases are likely to be only a small fraction of the true global incidence of dengue, which some estimate to be as high as 390 million cases annually, including asymptomatic infections.[7] With dengue transmission endemic in over 100 countries, predominantly low- and middle-income countries, the need for effective early triage and resource management is critical.[8]

Plasma leakage is a term describing the extravasation of fluid due to increased capillary permeability in dengue, typically seen around days 5–7 of fever (also referred to as the critical phase). It is an essential clinical criterion to define DHF[3] or SD[2]and therefore regardless of the WHO clinical classification, plasma leakage is the least common denominator to identify patients at risk of future life-threatening complications. Early identification and judicious fluid management during plasma leakage can significantly lower mortality rates.[9] Thus, if a patient's risk for future plasma leakage can be predicted during the early days of fever (within the first 96 hours), those with a higher risk can be prioritized for hospital admission. However, there is currently no validated early warning system to predict patients at risk of plasma leakage.

The complex interplay between viral factors, host genetics, and immune responses in dengue pathogenesis,[10] coupled with our incomplete understanding of disease mechanisms underpinning severe disease manifestations including plasma leakage, highlights the need for improved prognostic tools. Notably, severe clinical manifestations often coincide with viral clearance rather than peak viremia, underscoring the critical role of host immune mechanisms in disease progression.[11] Antibody-dependent enhancement (ADE), wherein non-neutralizing dengue-specific antibodies from prior heterotypic infections facilitate viral entry, further contributing to disease severity in secondary infection.[12–16] Consequently, there is a growing interest in the identification of host biomarkers predictive of dengue severity, enabling timely identification of high-risk patients and guiding targeted therapeutic interventions. Our recent systematic review and meta-analysis identified several early biomarkers (measured within the first 96 hours) that were consistently predictive of either DHF or SD. Elevated C-reactive protein (CRP), aspartate aminotransferase (AST), interleukin-8 (IL-8) and decreased albumin levels were predictive of DHF, while elevated vascular cell adhesion protein -1 (VCAM-1), syndecan-1, AST and CRP were predictive of SD with high certainty evidence (reported by more than one study and confirmed by meta-analyses). A further 44 and 28 biomarkers were also statistically significantly associated with DHF and SD respectively, but were reported in one study only.[5] However, there was minimal evidence for biomarkers that were predictive of plasma leakage as a stand-alone criterion.

The present study aimed to identify early circulating biomarkers predictive of plasma leakage, prioritizing biomarkers with high or low certainty evidence of association with DHF or SD from our systematic review (as plasma leakage is a common criterion to define both DHF and SD), as well as other related biomarkers as suggested by network and pathway analyses (see below).

Methods

Clinical samples

Stored human plasma samples and clinical data were sourced from the Colombo Dengue Study (CDS), an ongoing prospective observational cohort study in Sri Lanka administered by the University of Colombo in collaboration with the University of New South Wales (UNSW) Sydney, Australia. Details of CDS and several of its completed projects have been published previously.[17–20] Briefly, patients clinically suspected of having dengue fever are recruited from the National Hospital, Colombo, Sri Lanka, within the first 96 hours of fever according to the WHO clinical case definition of dengue. Plasma and buffy coats are collected on admission for research, and an aliquot is transferred in dry ice to UNSW Sydney for experiments. The diagnosis is confirmed using the NS1 antigen test at the bedside (one-step SD Bioline dengue NS1 antigen test, Alere SD, USA) and RT-qPCR (SuperScript III Platinum One-Step Quantitative RT-PCR System without Rox, Invitrogen, USA, cat#)11732–088). For logistical reasons, the latter is done retrospectively for all patients via batch processing. Hence, the treating physicians who are independent of the study team are unaware of RT-PCR results and treat all patients as presumptive dengue, which is the standard clinical practice in Sri Lanka.[21] All recruited patients are followed up daily to record the progression of the clinical illness, results of routine laboratory tests, and outcomes. The primary outcome recorded in CDS is plasma leakage, which is diagnosed as either a ≥20% increase in haematocrit compared to baseline, an absolute haematocrit ≥ 45% during any time of illness, or by detection of extravasated fluid in peritoneal or pleural cavities by bedside ultrasonography according to a protocol previously published.[22] All patients in this study were assessed for plasma leakage by both criteria and were considered as having plasma leakage if positive by at least one criterion. At the time of sample collection for biomarker assessment, none of the patients had plasma leakage. Occurrence of severe dengue and death are also observed as secondary outcomes.[19]

Ethics Statement

Ethics approvals for the study was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo (EC/17/080), and the Human Research Advisory Panel (Biomedical) of the University of New South Wales (HC220706). All patients gave written informed consent for the donation and storage of serum samples for research.

