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Cytokine and chemokine kinetics in natural human dengue infection as predictors of disease outcome

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Dengue is an important tropical disease with considerable global impact. Despite this, there remains an urgent need for reliable biomarkers to predict disease severity, as well as effective antiviral drugs and targeted treatments. In this study, we conducted a comprehensive profiling of 41 plasma mediators in patients with asymptomatic dengue (AD) and symptomatic dengue (SD), which includes mild dengue fever (DF) and severe dengue hemorrhagic fever (DHF). Our findings revealed that the levels of nearly all measured mediators were consistently lower in AD compared to SD patients, suggesting a potential protective cytokine response signature. Time-course cytokine analysis in SD shown significantly elevated levels of pro-inflammatory cytokines and chemokines associated with inflammation and viral clearance upon the acute phase, while various growth factors were elevated during the convalescence. Notably, we identified elevated IL-15 levels in DHF patients three days before fever subsidence, highlighting its potential as an early prognostic biomarker for severe disease outcomes. Furthermore, prolonged high levels of IL-8 and IP-10 in DHF during the critical period may contribute to dengue immunopathogenesis. This study advances the understanding of cytokine dynamics in the natural course of human dengue infection, providing valuable insights for the development of targeted treatments and prognostic biomarkers.

Dengue is one of the most important neglected tropical diseases in the world, with an estimated total of 390 million infections occurring annually[1]. Despite significant vector control efforts, the number of dengue cases reported by the World Health Organization (WHO) has increased more than eightfold over the past 2 decades. The disease is endemic in more than 100 countries across the globe as a consequence of steady geographical expansion of both *Aedes* vector mosquitoes and dengue viruses (DENV)[2]. Despite being a major public health concern, afflicted individuals typically have to rely on supportive care as there is no approved antiviral for dengue treatment. There are two licensed dengue vaccines to date, namely Dengvaxia, has limited efficacy and can be unsafe for children and DENV-naïve individuals[3] and Qdenga which has better safety profile but still has limited efficacy to DENV serotype 3 and 4[4].

Up to 75% of dengue patients have asymptomatic DENV (AD) infection and therefore receive little to no contact with healthcare providers during viremia[1]. These undetected infections pose challenges for disease surveillance and may contribute to continued virus circulation in endemic areas[5,6]. Meanwhile, a relatively small subset of patients incurs symptomatic DENV (SD) infection, accounting for approximately 100 million cases per year that can lead to hospitalization and/or mortality[1]. Within this subgroup, clinical manifestation can range from mild dengue fever (DF), severe dengue hemorrhagic fever (DHF), and life-threatening dengue shock syndrome (DSS)[7]. The clinical course of SD infection comprises 3 major phases: the febrile phase (2–7 days), critical or leakage phase (24–48 h), and convalescence phase (2–7 days). During the febrile phase, DF and

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DHF patients indistinguishably present with high fever, severe headache, muscle pain, nausea, and vomiting[8]. Following defervescence and a rapid decline in viremia, some DHF patients may experience plasma leakage and bleeding, which can subsequently lead to DSS. Timely supportive care during this critical phase can reduce fatality rates from up to 20% to less than 1%[9]. However, clinical warning signs of severe dengue (persistent vomiting, abdominal pain, lethargy or restlessness, irritability, and oliguria), as delineated by the WHO, can occur within just a few hours before the patient requires urgent life-saving interventions[10]. Moreover, while the WHO guidelines are highly specific, their sensitivity remains a concern. The DHF classification has been shown to have high specificity but unacceptably low sensitivity in detecting severe dengue cases[11,12]. These limitations present challenges in accurately identifying severe cases, underscoring the potential of cytokine biomarkers to improve classification and clinical management.

The initial overlap of clinical symptoms at presentation between DF and DHF/DSS patients during the febrile phase along with rapid development of severe dengue call for the identification of readily accessible biomarkers that can help diagnose, prognose, and monitor patients early on in the disease course to optimize patient outcome. Plasma mediators, including cytokines, chemokines, and growth factors, have long been recognized as promising predictors of dengue severity due to their involvement in both viral clearance and immunopathogenesis of severe disease[13–17]. Although no precise biomarker has been established, some studies suggest that certain cytokines, such as IL-8, IL-10, soluble urokinase plasminogen activator receptor (suPAR), and olfactomedin 4[18–21], may serve as potential markers for dengue severity. However, most studies investigating these mediators are cross-sectional and lack data on AD individuals, as they are notoriously difficult to identify during viremia. To our knowledge, only one group has thus far compared cytokine profiles between AD and SD cases during viremia, and their study focused exclusively on children with DENV1 infection from the DENFREE Cambodia cohort[22].

To address this gap, we comprehensively quantified 41 soluble plasma mediators, including pro-inflammatory and anti-inflammatory cytokines, chemokines, and growth factors that previously reported or potentially involved in dengue pathogenesis[13–15]. We compared these mediators in dengue-infected AD individuals with DF and DHF patients during viremia and further tracked their kinetics from the febrile to the convalescent phase in DF and DHF patients. All samples were derived from our well-characterized DENFREE (Dengue research Framework for Resisting Epidemics in Europe) Thailand-based prospective, longitudinal cohort[23]. Interestingly, we showed that most plasma mediators, including the antiviral type I interferon (IFN), were dampened in AD individuals as compared to DF and DHF patients. Furthermore, in the longitudinal SD cohort, several pro-inflammatory cytokines, anti-inflammatory cytokines, chemokines as well as antiviral cytokines were elevated during acute infection while various growth factors were increased during convalescence. Importantly, our results suggest that heightened IL-15 levels during the febrile phase strongly correlated with subsequent severe disease outcomes. Our study provides an assessment of plasma mediators as potential prognostic indicators for dengue severity that may help predict the need for early intervention aiming to reduce the likelihood of life-threatening complications and/or death.

Results

Asymptomatic infection exhibits lower plasma mediator levels compared to symptomatic infection

To investigate the differences in cytokine profiles between AD and SD infections, we quantified 41 plasma mediators in 32 DF, 10 DHF, and 5 AD cases using multiplex bead-based Luminex technology. SD samples collected 1–2 days before defervescence, as previously shown to exhibit the most active immune responses[24], were selected to compare the cytokine profiles with AD samples. To minimize potential confounding factors, we carefully matched the selected cases by DENV viral load (p value = 0.5172) (Fig. 1a), DENV serotype, age, and sex (Supplementary Table 1). We next visualized the overall cytokine profiles of AD, DF, and DHF cases using principal component analysis (PCA). The PCA results revealed a significant overlap between DF and DHF cases, while a clear distinction was observed between AD and SD infections (DF and DHF) (Fig. 1b). This indicates that cytokine profiles differ markedly between AD and SD, whereas the cytokine secretion patterns in DF and DHF patients are largely similar. To further understand this separation, a PCA biplot was generated to identify the mediators driving this variance (Fig. 1c). The first two principal components (PC1: 36.2%, PC2: 15.6%) accounted for 51.8% of the total variance, with eigenvalues greater than 1. Cytokines such as IP-10, IL-10, MCP-1, MIP-1α, IL-8, IL-1α, IL-1RA, TNF-α, and IL-15 were strongly associated with SD, as evidenced by their positive correlation with PC2. In contrast, GRO, EGF, and PDGF-AA were more abundant in AD, corresponding to their negative correlation with PC2 (Fig. 1c and Supplementary Fig. 1a).

