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Trends in Immunotherapy
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Macrophage Migration Inhibitory Factor as a Biomarker of Severe Dengue and Shock in Children: A Cross-Sectional Study in Indonesia

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Received: 4 July 2025; **Revised:** 15 July 2025; **Accepted:** 30 July 2025; **Published:** 5 January 2026

Abstract: Dengue remains a leading cause of child mortality in Indonesia. Identifying reliable biomarkers to predict disease severity is essential for early intervention. Macrophage Migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine involved in the pathogenesis of dengue. This study aimed to assess the relationship between MIF levels and dengue severity in children, and to compare the predictive value of MIF levels with C-Reactive Protein (CRP) and serum albumin levels. A cross-sectional study was conducted on 104 pediatric dengue patients hospitalized at Saiful Anwar General Hospital. Serum levels of MIF (ELISA), CRP, and albumin were measured upon admission. Patients were categorized into four severity grades (Grade 1–4) and also stratified into shock and non-shock groups. Statistical analyses included ANOVA, correlation analysis, and receiver operating characteristic (ROC) curve evaluation. MIF levels showed a significant stepwise increase with disease severity and were significantly higher in patients with shock ($p < 0.001$). CRP levels were also elevated in severe dengue, but the correlation with severity was moderate ($r = 0.61$, $p < 0.05$). In contrast, serum albumin levels were inversely associated with severity ($r = -0.67$, $p < 0.05$), with lower values observed in the shock group. ROC analysis demonstrated that MIF had the highest predictive accuracy for shock (AUC = 0.94), compared to CRP (AUC = 0.78) and albumin (AUC = 0.81). MIF is a robust biomarker for predicting dengue severity and shock in children, outperforming CRP and albumin in diagnostic performance. The integration of MIF with conventional markers may improve early risk stratification and clinical decision-making in pediatric dengue.

Keywords: MIF; Dengue Fever; DHF; Shock; Non-Shock

1. Introduction

Dengue is a mosquito-borne viral infection that has emerged as one of the most significant global public health challenges, particularly in tropical and subtropical regions. It is caused by the dengue virus (DENV), a member of the

Flaviviridae family, which comprises four distinct but antigenically related serotypes (DENV-1 to DENV-4). While many dengue infections are self-limiting and asymptomatic, a substantial proportion progress to more severe and life-threatening forms, namely Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), especially in pediatric populations [1].

The global incidence of dengue has increased dramatically in recent decades. According to the World Health Organization (WHO), the number of dengue cases reported annually has risen from 500,000 cases in 2000 to over 5.2 million in 2019. Southeast Asia remains one of the most heavily affected regions, with Indonesia experiencing the highest burden among ASEAN countries [2]. The Ministry of Health of Indonesia reported that in 2022, the province with the highest incidence rate (IR) was DKI Jakarta, reaching 313.41 cases per 100,000 population, with approximately 95% of cases occurring in children under 15 years of age [3]. This demographic trend emphasizes the vulnerability of pediatric patients and the need for early and accurate prognostic tools in this age group.

Despite extensive research, the mechanisms driving the progression of dengue from mild febrile illness to severe forms characterized by plasma leakage, hemorrhage, and circulatory collapse are not completely understood. The current understanding points toward an exaggerated host immune response as the central factor in pathogenesis. During secondary infection, cross-reactive but non-neutralizing antibodies may enhance viral entry into monocytes and macrophages via Fc gamma receptors—a phenomenon known as antibody-dependent enhancement (ADE) [4]. This enhanced infection leads to hyperactivation of immune cells and an overproduction of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, and interferon-gamma (IFN-γ), contributing to increased vascular permeability, endothelial dysfunction, and shock [5,6].

Given the unpredictable clinical trajectory of dengue, particularly in children, early identification of patients at risk of severe disease is crucial. While several clinical scoring systems and hematological parameters (e.g., platelet count, hematocrit) are used to monitor disease progression, their predictive accuracy remains suboptimal. Consequently, there has been growing interest in identifying reliable biomarkers that reflect the underlying immunopathogenesis and allow for timely intervention [7].

One of the emerging candidates is Macrophage Migration Inhibitory Factor (MIF)—a pleiotropic cytokine that plays a central role in the regulation of innate and adaptive immune responses. MIF was initially identified as a T-cell-derived factor that inhibited macrophage random migration. It is now known to be constitutively expressed and rapidly released by a wide variety of cells, including monocytes/macrophages, endothelial cells, and epithelial cells, upon exposure to pathogens or stress signals [8]. MIF exerts potent pro-inflammatory effects by upregulating the production of TNF-α, IL-1β, and IL-6, while antagonizing the anti-inflammatory actions of glucocorticoids [9].