Groups and sample size calculation

Since plasma was collected within the first 96 hours of fever, the study design allowed a nested case-control comparison of dengue patients who developed plasma leakage vs. those who did not, further stratified into those with primary or secondary dengue infection. Anti-dengue IgG was measured in all included samples using ELISA (Euroimmune, Lubeck, Germany) as published previously,[20] and given that the plasma was collected within the first 96 hours of fever, any anti-dengue IgG present was attributed to a previous dengue infection. Thus, four age and sex-matched groups were defined as follows: a) primary dengue with plasma leakage (PD+ PL+), b) primary dengue without plasma leakage (PD+ PL-), c) secondary dengue with plasma leakage (PD- PL+), and d) secondary dengue without plasma leakage (PD- PL-). Assuming the incidence of plasma leakage in dengue to be 35%,[23] for an expected odds ratio of 3 in a case-control comparison (with 95% confidence and 0.8 power), the estimated sample size was n=51 per group.[24]

In addition to the primary outcome of plasma leakage, early biomarker associations with SD (severe outcome) were also explored. There were no deaths reported in CDS.

Biomarker selection

The selection of biomarkers was guided by the findings of our recent systematic review.[5] Since plasma leakage is an essential feature in both DHF and SD, four associated biomarkers were included (VCAM-1, Syndecan-1, IL-8, IP-10) that were confirmed with high certainty evidence in the meta-analysis as associated with either of the DHF or SD outcomes, as well as seven other biomarkers that were associated with either DHF or SD in a single study only (ICAM-1, IL-33R, TREM-1, SAA, Ferritin, Fas-L, uPAR). Several other similar biomarkers (associated with SD or DHF in a single study) were not available from the commercial reagent provider (Thermo Fisher Scientific, Waltham, MA, USA) and for these a further 23 replacement biomarkers were included based on network and pathway analysis using String and QIAGEN Ingenuity Pathway Analysis (IPA) databases (CCL3, CCL11, CCL2, CXCL11, CXCL9, CHI3L1, GM-CSF, G-CSF, HGF, CD44, Leptin, IL-1α, IL-1β, IL-4, IL-10, 1L-17A, IL-1RA, IL-6, IL-18, IL-13, VEGF-A, TNF-α, IFN-γ). This pathway analysis revealed functional relationships connecting these proteins to additional molecules involved in dengue pathogenesis, seeking to ensure a comprehensive coverage of key molecular processes in severe dengue progression. Collectively all samples were analyzed using a 34-Plex custom-made panel on the ProcartaPlex™ platform (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions using a Luminex MAGPIX instrument (Luminex Corporation, Northbrook, IL, USA). The instrument was calibrated with MAGPIX Calibration and Performance Verification Kits (Millipore, Burlington, MA, USA). xPONENT software (Luminex) was used to obtain data. Data were analyzed using Multiplex Analyst software version 5.1 (Merck, Darmstadt, Germany) as the Median Fluorescent Intensity (MFI) using spline curve-fitting for calculation of analyte concentrations. The biomarker VCAM-1 levels were mostly above upper limit of detection in the Luminex assay and so this analyte was re-measured in all samples at a 1:2500 dilution using the DuoSet Human VCAM-1 ELISA kit (R&D Systems, Minneapolis, USA). The full list of biomarkers analyzed is detailed in Supplementary Table S1.

Statistical Analysis

The distributions of biomarker concentration across samples were tested for normality using the Kolmogorov-Smirnov test and were compared between the above-mentioned groups using non-parametric Mann-Whitney U test and median comparisons to identify statistically significant associations with plasma leakage (univariate analysis). When readings for a minority of samples were above or below the detection limit for the particular analyte (censored data), the values were imputed using the Multiplicative Replacement Strategy[25] provided less than 40% of samples per biomarker had censored data. The distributions for biomarkers which were statistically significantly associated with plasma leakage were then log-transformed, tested for collinearity using Spearman's correlations, and then subjected to logistic regression to identify independent biomarker associations with plasma leakage. Four biomarkers that had censored data for > 40% of samples were still tested for statistical significance but as categorical variables (biomarker detectable or not) using chi-square tests, and those that were statistically significant were included in the logistic regression as categorical variables. The analysis was first done for groups with and without plasma leakage and then repeated in a subgroup analysis where patients were further classified as having primary or secondary dengue. Statistical analyses were done with GraphPad Prism v10 and SPSS v28 (IBM, USA) and statistical significance was set at $p < 0.05$. The p-value was not corrected for multiple comparisons because the selection of biomarkers was guided by a systematic review and all selected biomarkers had direct or indirect prior evidence for association with SD or DHF for which plasma leakage is a pre-requisite.