Further analysis of individual plasma mediator levels across the three disease outcomes revealed that AD samples had significantly lower concentrations of TNF-α, IL-15, and MIP-1 α (Fig. 1d), as well as IL-10 (previously reported by Sungnak et al. under revision) (Fig. 1f), IL-8, and IP-10 (Fig. 1g), compared to both DF and DHF. Additionally, AD cases exhibited downregulation of IL-1α and IFN-α2 (previously reported by Sungnak et al. under revision) (Fig. 1e), IL-1RA, and Eotaxin. However, statistically significant differences were only observed between AD and DF for IL-1α and IFN-α2, and between AD and DHF for IL-1RA and Eotaxin (Fig. 1d–f). Baseline levels of certain cytokines in non-infected healthy individuals (HI) compared to AD and SD cases were provided in the supplementary Fig. 2. No significant differences were observed for other mediators among the severity groups (Supplementary Fig. 3). In summary, these findings demonstrate that differential levels of specific cytokines in AD distinguish it from SD, suggesting that cytokine profiles play a crucial role in determining the clinical outcomes of dengue infection.

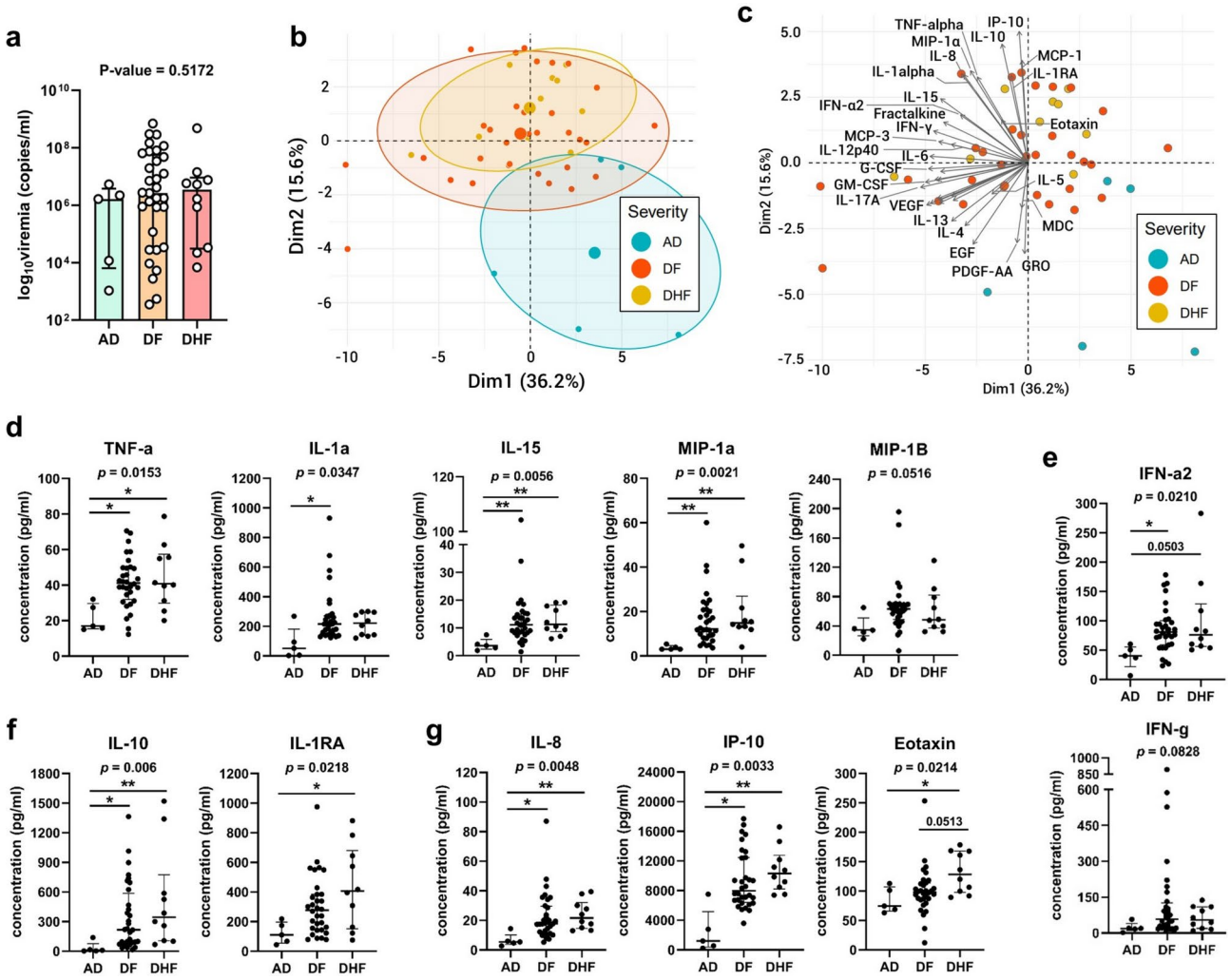

Fig. 1. Asymptomatic infection has lower cytokine secretion than symptomatic infection. (**a**) Comparable viremia level between AD (n = 5), DF (n = 32) and DHF (n = 10) sample. (**b**) PCA and (**c**) PCA biplot of the total 41 humoral mediators clustered by severity (Total n = 47). Each circle corresponds to one patient. (**d**) Levels of pro-inflammatory cytokines: TNF-α, IL-1α, IL-15, MIP-1α, and MIP-1β. (**e**) Level of antiviral cytokines: IFN-α2 and IFN-γ. (**f**) Levels of anti-inflammatory cytokines: IL-10 and IL-1RA. (**g**) Levels of chemokines: IL-8, IP-10, and Eotaxin. Each dot represents the level of cytokine from each sample. Kruskal–Wallis test was used to compare 3 groups of independent data (*p* value indicated in each graph). Kruskal–Wallis statistics with Dunn's multiple comparisons test were used to compare between 2 groups indicated. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Median and interquartile ranges were shown in each group.

Distinct mediator profile in acute and convalescent phases of symptomatic dengue infection

We further investigated the kinetics of cytokine response in a longitudinal cohort of SD patients. For this analysis, we selected 100 samples collected from 15 DF and 22 DHF patients at four different time points (Supplementary Tables 2–4). The first time point, denoted as "D-3", corresponds to the febrile phase when patients exhibited high fever and symptoms of dengue infection, occurring three days before defervescence. The second time point, denoted as "D0", marks the day of fever resolution (defervescence). The third and fourth time points were collected during the convalescent phase, with samples taken at 2 weeks (F1) and 2 months (F2) after recovery and hospital discharge (Fig. 2a). PCA was performed to visualize the overall cytokine profile across these time points (Fig. 2b–c) and between disease severities (Fig. 2d). The results revealed a clear separation between samples from the acute phase of infection (D-3 and D0) and the convalescence phase (F1 and F2) (Fig. 2b), indicating distinct cytokine profiles between the two stages. Within the acute phase, there was some separation between samples of D-3 and D0, though there was some overlap between these two time points. In contrast, samples from F1 and F2 appeared scattered in the lower quadrants of the PCA plot without clear separation between these two convalescent time points. The PCA biplot highlighted that cytokines such as IP-10, IL-10, TNF-α, MCP-1, IL-8, MIP-1α, IL-1RA, and IL-15 were positively correlated with the upward direction of PC2, indicating their elevated levels during the acute phase of infection (Fig. 2c and Supplementary Fig. 1b). Conversely, PDGF-AA, RANTES, sCD40L, PDGF-AB/BB, VEGF, and EGF were more abundant during convalescence, contributing to