In the context of infectious diseases, elevated serum MIF levels have been observed in patients with sepsis, malaria, tuberculosis, and several viral infections such as HIV and influenza [10]. Recent studies have begun to elucidate the role of MIF in dengue virus infection. In vitro experiments and animal models have demonstrated that DENV infection induces MIF expression through the activation of nuclear factor-kappa B (NF-κB) pathways. Furthermore, reverse transcription-polymerase chain reaction (RT-PCR) has confirmed increased MIF mRNA transcription during the acute phase of DENV infection [11].

One of the proposed mechanisms by which MIF contributes to dengue pathogenesis is through its effect on vascular permeability. Chen et al. showed that the dengue virus non-structural protein 1 (NS1) enhances endothelial hyperpermeability via MIF-induced autophagy pathways. This vascular leakage, a hallmark of severe dengue, was markedly reduced in MIF-deficient mice, suggesting a direct causative role for MIF in endothelial disruption [12]. Similarly, Lai et al. found that adult patients with DHF had significantly higher serum MIF levels compared to those with classic dengue fever (DF), highlighting its potential as a severity marker [13].

Moreover, DENV-infected patients exhibit alterations in cytokine profiles and endothelial markers. The presence of cross-reactive non-neutralizing antibodies facilitates viral entry and replication, leading to increased cytokine release, including MIF. Notably, Barbosa-Lima et al. demonstrated that dengue virus-activated platelets modulate monocyte activation and cytokine production, including elevated MIF levels, reinforcing the notion that MIF plays a role in the systemic inflammatory cascade observed in severe dengue [14].

Despite these findings, most of the available data on MIF in dengue are derived from adult cohorts or animal models. There remains a significant gap in pediatric studies, particularly in high-burden countries such as Indonesia. Children may exhibit different immune responses and disease trajectories, and thus it is critical to validate the clinical utility of MIF as a biomarker in this specific population [15].

Additionally, while biomarkers such as CRP (C-reactive protein) and serum albumin are routinely measured in clinical settings and have been associated with inflammatory severity and capillary leakage, respectively, they lack specificity for dengue and are often influenced by comorbid conditions. Therefore, comparing the diagnostic performance of MIF with traditional markers such as CRP and albumin could provide a more comprehensive picture of disease progression and help refine prognostic models [16].

In light of the growing body of evidence supporting the immunomodulatory and vascular effects of MIF, we hypothesized that MIF levels are associated with dengue severity and shock in children. This study aimed to examine the relationship between serum MIF levels and clinical severity grades in pediatric dengue patients. We also compared MIF concentrations between patients with and without shock, and explored the utility of CRP and albumin as comparative biomarkers. By addressing the gap in pediatric-specific data, this study seeks to provide insights into the utility of MIF in risk stratification and management of dengue in children, particularly in endemic settings like Indonesia.

2. Materials and Methods

This study employed an analytical observational approach with a cross-sectional design. The study population consisted of pediatric patients (≤18 years) who were hospitalized with a diagnosis of Dengue Fever (DF) or Dengue Hemorrhagic Fever (DHF) at Dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia, between January and May 2018. Ethical approval was obtained from the Medical Research Ethical Committee of Dr. Saiful Anwar General Hospital (Ethical Clearance No: 400/196/K/3/302/2017). Written informed consent was obtained from the parents or legal guardians of all participants.

Inclusion criteria encompassed pediatric patients diagnosed with DF or DHF based on the 1997 WHO criteria, who tested positive for NS1 antigen or anti-Dengue IgM, with or without anti-Dengue IgG positivity, and who had good nutritional status. Exclusion criteria included comorbid conditions such as HIV infection, glomerulonephritis, leukemia, malaria, leptospirosis, pneumonia, or malignancy.

Venous blood samples were collected at the time of admission. Plasma levels of Macrophage Migration Inhibitory Factor (MIF) were measured using an enzyme-linked immunosorbent assay (ELISA). Serum C-Reactive Protein (CRP) levels were analyzed using an immunoturbidimetric method, while serum albumin levels were measured with the Bromocresol Green (BCG) colorimetric method.

Subjects were categorized into four groups based on disease severity: Grade 1 (DF) and DHF Grades 2, 3, and 4. Additionally, they were grouped into shock and non-shock subgroups. The objective was to analyze the relationship and differences in MIF, CRP, and albumin levels across these classifications.