Sensitivity analysis

We conducted two sensitivity analyses: the first analysis addressed a timing discrepancy in 23 subjects. These patients were admitted within 96 hours of fever onset, but their blood samples were collected up to 110 hours post-onset of fever. We repeated our analysis by excluding these patients to assess the impact of this delay. The second sensitivity analysis stratified results by day of fever, allowing the assessment of robustness of findings across different stages of illness progression in the first 4 days of fever instead of considering this period to be homogenous.

Role of funders

Funders had no role in study design, data collection, data analyses, interpretation, or writing of the reports.

Results

Plasma from 228 participants was included, and the minimum sample size for each subgroup was met for the main outcome of plasma leakage: PD+PL- (n=54), PD+PL+ (n=53), PD-PL+ (n=61), PD-PL- (n=60). Presence of PL was determined based on haematocrit criterion in 49 patients, by ultrasound in 28 patients and by both criteria in 37 patients. The groups were similar in age and gender distribution as shown in Table 1. Four biomarkers (IL-6, IL-8, Fas-L, and TREM-1) were treated as categorical variables in our analysis, as > 40% of data fell below the lower detection limit. A fifth biomarker (VCAM-1) which had > 40% data above the upper detection limit was re-assessed with a commercial ELISA kit. The biomarker concentrations for all samples are provided in Supplementary Data file S1. Only 24 patients met the definition for the secondary outcome of SD

Table 1
Comparison of demographic variables across the analyzed groups.

Group	Number (%) or Mean		P-value[a]
	With plasma leakage	Without plasma leakage	
All patients	114	114	
Females	35.7%	36.8%	0.88
Mean age (in years)	31.5	32.9	0.77
Day of sampling	2.7	2.9	0.99
Primary dengue patients	53	54	
Females	20.7%	22.2%	0.85
Mean age (in years)	27.6	28.7	0.92
Day of sampling	2.6	2.9	0.98
Secondary dengue patients	61	60	
Females	49.2%	50%	0.92
Mean age (in years)	34.8	36.6	0.63
Day of sampling	2.8	2.9	0.98

[a] Independent sample T-test or z-test for proportions. No comorbidities were reported for the patients except for one patient with diabetes enrolled in secondary dengue within the plasma leakage group.

within the available CDS samples, and they were compared against others for differences in early biomarker profiles. All these SD patients had secondary dengue.

When biomarker concentrations were compared in univariate analyses between those who developed plasma leakage and others (regardless of prior dengue exposure), IL-33R, ICAM-1, VCAM-1, CCL11, ferritin, and HGF showed statistically significant differences ($p < 0.05$, Fig. 1a-f) either by median comparison or by Mann Whitney U test (which compares concentration distributions between the two groups). Additionally, Fas-L was detected in statistically significantly more instances in the group with plasma leakage (chi-square test). Of all these associations, only elevated VCAM-1 (OR:3.289, 95% CI: 1.090–9.926, $p < 0.05$) and IL-33R (OR: 2.677, 95% CI: 1.244–5.856, $p < 0.05$) were independently associated with an increased risk of plasma leakage, while CCL11 elevation (OR: 0.166, 95% CI: 0.057–0.483, $p < 0.05$) was associated with a decreased risk of plasma leakage when tested with logistic regression (Supplementary Table S2, Fig. 2).

When samples were further stratified according to prior dengue exposure, in patients with primary dengue, the univariate analysis identified statistically significant differences in SAA, CHI3L1,

IL-33R, Ferritin, CD44, CXCL11, TNF-alpha, ICAM-1, and CCL2 levels ($p < 0.05$). Subsequent logistic regression (which excluded CD44 due to collinearity with TNF-alpha) revealed that elevated CXCL11 (OR: 0.130, 95% CI: 0.038–0.447, $p < 0.05$) was independently associated with a decreased risk of plasma leakage in this subgroup (Supplementary Table S3, Fig. 2).