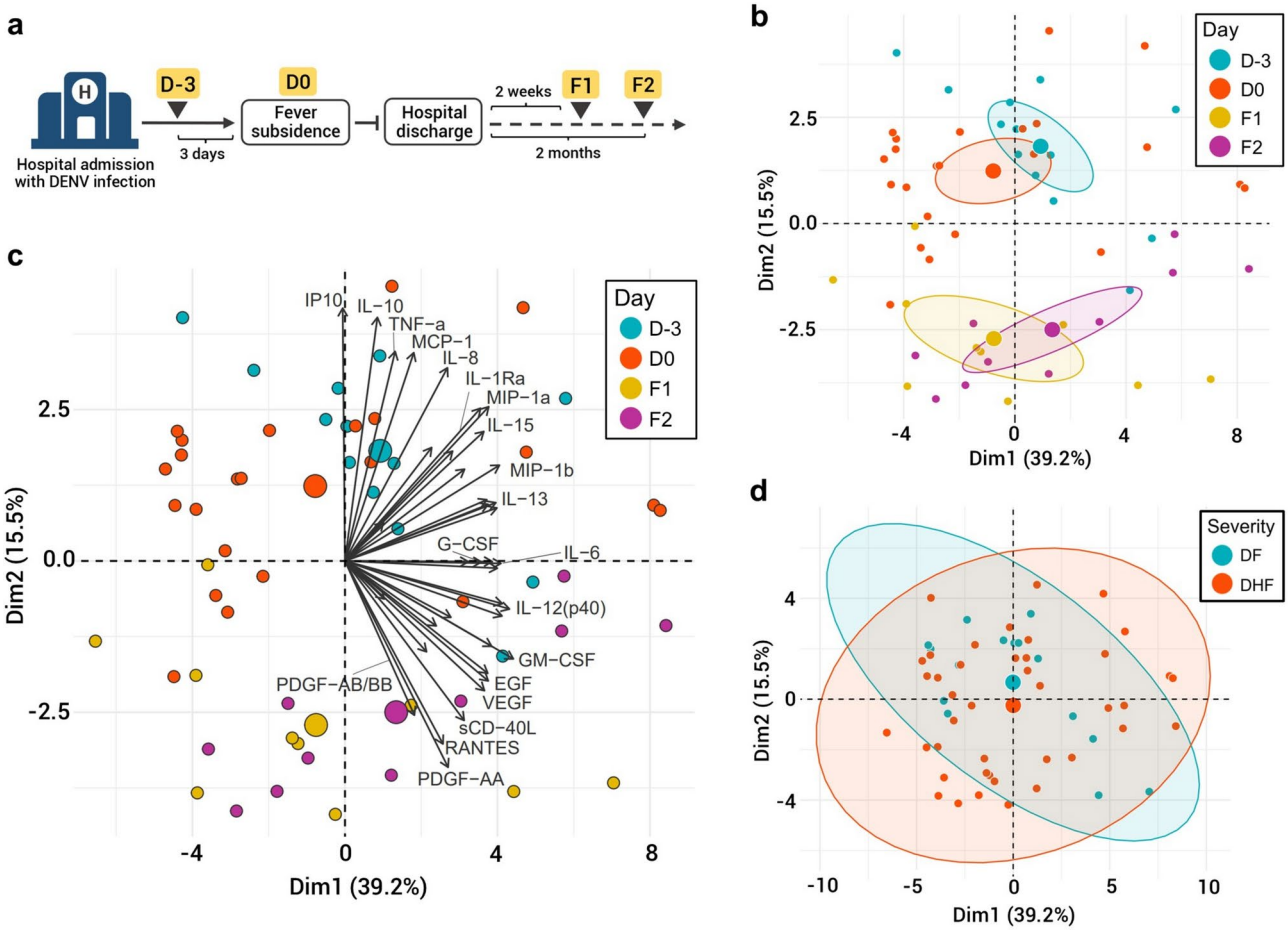

Fig. 2. Overall expression of the 41 cytokines over the time course in symptomatic DENV infection. (**a**) Diagram shows the 4 time points analyzed during the course of DENV infection. (**b**) Principal component analysis (PCA) plot and (**c**) PCA Biplot of total 55 dependent samples clustered by timepoints. (**d**) PCA plot clustered by severities; DF and DHF. (DF n = 15, DHF n = 41).

the downward direction on PC2, reflecting their increased secretion during recovery compared to the acute phase (Fig. 2c). Interestingly, while the cytokine profiles were clearly separated by time points (acute vs. convalescent), no distinct separation was observed between DF and DHF cases in the overall PCA plot (Fig. 2d). This is likely due to the similarity in overall cytokine secretion patterns between DF and DHF patients, suggesting that while cytokine expression shifts significantly over the course of infection, these shifts do not strongly differentiate between the disease severities of DF and DHF.

Cytokines associated with acute and convalescent phase of SD infection

To further investigate the role of individual cytokine contributions during acute DENV infection, we analyzed the levels of each cytokine across four timepoints (Fig. 3). Among the 41 cytokines measured, 14 exhibited peak levels during the acute phase (D-3 and D0), followed by a gradual decrease to the lowest levels during convalescence (F1 and F2), consistent with the PCA results (Fig. 2c). These 14 cytokines included proinflammatory cytokines (TNF-α, IL-1α, IL-6, IL-15, MIP-1α, and MIP-1β), anti-inflammatory cytokines (IL-1RA and IL-10), chemokines (IL-8, MCP-1, IP-10, and Fractalkine), as well as antiviral cytokines (IFN-γ and IFN-α2). The reduction in cytokine levels over the course of dengue infection was statistically significant when data from both DF and DHF patients were combined (Fig. 3, leftmost column) (Supplementary Table 5) and similar significance was observed in the DHF (Fig. 3, second column). However, for DF patients, the differences were generally not statistically significant (Fig. 3, third column), likely due to the limited sample size. Similar trends were observed when analyzing matched paired data from the same individual across timepoints (Fig. 3, rightmost three columns), reinforcing the observed cytokine dynamics throughout the progression of dengue infection.

Among the 14 cytokines highlighted, IL-15, IL-1RA, MIP-1β, Fractalkine, IFN-γ, and IFN- α2 reached their peak concentrations during the febrile phase. Their levels were significantly elevated at D-3 compared to D0, with no significant changes observed between D0 and the follow-up timepoints F1, and F2. Conversely, TNF-α, IL-10, IL-8, MCP-1, and IP-10 exhibited significant increases at both D-3 and D0 timepoints compared to the follow-up phase (Fig. 3).