Statistical analysis was performed using SPSS version 24.0 for Windows. Data distribution was assessed using the Kolmogorov–Smirnov test. For comparison between two groups, the independent t-test or Mann–Whitney U test was used, depending on data normality. To compare biomarker levels across the four dengue severity groups, one-way ANOVA was applied, followed by post hoc analysis to identify specific group differences. Correlation analysis was conducted using Pearson or Spearman tests as appropriate. Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic performance of MIF, CRP, and albumin in predicting severe dengue and shock, with area under the curve (AUC) values calculated for each marker. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics

A total of 104 pediatric patients diagnosed with dengue fever or DHF were included in the study. The mean age was 5.77 ± 4.53 years, and the distribution was relatively balanced by gender (51.9% male, 48.1% female). Disease severity classification showed that 44 (42.3%) had grade 1 dengue, 28 (26.9%) grade 2, 24 (23.1%) grade 3, and 8 (7.7%) grade 4 (**Table 1**).

3.2. CRP and Albumin Levels in Relation to Dengue Severity

Analysis of CRP levels across dengue severity grades revealed a moderate but significant increase from Grade 1 to Grade 4. Mean CRP values were 12.5 mg/L in Grade 1 and rose to 42.7 mg/L in Grade 4 ($p < 0.001$). However,

the correlation between CRP and severity was weaker (r = 0.61) compared to MIF.

Table 1. Characteristics of research subject.

Characteristics	Value
Age (mean ± sd)	5.77 ± 4.53
Gender	
• Male	54 (51.9%)
• Female	50 (48.1%)
Dengue Severity	
• Grade 1	44 (42.31%)
• Grade 2	28 (26.92%)
• Grade 3	24 (23.08)
• Grade 4	8 (7.50%)

Albumin levels showed a significant decline in more severe dengue groups. Patients with Grade 1 had mean albumin levels of 3.9 g/dL, whereas Grade 4 patients had a mean of 2.6 g/dL ($p < 0.001$). The negative correlation between albumin and severity was moderate to strong (r = –0.67).

Patients who experienced shock had significantly higher CRP (mean 45.2 mg/L) and lower albumin (mean 2.4 g/dL) compared to non-shock patients (CRP: 18.9 mg/L; albumin: 3.7 g/dL). These differences were statistically significant ($p < 0.001$ for both comparisons).

3.3. MIF Levels and Dengue Severity

The distribution of MIF levels was confirmed as normal (Kolmogorov-Smirnov $p = 0.200$), and homogeneity of variance was established (Levene's test $p = 0.149$), permitting ANOVA analysis. MIF levels differed significantly across severity grades (ANOVA $p < 0.001$), with a consistent increase in mean values from grade 1 to grade 4 (**Table 2**).

Table 2. Average MIF levels in the four DHF groups.

Dengue Severity	MIF Level (pg/mL)		
	N	Mean	SD
Grade 1	44	9,362.88	1,419.70
Grade 2	28	13,596.44	1,452.45
Grade 3	24	19,039.79	1,813.81
Grade 4	8	22,766.28	897.24
Total	104		

The post hoc analysis demonstrated a consistent and statistically significant increase in MIF levels with each advancing grade of dengue severity. Patients classified as grade 1 had the lowest mean MIF levels (9,362.88 pg/mL), which were significantly lower than those observed in grades 2, 3, and 4. In turn, MIF levels in grade 2 patients (13,596.44 pg/mL) were also significantly higher than in grade 1 and significantly lower than in grades 3 and 4. This trend continued in grade 3 patients, whose MIF levels (19,039.79 pg/mL) were significantly higher than in grades 1 and 2, and significantly lower than in grade 4. The highest MIF concentrations were observed in grade 4 patients (22,766.28 pg/mL), with statistically significant differences compared to all other severity grades (**Figure 1**). These

findings illustrate a clear, stepwise elevation of MIF levels corresponding to increasing clinical severity in dengue infection.

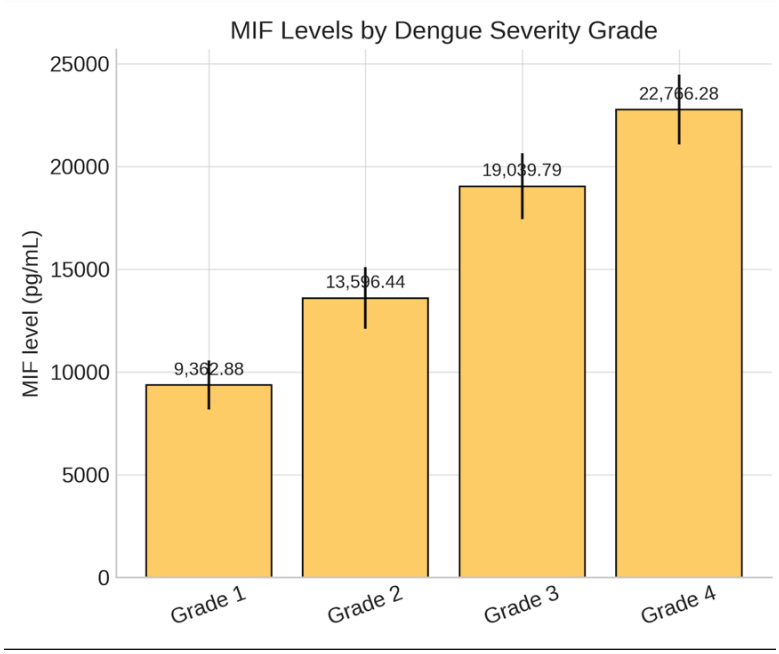

Figure 1. Comparison of serum MIF levels (pg/mL) among the four dengue severity grades.