For secondary dengue infections, a higher number of biomarkers showed statistically significant differences in univariate analysis, including IL-4, IP-10, SAA, CCL11, G-CSF, IFN-γ, CD44, CXCL11, ICAM-1, VCAM-1, IL-1β, IL-10, IL-13, and HGF ($p < 0.05$). The logistic regression which excluded IL-13 due to collinearity with IL-1β and IFN-γ, showed that a higher VCAM-1 concentration (OR: 32.979, 95% CI: 2.429–447.708, $p < 0.05$) was independently associated with an increased risk of plasma leakage, and a higher SAA concentration was associated with a decreased risk (OR: 0.031, 95% CI: 0.002–0.433, $p < 0.05$) (Supplementary Table S4, Fig. 2).

For the secondary outcome of SD, nine biomarkers (SAA, IL-10, CHI3L1, Ferritin, CCL2, uPAR, TNF-α, VGF-A, VCAM-1) showed statistically significant associations in univariate analysis ($p < 0.05$), and in the logistic regression, increased VCAM-1 (OR: 7.190, 95% CI: 1.011–51.159 $p < 0.05$) and Ferritin (OR: 3.630, 95% CI: 1.183–11.142, $p < 0.05$) levels were independently associated with an increased risk of severe dengue, while increased SAA (OR: 0.027, 95% CI: 0.003–0.255, $p < 0.05$) and CCL2 (OR: 0.137, 95% CI: 0.032–0.598, $p < 0.05$) levels were associated with a decreased risk (Supplementary Fig. S1 and Supplementary Table S5). A full list of biomarkers that had statistically significant associations with plasma leakage in the univariate analyses and their prior associations with SD, DHF or plasma leakage according to literature is summarized in Table 2.

In sensitivity analyses, when the 23 individuals that were sampled between 96–110 hours post-fever onset were excluded, the results did not change for the outcome of plasma leakage. By contrast, none of the biomarkers retained statistically significant associations in logistic regression for the SD outcome (Supplementary Table S6). The second sensitivity analysis that separated results based on day of fever, found that on day 3 of fever VCAM-1, IL-33R and CCL11 were independently and statistically significantly associated with plasma leakage after logistic regression, while for day 4 samples, only IL-33R showed a similar association (Supplementary Table 6). Analysis of samples from days 1 or 2 of fever or further stratification of day 3 or 4 samples according to prior dengue exposure was not done as there were inadequate numbers (< 30 patients) in at least one of the groups.

Discussion

This guided, well-powered analysis of early circulating biomarkers in dengue infection identified multiple proteins that were independently associated with either an increased or decreased risk of subsequent plasma leakage, while adjusting for the confounding effects of sex, age and prior dengue exposure. The analysis was inadequately powered to identify early biomarkers predictive of SD, which may also explain the inconsistencies between the main analysis and the sensitivity analysis for this outcome. Hence the results cannot be interpreted for this outcome.

CDS uses plasma leakage instead of SD as the preferred outcome to predict for several reasons. Therefore, in this study also, plasma leakage was selected *a priori* as the primary outcome. Many prospective cohorts on dengue have reported on SD as the primary outcome, after the publication of the WHO 2009 clinical classification. However, we argue that to triage patients early in the illness course for hospital admission or for prolonged hospital stay, prediction of the risk of plasma leakage is a better criterion than the risk of SD or death. Firstly, SD is characterized by one or more of "severe" plasma leakage, "severe" bleeding and/or "severe" organ impairment,[2] all of which are subjectively determined and hence prone to observer bias. Secondly, a patient with WHO defined SD is very ill and must be managed in an intensive care unit (ICU) due to severe hemorrhage and organ impairment. Ideally, all patients must be prevented from reaching this stage instead of predicting its likelihood. Finally, a subset of SD may actually arise due to medical mismanagement (failure to identify plasma leakage or overzealous fluid management) which cannot be predicted in early infection. Using the presence of plasma leakage alone as the preferred outcome to predict avoids these shortcomings. Almost all patients with SD would have had preceding plasma leakage and correct identification and management of plasma leakage would have prevented them from deteriorating to the undesired state of SD.[2,3,8,26,27] Furthermore, plasma leakage is mostly a pathophysiological phenomenon independent of medical mismanagement and can be measured by objective criteria with less inter-observer bias. Finally, the incidence of plasma leakage in dengue is between 35 −38% of all hospitalized dengue patients[23] which allows adequate power in prospective cohorts to identify risk associations. The DHF category in the WHO 1997 classification and the "dengue with warning signs" category in the WHO 2009 classification are also potential alternative outcomes that can be used for early warning systems, but