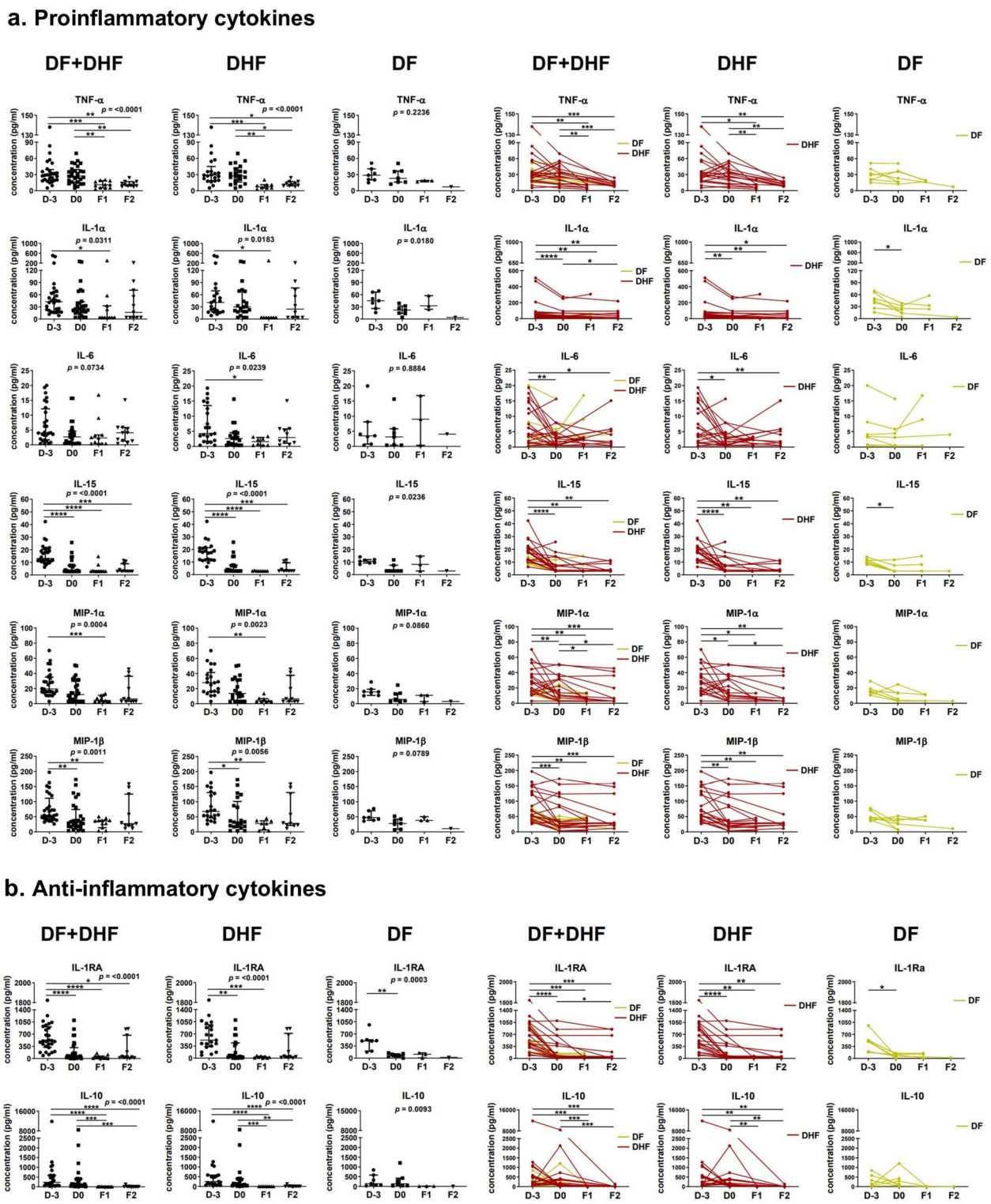

Fig. 3. Cytokines associated with acute dengue infection. The level of (**a**) proinflammatory cytokines, (**b**) Anti-inflammatory cytokines, (**c**) Chemoattractant cytokines and (**d**) Antiviral cytokines. Each dot represents the level of cytokine from each sample. The 3 left columns include all samples, Kruskal–Wallis test was used to compare 4 groups of independent data (*p* value indicated in each graph). Kruskal–Wallis statistics with Dunn's multiple comparisons test were used to compare between 2 groups indicated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Median and interquartile ranges were shown in each group. Left most column: combined DF and DHF, second column: DHF only, third column: DF only. The 3 right columns include only dependent data from the same patients (each line connects between paired samples). Wilcoxon matched-pairs signed rank test was used to compare between 2 groups indicated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The 4th column: combined DF and DHF, 5th column: DHF only, 6th column: DF only.

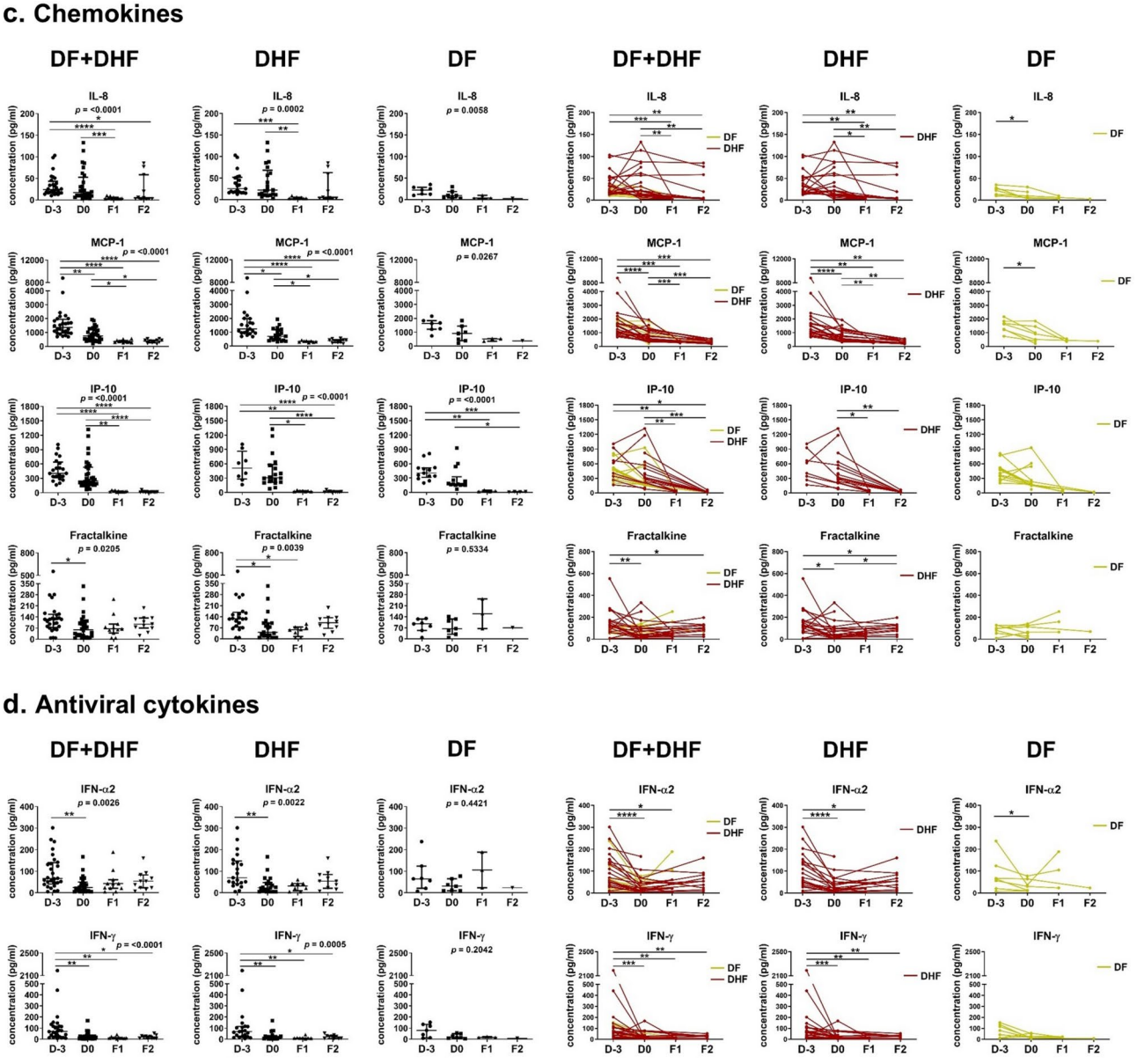

Figure 3. (continued)

In contrast, the levels of 7 mediators showed a significant elevation during the convalescence compared to the acute phase (Fig. 4) (Supplementary Table 5), consistent with the PCA results (Fig. 2c). Among these cytokines, 5 were growth factors, including epidermal growth factor (EGF), fibroblast growth factors (FGF2), granulocyte–macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor-AA and AB/BB (PDGF-AA, PDGF-AB/BB). The remaining 2 cytokines were RANTES (CCL-5), and soluble CD40 ligand (sCD40L).