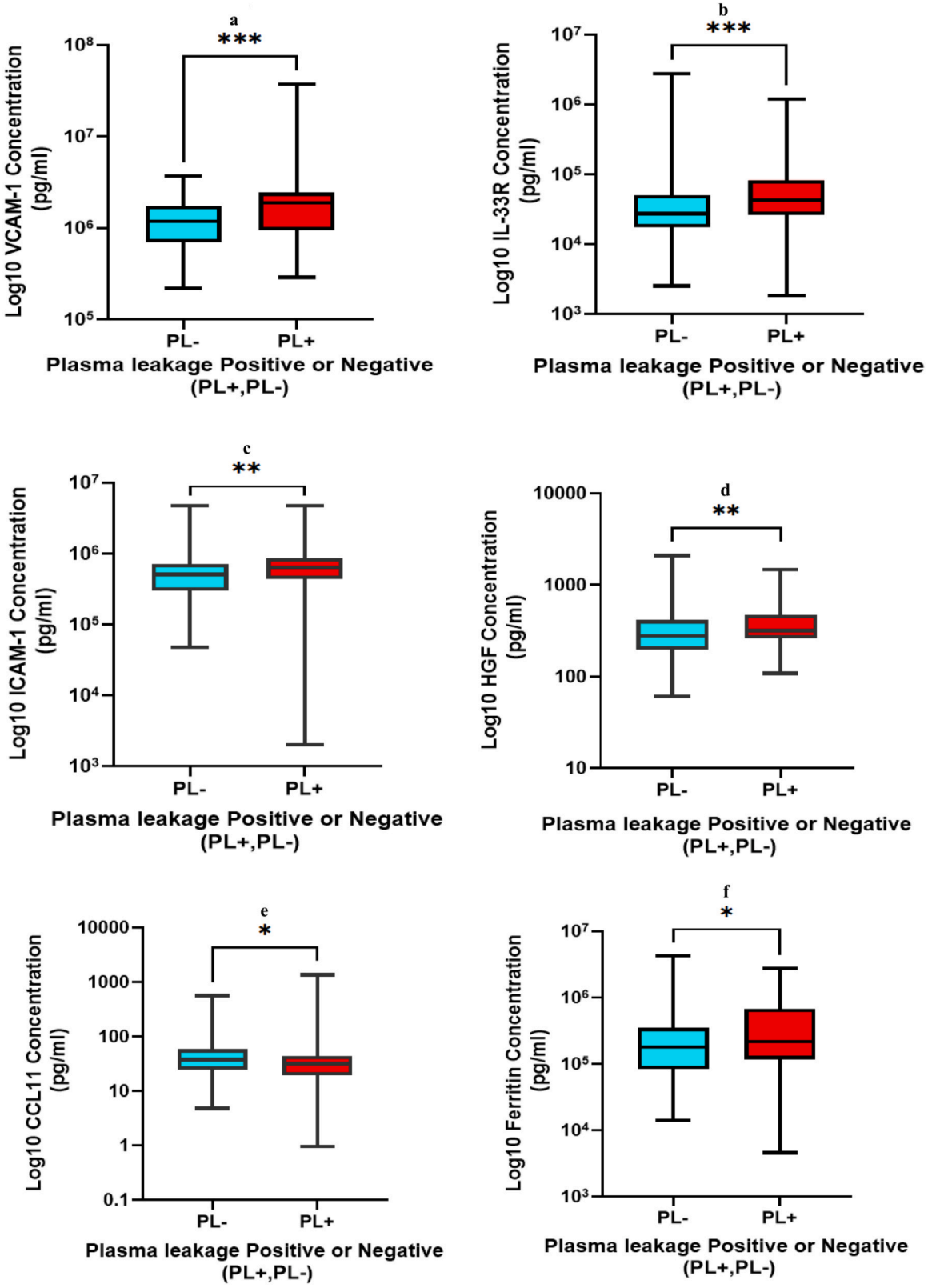

Fig. 1. a-f. Statistically significant biomarkers associated with plasma leakage (all patients) in univariate analysis. Box plots show log10-transformed concentrations (pg/ml). VCAM-1, IL-33R, ICAM-1, HGF, and ferritin levels which were significantly higher in PL+ patients, while CCL11 level was lower. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). VCAM-1: Vascular cell adhesion molecule 1; IL33R: Interleukin-33 receptor; ICAM-1: Intercellular Adhesion Molecule 1; HGF: Hepatocyte growth factor; CCL11: C-C motif chemokine 11.

these are complex diagnoses that must have plasma leakage plus several other criteria to be diagnosed. This again increases the risk of inter-observer bias.

Our analysis revealed a significant association between IL-33R and plasma leakage in patients with dengue infection. This finding is particularly noteworthy as IL-33R (ST2) maintained its significant

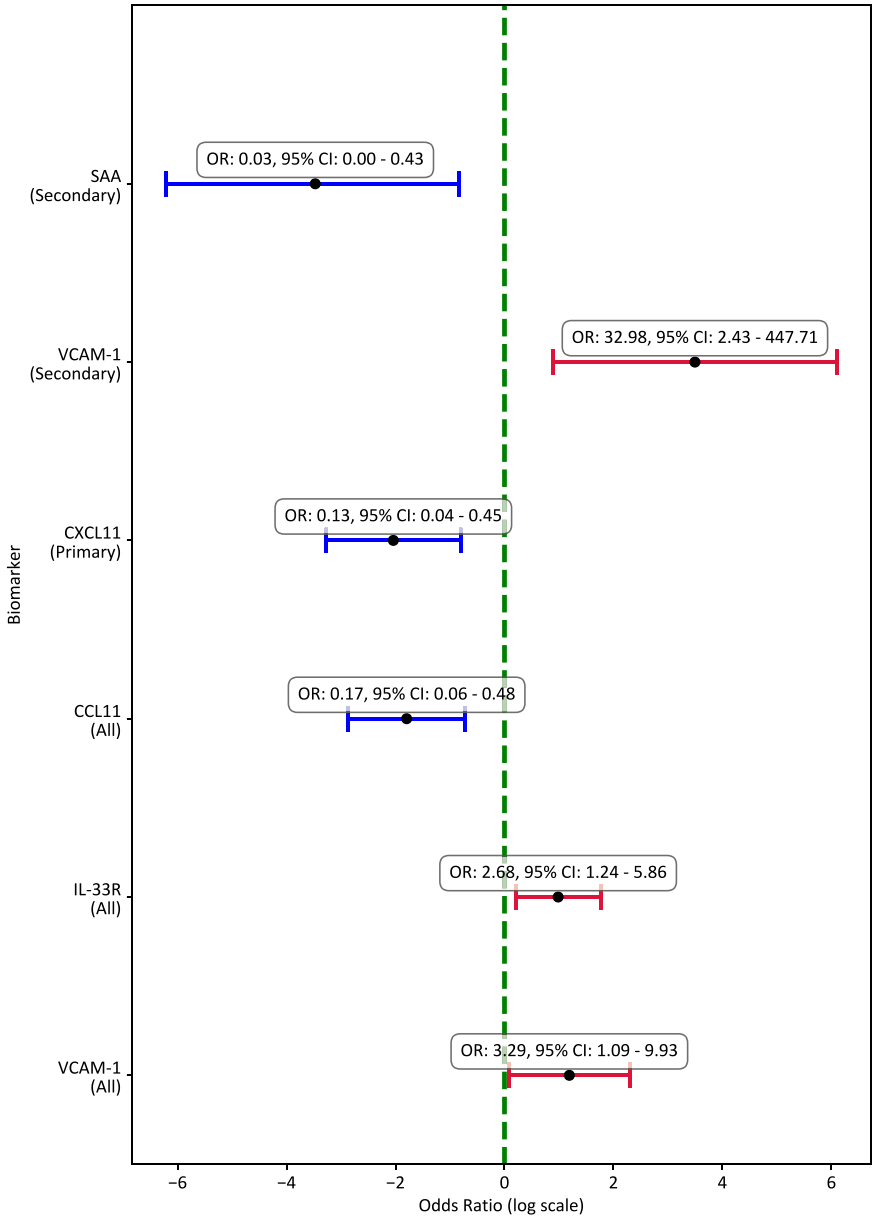

Fig. 2. Forest plot of biomarkers associated with plasma leakage in dengue. Forest plot showing the associations between various biomarkers and the risk of plasma leakage (PL) in dengue patients. The plot displays odds ratios (ORs) with 95% confidence intervals for each biomarker across different patient groups. ORs > 1 indicate an increased risk of PL, while ORs < 1 indicate a decreased risk. The vertical dashed line represents an OR of 1 (no association). Biomarkers are grouped by patient category: all patients, primary dengue only, and secondary dengue only. VCAM-1: Vascular cell adhesion molecule 1; IL33R: Interleukin-33 receptor; CCL11: C-C motif chemokine 11; CXCL11: C-X-C motif chemokine 11; SAA: Serum amyloid A.