Notably, most of these cytokines exhibited their lowest levels at D0 and reached their highest levels at F2, suggesting their potential role in recovery from the infection. This increase was statistically significant when combining data from both DF and DHF patients (Fig. 4, leftmost columns) and in the DHF subgroup (Fig. 4, second column). However, in the DF subgroup, the differences were mostly not statistically significant (Fig. 4, third column), likely due to the small sample size. Similar trends were observed in matched paired data across time points (Fig. 4, last three columns). These findings provide valuable insights into the dynamic changes in cytokine levels during the course of symptomatic dengue infection, offering a better understanding of the immune response at different stages of the disease.

Cytokines associated with dengue severity

To better understand the role of soluble immune mediators in severe dengue pathogenesis and to identify potential mediators for predicting disease severity, cytokine concentrations between DF and DHF patients were compared at four time points: D-3, D0, F1, and F2. We found that the concentrations of three cytokines, IP-10, IL-15, and IL-8 (previously reported by Opasawatchai et al.[25]), were significantly elevated in DHF patients

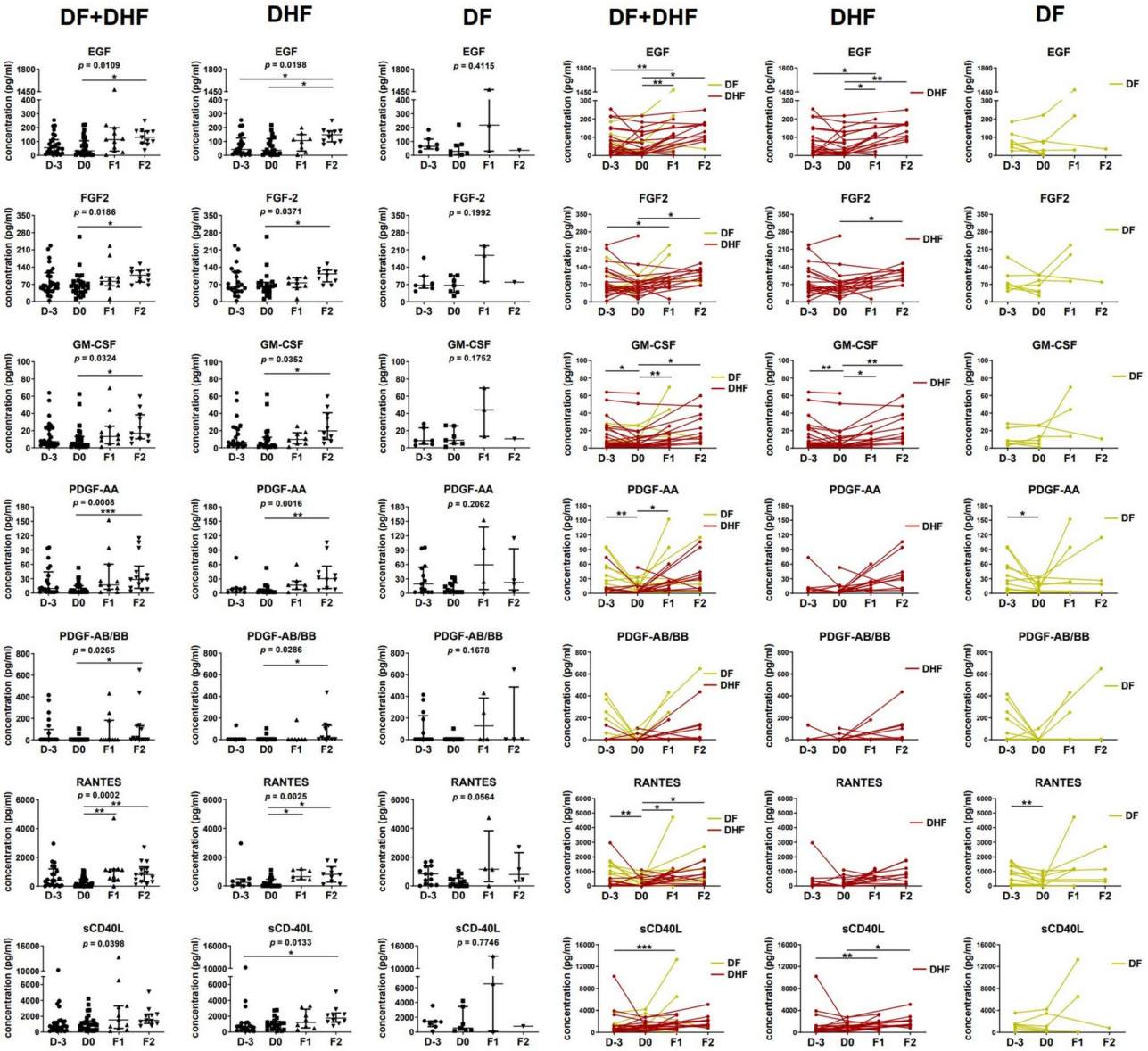

Fig. 4. Cytokines associated with convalescent dengue infection. Each dot represents the level of cytokine from each sample. The 3 left columns include all samples, Kruskal–Wallis test was used to compare 4 groups of independent data (p value indicated in each graph). median with interquartile range were shown in each group. Kruskal–Wallis statistics with Dunn's multiple comparisons test were used to compare between 2 groups indicated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Left most column: combined DF and DHF, second column: DHF only, third column: DF only. The 3 right columns include only dependent data from the same patients (line connect between paired samples). Wilcoxon matched-pairs signed rank test was used to compare between 2 groups indicated. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The 4th column: combined DF and DHF, 5th column: DHF only, 6th column: DF only.

compared to DF patients (Fig. 5a–b). At the D-3 time point, IL-15 levels were higher in DHF patients (median 17.79 pg/ml, IQR 11.69–21.38) than in DF patients (median 10.58 pg/ml, IQR 9.2–11.96) (Fig. 5a). Similarly, at D0, the levels of IL-8 and IP-10 were higher in DHF patients (IL-8: median 22.32 pg/ml, IQR 11.05–68.43; IP-10: median 309.9 pg/ml, IQR 211.8–585.4) compared to DF patients (IL-8: median 9.46 pg/ml, IQR 4.29–18.87; IP-10: median 176.4 pg/ml, IQR 157.4–327.3) (Fig. 5b). No significant differences in cytokine concentrations were observed between DF and DHF during the convalescent phase.