relationship with plasma leakage across all sensitivity analyses. IL-33 (belonging to the IL-1 superfamily) induces T helper cells, mast cells and eosinophils to produce cytokines favouring a humoral immune response.[28] IL-33R, expressed in various cell types including T helper 2 cells, mast cells, and endothelial cells, appears to also have a regulatory role in this process. The correlation of IL-33R with plasma leakage suggests the potential importance of this pathway in the pathophysiology of dengue-induced vascular leakage and highlights its possible utility as a reliable indicator of disease progression. Other authors have also observed an early rise in soluble IL-33R in patients progressing to SD or DHF (there are no prior data for plasma leakage alone).[29–31]

VCAM-1 is another biomarker that showed an independent positive association with the risk of plasma leakage across all patients, particularly within those with secondary dengue. VCAM-1 is a cell adhesion molecule that mediates leukocyte adhesion to the vascular endothelium.[32] It is therefore plausible that in patients who eventually develop plasma leakage, there is an earlier endothelial cell activation plus a dominant Th2-driven immune response to infection. Consistent with our observations, prior research has also noted early elevation of VCAM-1 levels in patients progressing to SD, DHF, or plasma leakage.[33–36] Our data suggests that the association of VCAM-1 may be specific to secondary dengue, but because an adult population living in an endemic area would typically be dominated by those with prior exposure, this association may still be observed when all patients are considered regardless of their prior dengue exposure. It is important to note that in most resource-limited settings, prior dengue exposure is not assessed due to the costs of testing, and self-reported dengue exposure is also not a reliable indicator of actual prior infection.[20] Thus, from the perspective of real-world clinical use, biomarker associations for plasma leakage which had not been adjusted for past dengue exposure are important. Yet

Table 2
Biomarkers that were statistically significantly associated with plasma leakage in univariate analysis in this study (Moallemi et al., 2025) and their prior associations with SD/ DHF, or PL according to literature from human clinical studies.

Biomarker	Evidence from literature (associated statistically significantly with DHF/SD, or PL)	Results in Moallemi et al 2025.
ICAM-1[a,b,c]	Elevated in DHF/SD[44,45]	Elevated in PL (all, primary, secondary)
IL-33R[a,b]	Elevated in DHF/SD (all and secondary)[29–31]	Elevated in PL (all, primary)
Ferritin[a,b]	Elevated in DHF/SD[46,47]	Elevated in PL (all, primary)
VCAM-1[a,c]	Elevated in DHF/SD[33–35]Elevated in PL[36]	Elevated in PL (all, secondary)
CCL11[a,c]	None	Decreased in PL (all, secondary)
HGF[a,c]	Elevated in DHF/SD[48,49] Elevated in PL (all and secondary)[36]	Elevated in PL (all, secondary)
SAA[b,c]	Elevated in DHF/SD (all and primary)[42,43]	Elevated in PL (primary) Decreased in PL (secondary)
CD44[b,c]	None	Elevated in PL (primary) Decreased in PL (secondary)
CXCL11[b,c]	None	Decreased in PL (primary) Elevated in PL (secondary)
CHI3L1[b]	None	Elevated in PL (primary)
CCL2[b]	Elevated in DHF/SD[49,50]	Elevated in PL (primary)
TNF-α[b]	Elevated in DHF/SD[49,51]	Elevated in PL (primary)
IL-4[c]	Elevated in DHF/SD[51,52] Decreased in DHF/SD (primary)[42]	Elevated in PL (secondary)
IP-10[c]	Elevated in DHF/ SD[45,50] Elevated in PL (all and secondary)[36] Decreased in DHF/SD (primary)[42]	Decreased in PL (secondary)
G-CSF[c]	Decreased in DHF/SD (primary)[42] Elevated in DHF/SD (all)[49]	Elevated in PL (secondary)
IFN-γ[c]	Elevated in DHF/SD[51,52] Decreased in DHF/SD (all and primary)[42,53]	Elevated in PL (secondary)
IL-1β[c]	Elevated in DHF/SD[49] Decreased in DHF/SD (primary)[42]	Elevated in PL (secondary)
IL-10[c]	Elevated in DHF/SD[51]	Elevated in PL (secondary)
IL-13[c]	Elevated in DHF/SD[52,54]	Elevated in PL (secondary)

In vitro studies, and studies using animal models, and biospecimens other than serum or plasma were excluded. For biomarkers highlighted in green there is no prior evidence of an association with adverse dengue outcomes in literature.
DHF: Dengue Hemorrhagic Fever, SD: Severe Dengue, PL: Plasma Leakage.
[a] Statistically significant association for all patients.
[b] Statistically significant association for patients with primary dengue only.
[c] Statistically significant association for patients with secondary dengue only.

from a pathophysiological perspective, the "true" association of a biomarker for plasma leakage cannot be differentiated unless the confounding effect of prior dengue exposure is adjusted for. Hence, in this study, we performed a two-tiered analysis with and without considering prior dengue exposure.

Several biomarker concentrations were negatively associated with the risk of plasma leakage: CCL11 in all patients, CXCL11 in those with primary dengue, and SAA in secondary dengue. CCL11 is an eosinophil chemotactic factor (eotaxin-1) which has been shown to be negatively associated with plasma leakage in previous *in vitro* study using HUVEC (Human Umbilical Vein Endothelial Cell) in monolayer permeability assays.[37] A study from Malaysia also demonstrated reduced CCL11 levels during the dengue febrile phase (pre-plasma leakage) in patients who later developed dengue with warning signs which includes plasma leakage as a pre-requisite (WHO 2009 clinical classification).[38] Studies show that upon dengue virus infection, CXCL11 levels are elevated. This potent chemokine, secreted by endothelial cells and induced by IFN-γ, acts as a chemoattractant for activated T cells and natural killer cells, thereby contributing to the inflammatory response in dengue.[39,40] While these studies have noted the increase in CXCL11 gene expression and serum concentrations during acute dengue infection,[39–41] the potential role of CXCL11 as an early predictor of severe outcomes is largely unexplored. Serum amyloid A (SAA) are apolipoproteins and acute phase reactants produced by liver. Like CRP, SAA levels also rise rapidly within the first hours of inflammation. Literature on SAA levels and the risk of adverse outcomes in dengue is limited. A proteome study showed an early and significant SAA elevation in patients who eventually developed DHF.[42] However, this study only included primary dengue patients, while our observations were statistically significant for patients with secondary dengue infection only. Another study in India showed that SAA2 levels were elevated significantly during the critical phase in the severe dengue group (vs. others) but not during the febrile phase.[43].

This study has several limitations. Firstly, the patients were recruited from a single hospital in Sri Lanka which limits the generalizability of interpretations. However, since the biomarkers were already selected from the pre-screening process of a systematic review, some of them have already been associated with either DHF or SD by other authors. Secondly, we could not test all biomarkers that had a significant association with either DHF or SD in the systematic review in this analysis because they were not available in the panels offered by the manufacturer. This was mitigated by performing a network and pathway analysis to identify replacement biomarkers that were available and were expected to correlate with the levels of missing biomarkers given their proximity in metabolic pathways. Thirdly, four biomarkers had below detection limit censored data for more than 40% of the samples; therefore, they were only used as categorical variables ignoring the absolute value. This ensured that all the data were used but without compromising the statistical power of the analysis. Fourthly, the systematic review on which the biomarkers were selected only reported associations for SD or DHF. Given that clinical categories of plasma leakage, SD, and DHF are not synonymous, these results cannot be incorporated to our previous systematic review to generate better-quality data for biomarker associations. Yet in our opinion, it is important to find biomarker associations for plasma leakage instead of DHF or SD as mentioned above, and while performing the systematic review we identified this as a significant knowledge gap to address. Finally, this analysis was dominated by samples from days 3 and 4 of fever, and results observed from an adequately powered analysis using samples obtained exclusively in days 1 or 2 of fever, may differ.

Conclusion

Our study identified novel biomarkers (CCL11 and CXCL11 in plasma) and confirmed previously reported markers (VCAM-1) associated with the risk of plasma leakage in dengue infection. We also demonstrated the potential utility of IL-33R as a biomarker specifically for plasma leakage, expanding on its previously reported associations with DHF and SD. Notably, the biomarker profiles differed between primary and secondary dengue infections, with a higher number of differentially expressed biomarkers observed in secondary cases. This study focused on plasma leakage as the primary endpoint, addressing a critical knowledge gap in identifying biomarkers directly linked with the probability of plasma leakage.

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Author contributions

Concept and design: SM, CR, AL.
Sample and data collection: CS, PW, DF, SR.
Funding acquisition: CR, DF, AL.
Experiments: SM, NT.
Data analysis: SM, CR.
Interpretation of results: SM, CR, AL.
Writing – original draft: SM, CR.
Writing – review & editing: All authors.
Final approval: All authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106401.

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