When examining the trend of IL-8 and IP-10 over the course of SD infection, both of these cytokines were already elevated at D-3, with no distinct difference between DF and DHF patients at this time point (Fig. 3c). However, the sustained high levels of these cytokines in DHF patients suggest their association with severe hemorrhage during the critical phase. In contrast, DF patients experienced a more rapid decline in IL-8 and IP-10 levels by D0.

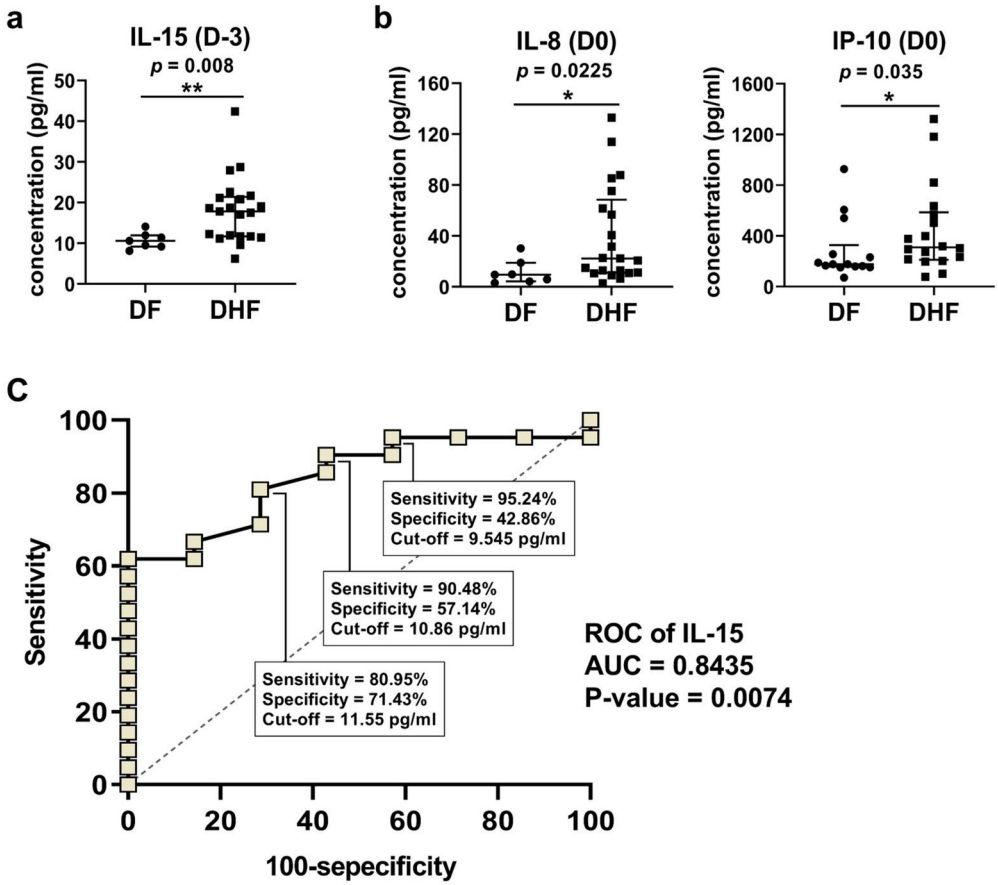

Fig. 5. Cytokines are associated with dengue severity. (**a**) Level of IL-15 at D-3 time-point. (**b**) Levels of IL-8 and IP-10 at D0 time-point. Each dot represents the level of cytokine from each sample. The Mann Whitney test was used to compare the difference between DF and DHF samples (*p* value indicated in each graph). Median and interquartile ranges are shown in each plot. (**c**) ROC curve of IL-15 level between DF and DHF samples at D-3 time-point. AUC; area under the curve, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

The significant increase in IL-15 levels in DHF patients, observed 3 days before defervescence, suggests that IL-15 could serve as a prognostic marker for identifying patients at risk of developing DHF during the early febrile phase. Notably, the receiver operating characteristic (ROC) plot for IL-15 at the D-3 time point showed an area under the curve (AUC) near 1 (0.8435) with a significant *p* value of 0.0074 (Fig. 5c). Using a cut-off value of 9.545 pg/ml for IL-15, the sensitivity was high at 95.24%, although the specificity was lower at 42.46% (Extended Data Table 1). Increasing the cut-off to 10.86 and 11.55 pg/ml improved specificity to 57.14% and 71.43%, respectively, while sensitivities decreased to 90.48% and 80.95%. In summary, our findings suggest that IL-15 could serve as an early predictive biomarker for severe dengue, particularly for identifying patients at risk of progressing to DHF.

Discussion

A major challenge in managing DENV infections is the lack of reliable predictors for disease severity, as well as the absence of early biomarkers that can predict disease progression. In this study, we measured the levels of 41 plasma mediators, which are crucial in both innate and adaptive immune responses and have previously been associated to DENV infection[13–15]. The study included patients with three distinct disease outcomes (AD, DF, and DHF), recruited from the well-defined DENFREE Thailand cohort, and encompassed four key time points throughout the course of SD infection to provide a comprehensive understanding of dengue immunopathology and identify potential biomarker.

Our cross-sectional findings revealed a global dampening of plasma mediators in AD compared to SD patients (DF and DHF). This supports the hypothesis that AD cases regulate cytokine secretion more efficiently, enabling viral clearance without excessive immunopathology. The findings align with our previous study on the same cohort, which showed a lower viral decay rate in AD cases[23]. Therefore, we suggest that the balanced immune response observed in AD potentially limits immunopathology and symptoms.

Our finding may also support several studies linking cytokine storms, characterized by excessive soluble mediator production, to dengue pathogenesis[13,26]. Notably, the elevated levels of anti-inflammatory cytokine IL-10 in SD patients have been identified as a hallmark of severe dengue infection[14,19,20]. Previous studies suggested that elevated plasma IL-10 may be associated with plasmablast differentiation[22,27], linking it to antibody-

dependent enhancement, a well-known mechanism in dengue pathogenesis. Moreover, the concentrations of IL-10 in severe DHF patients were found to be 25 times higher than in severe COVID-19 patients[28], underscoring its potential role in disease severity. Beyond IL-10, prolonged high concentrations of inflammatory cytokines have been linked to severe dengue, in contrast to the rapid decline seen in mild dengue cases[29]. Moreover, elevated TNF-α and IL-6 levels, in particular, have been implicated in vascular leakage and are thought to play critical roles in dengue pathogenesis[30–32]. Meanwhile, the secretion of anti-inflammatory cytokines such as IL-1RA and IL-10 during the acute phase may help counterbalance inflammatory activities, as evidenced by the positive correlation between IL-10 levels and pro-inflammatory cytokines, including IL-6 and IL-8[33]. In summary, SD is characterized by excessive cytokine production, and this dysregulation likely contributes to a spectrum of outcomes, ranging from mild symptoms to severe disease.

Although we observed a global dampening of cytokines in AD compared to SD patients, a study from the DENFREE Cambodia cohort showed contrasting results, which reported significantly elevated levels of IFN-γ, IL-2, IL-12, and IL-23 in AD cases[22]. However, the median levels of these cytokines in both AD and SD patients were relatively low (< 5 pg/ml), and the differences between the groups were only 1–5 pg/ml. This raises questions about the biological significance of such small variations in cytokine levels. Additionally, other cytokines, including IL-8, IL-15, TNF-α, IL-6, CCL3, and CCL4, exhibited similar levels in both AD and SD patients in that study[22]. The discrepancies between our cytokine profiles and those reported in previous research may arise from differences in study cohorts, such as the age range of donors (children vs. adults), the timing of sample collection relative to the course of infection, or the detection limits of the assays used.

In our longitudinal analysis, we observed significant shifts in plasma mediator levels during the febrile, defervescence, and convalescent phases of SD. Our findings are consistent with a study by Chng et al., which measured the levels of 65 cytokines and observed distinct cytokine profiles between the acute and convalescent phases[34]. Both studies reported elevated levels of IFN-γ, IL-1β, IP-10, and MCP-1 during the acute phase. However, our findings during the convalescent phase differed, with Chng et al. reporting an increase in Eotaxin-2 (CCL24), while we identified elevated levels of several growth factors. Similarly, our findings on anti-inflammatory cytokines, IL-10 and IL-1RA, align with those of a recent study by Vuong et al., which reported increased levels of these cytokines during the acute phase of infection (1–3 days after disease onset), followed by a decline during the convalescent phase (10 days after disease onset)[35].

During the acute phase of SD infection, we observed a broad upregulation of cytokines involved in inflammatory responses (TNF-α, IL-1α, IL-6, IL-15, MIP-1α, and MIP-1β), antiviral activity (IFN-γ, IFN-α2), anti-inflammatory control (IL-10, IL-1RA), and chemokines (IL-8, MCP-1, IP-10, and Fractalkine). Previous studies have shown that increasing levels of interferons are associated with the decline of DENV viral load, suggesting their role in viral elimination and heightened cellular antiviral responses during symptomatic infection[36,37]. Other cytokines, such as IL-8, has been shown to promote neutrophil activation during acute dengue infection, leading to the formation of neutrophil extracellular traps (NETs)[25]. Elevated levels of IP-10 have been linked to the recruitment of CXCR3-bearing cells, such as activated T cells and NK cells, which amplify the inflammatory response[38]. Similarly, MIP-1α and MIP-1β, which are predominantly produced by macrophages, exhibit chemotactic and pro-inflammatory activity[39], contributing to the inflammatory milieu upon acute dengue infection.

Conversely, during the convalescent phase, we observed elevated levels of several growth factors and other mediators that may play a role in recovery following DENV infection. One such mediator, sCD40L, has been shown to enhance platelet activation, leading to the release of platelet granule-derived mediators such as TGF-β, EGF, FGF, PDGF, RANTES, VEGF, IL-8, and PF4 (CXCL4)[40]. These factors are critical for endothelial cell expansion and extracellular matrix formation, promoting wound healing[41–43]. Given that plasma leakage is a hallmark of dengue pathogenesis, we hypothesize that platelet activation and the secretion of these cytokines may promote endothelial repair during recovery. Interestingly, reduced levels of platelet-derived growth factor have been frequently observed in severe dengue cases[44]. However, platelet activation has also been reported during the acute phase, where activated platelets exhibit apoptotic characteristics, resulting in thrombocytopenia[45], a key signs of bleeding manifestations. These contrasting findings underscore the need for further studies to clarify the role of sCD40L and its association with disease pathogenesis.

Although the cytokine profiles in DF and DHF were generally similar, we observed increased IL-15 levels during the early febrile phase in DHF, which may serve as a promising predictive biomarker for disease severity. IL-15, an inflammatory cytokine with a broad range of biological functions, primarily involves the activation and survival of CD8 T cells and NK cells[46]. However, few studies have examined the role of IL-15 in the context of DENV infection, with some suggesting a protective role, which contrasts with our findings. One study linked increased IL-15 during the acute phase to the frequency and cytotoxic activity of NK cells[47], while another study found higher IL-15 production prior to DENV exposure was associated with subclinical symptoms upon subsequent infection[48]. Interestingly, IL-15 can also induce the secretion of IL-8 from neutrophils and monocytes[49,50], potentially contributing to the elevated IL-8 levels observed during the febrile phase. This dual role of IL-15, both as a predictive biomarker and a contributor to inflammation, warrants further investigation to better understand its impact on disease progression and to support its potential use as a biomarker.

We also observed differing levels of IL-8 and IP-10 between DF and DHF during the critical phase, suggesting that prolonged high levels of these cytokines may be associated with dengue immunopathogenesis and could be potential targets for treating severe disease. Elevated IP-10 levels are associated with higher levels of atypical lymphocytes and T cell apoptosis in severe dengue[51,52], and it has been shown to inhibit endothelial cell proliferation and disrupt angiogenesis, which supports its role in causing plasma leakage[53,54]. Consistently, the level of IP-10 has been shown to increase in patients with significant plasma leakage[55]. However, a protective role for IP-10 has also been suggested, as the absence of IP-10 and its receptor leads to reduced effector T cell recruitment and increases the mortality rate in dengue-infected mice[56]. Additionally, one study demonstrated

that high IP-10 levels can prevent DENV infection by inhibiting viral binding to heparan sulfate molecules[57]. Furthermore, McCracken's study showed that downregulation of IP-10 transcripts during mosquito probing promotes DENV spread and viral replication in vivo, highlighting the role of IP-10 in DENV suppression[58].

Despite the limitations of our study, such as the lack of healthy controls for all cytokines and the absence of dengue shock syndrome (DSS) patients, our work addresses important gaps in understanding the soluble immune response during dengue infection. The small sample size, particularly for DF patients during convalescence, limits the generalizability of our findings. Further studies, including in vitro and in vivo experiments, are needed to validate these findings, particularly the role of IL-15 in dengue, and to develop reliable predictive biomarkers. Additionally, future studies incorporating healthy controls to establish baseline cytokine levels would be valuable. This would also help clarify the persistently high levels of certain cytokines observed at the two-month follow-up. Moreover, the predictive value of IL-15, along with other potential biomarkers such as IL-8, IL-10, suPAR, and olfactomedin 4 identified in various studies[18–21], should be validated across multiple independent cohorts to establish their reliability. Future studies utilizing machine learning approaches, along with independent cohort validation involving larger sample sizes and comprehensive clinical parameters, will be essential for designing and developing a robust disease severity prediction model.

In summary, this work highlights the soluble immune signature in asymptomatic dengue (AD) and the cytokine profile throughout the course of symptomatic dengue (SD) infection (Supplementary Fig. 4). We suggest that the limited secretion of multiple cytokines is a key feature of AD, while excessive cytokine secretion is characteristic of symptomatic disease. Prolonged high levels of IL-8 and IP-10 during the critical phase may be associated with hemorrhagic manifestations in DHF patients. Additionally, growth factors and platelet activation may play an essential role in the recovery of endothelial integrity during the convalescent phase. Finally, we propose that IL-15 could serve as a valuable biomarker for predicting disease severity. Ultimately, these findings improve our understanding of the cytokine response in DENV infection and lay the foundation for biomarker development and better treatment strategies.

Method

Ethics statement

The study was conducted according to the principles expressed in the Declaration of Helsinki. Approval was granted by the Institutional review board (IRB) of faculty of Medicine, Vajira hospital (No.015/12), the Faculty of Tropical Medicine, Mahidol university (TMEC 13-041), and Faculty of Medicine, Ramathibodi hospital, Mahidol University (MURA2016/219 and MURA2019/603). Informed consents were provided by all enrolled participants or their legal guardians.

Study population

Dengue patients and their household members were recruited under the DENFREE Thailand cohort from 2 study sites: Bangkok (Vajira hospital and Faculty of Tropical Medicine) and Tak (Tasongyang hospital)[23]. Criteria to include SD cases were (1) the presence of DENV RNA detected by reverse transcription real time PCR (RT-PCR) or NS1 antigen or anti-DENV IgM detected by rapid screen test (SD, Korea), (2) high fever (≥ 38 °C), and (3) presenting of at least 2 clinical symptoms of dengue illness (severe headache, retro-orbital pain, muscle pain, joint pain, rash, or bleeding manifestation). Severity of SD patients was classified into DF and DHF according to the WHO1997 criteria[7]. Hemagglutination inhibition (HI), focus reduction neutralization test (FRNT), and IgM/IgG ratio assessed by ELISA of paired acute and convalescent plasma samples were used to classify primary and secondary dengue infections based on the WHO 1997 criteria.

SD patients were recruited into our DENFREE Thailand cohort upon hospital admission. EDTA blood samples were collected daily during the febrile phase until the day of defervescence, which was uniformly designated as Day 0 (D0). To systematically account for time-course variations during the febrile phase in SD, we retrospectively designated samples as 1 day before defervescence (D-1), 2 days before defervescence (D-2), and progressively earlier time points accordingly. Blood samples were also collected from SD patients during the convalescent phase and at two follow-up time points (F1 and F2).

Household members of the SD index cases were recruited within 1–2 days after the enrollment of SD patients. Participants who were positive for DENV RNA as detected by RT-PCR and absent of clinical symptoms for 2 weeks upon follow-up were recruited as AD cases. EDTA blood was collected only once during the viremic phase. Household members who tested negative for dengue viral RNA were recruited as non-infected HI cases. EDTA plasma was separated from blood cells and stored at − 80 °C until further analysis.

Cross-sectional study

Five AD cases in which plasma samples were available were selected from the cohort to compare the cytokines levels with 32 DF and 10 DHF cases (Supplementary Tables 1 and 3). SD samples collected during 1–2 days before fever subsided (D-1 and D-2) as previously showed to exhibit active systemic immune response upon the acute phase[24] were used. We excluded SD without plasma samples at D-1 and D-2. AD samples were selected based on plasma sample availability, and individuals without available samples were excluded. Viremia level, age, and sex among the 3 severity groups were not significant different (Fig. 1a, Supplementary Table 1). While most SD cases were secondary infection, only 2 DF patients were presented with primary infection. The majority of infected DENV were serotype 3 and 4. One DF patient was presented with co-infection of serotype 3 and 4 and no serotype 2 infection was presented (Supplementary Table 1).

Longitudinal study

A total of 100 samples from 15 DF and 22 DHF patients across four different time points were included in this study (Supplementary Tables 2 and 4). To ensure that all samples were collected at comparable stages of

disease progression, we selected samples from three days before defervescence (D-3) as the first time point in this longitudinal study. The second time point was the day of defervescence (D0). The third and fourth time points were at two weeks (F1) and two months (F2) post-hospital discharge.

We included SD samples from patients whose plasma samples at least at two time points: D-3 and D0, were available. We excluded patients who did not have blood samples at both of these time points. During convalescence, most patients provided at least one follow-up sample (either F1 or F2), while some participants did not provide follow-up samples (no F1 or F2). (Supplementary Table 4).

The percentage of male in both infection severity was approximately 50% which indicates no sex bias between 2 groups (Supplementary Table 2). There was no significant difference between the age of the DF and DHF patients. The majority of all patients had secondary dengue infection; 86.6% (n = 13) in DF and 95.5% (n = 21) in DHF. In the DF group, most patients were infected with DENV serotype 4 (73.7%, n = 11) followed by serotype 3 (20%, n = 3) while the rate of serotype 4 infection was equal to the serotype 3 infection in the DHF group (36.4%, n = 8). Additionally, DHF patients infected with DENV serotype 1 and 2 were recruited in this study, whereas there were no DF patients with DENV serotype 1 and 2 infections.

Viral load detected by qRT-PCR during the febrile day showed no significant difference between DF and DHF patients (p = 0.4316) (Supplementary Table 2). The viremia level was markedly decreased upon the defervescence when compared with those at a febrile day in both DF ($p < 0.0001$) and DHF patients ($p < 0.0001$). Also, there was no significant difference of the viral load during the defervescence when compared between DF and DHF (and $p = 0.6808$).

Cytokine measurement

Levels of 41 cytokines, chemokines and growth factors were measured using MILLIPLEX® MAP 38-plex and 4-plex human cytokine/chemokine magnetic bead panels on Luminex xMAP® platform (Merck KGaA, Germany). The 41 mediators includes EGF, FGF, Eotaxin, TGF-α, G-CSF, Flt-3L, GM-CSF, Fractalkine, IFN-α2, IFN-γ, GRO, IL-10, MCP-3, IL-12P40, MDC, IL-12P70, IL-13, IL-15, sCD40L, IL-17A, IL-1RA, IL-1α, IL-9, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, TNF-β, VEGF, PDGF-AA, PDGF-AB/BB, and RANTES. Plasma collected using EDTA as an anticoagulant were used. Samples were thawed completely, mixed well, and centrifuged prior to the experiment. The immunoassay was performed according to the manufacturer's protocol. Each run contained quality controls and standard human cytokine reagents. The immunoassay plates were read on a Luminex MAGPIX® instrument with xPONENT® 4.2 software (Merck KGaA, Germany) (https://int.diasorin.com/en/luminex-ltg/reagents-accessories/software) for data acquisition and cytokine concentration analysis. The raw cytokine data was provided in Extended Data Table 2.

Statistical analysis

Cytokine concentrations falling below the detection limit were adjusted to the lower detection limit minus 0.1, while concentrations exceeding the detection limit were set to the upper detection limit plus 0.1. Principal component analysis (PCA) was performed using Rstudio (https://cran.rstudio.com/) (R version 4.0.4)[59] with the FactoMineR package[60] version 2.4 (https://cran.r-project.org/web/packages/FactoMineR/index.html). Raw cytokine levels from 56 samples composed of 15 DF and 41 DHF, which complete 41 cytokine data, were log-transformed before the PCA calculation. GraphPad Prism PRISM version 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) (https://www.graphpad.com/) was used to perform the statistical analysis of each cytokine. The kinetics of individual cytokines were analyzed using non-parametric Kruskal–Wallis test with Dunn's multiple comparison test. The Wilcoxon matched pairs signed rank test was used to analyze the dependent data from the same patients between different timepoints. The difference of each cytokine level between DF and DHF patients was calculated by non-parametric Mann-Whitny U test. The findings are reported as median and interquartile range (IQR) and represented as dot graphs. A p value less than 0.05 was considered statistically significant.

Data availability

The raw cytokine data and the ROC plot analysis results for IL-15 at the D-3 time point are provided in the extended data table files.

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Author contributions

Conceptualized and designed the study: P.M., N.J. Clinical samples: DENFREE Thailand, W.C., W.T., P.M. Methodology: N.J., W.C. Data analysis and interpretation: N.J., P.M., V.V., W.S., V.C. Wrote manuscript: N.J., P.M., V.V. All authors have reviewed and approved the submitted manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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