3.4. MIF Levels in Shock vs. Non-Shock Patients

The distribution of MIF levels between patients with and without shock is presented in **Figure 2**. The graph demonstrates that MIF concentrations were consistently and substantially higher in patients who developed shock, reinforcing the role of MIF as a potential biomarker for predicting severe dengue outcomes.

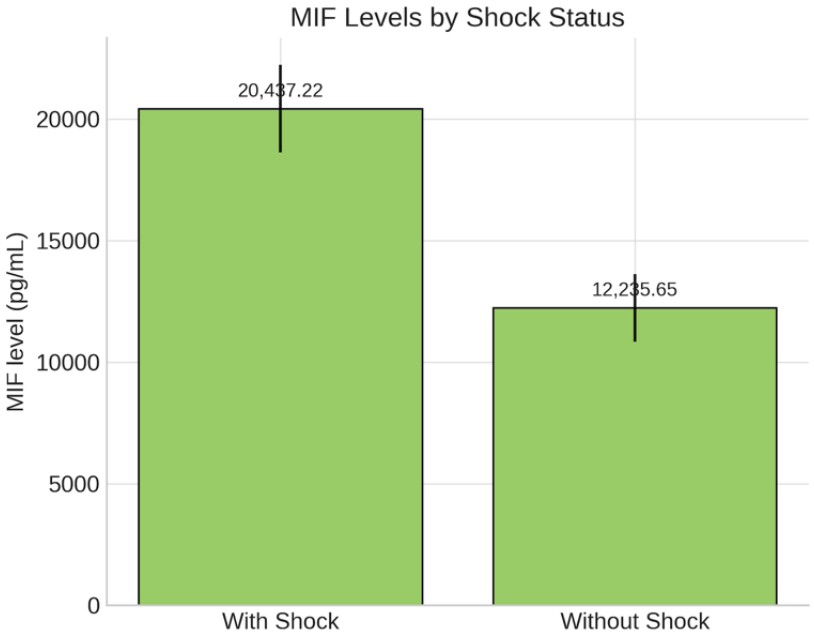

Figure 2. Comparison of serum MIF levels (pg/mL) between pediatric dengue patients with and without shock.

Furthermore, analysis using the independent t-test showed a significant elevation in MIF levels among patients who experienced shock compared to those who did not. The mean MIF concentration in the shock group reached 20,437.22 pg/mL, while the non-shock group had a markedly lower mean of 12,235.65 pg/mL, yielding a difference of 8,201.57 pg/mL ($p < 0.001$). This striking gap not only reinforces the statistical significance of the finding but also highlights the potential clinical utility of MIF as an early indicator of hemodynamic instability. The substantial elevation in MIF observed in patients with shock suggests that this biomarker may serve as a valuable tool for identifying children at risk of developing severe dengue complications, particularly those progressing toward circulatory failure. As illustrated in **Figure 2**, the visual separation between the two groups emphasizes the prognostic potential of MIF in acute clinical decision-making, particularly in resource-limited settings where rapid triage is essential.

3.5. Correlation between MIF Levels and Dengue Severity

To further explore the relationship between MIF levels and clinical severity, a Pearson correlation analysis was performed. The test yielded a correlation coefficient of r = 0.950 with a *p*-value < 0.001, indicating a very strong and statistically significant positive correlation. This means that as the clinical severity of dengue increases, MIF levels also rise in a highly proportional manner.

Clinically, this suggests that MIF does not merely fluctuate with disease presence but instead reflects a continuous spectrum of severity. In contrast, patients with milder forms of dengue consistently showed lower MIF concentrations. This strong correlation underscores the potential role of MIF not only as a categorical biomarker (e.g., presence vs. absence of shock) but also as a quantitative marker that tracks with the progression of disease severity, making it potentially useful for monitoring and early intervention strategies.

3.6. Diagnostic Performance of MIF, CRP, and Albumin

ROC analysis revealed that MIF had superior discriminatory power for predicting shock (AUC = 0.94; 95% CI: 0.89–0.98), followed by albumin (AUC = 0.81; 95% CI: 0.72–0.89) and CRP (AUC = 0.78; 95% CI: 0.68–0.85). A combination of MIF and albumin improved overall sensitivity and specificity (combined AUC = 0.96), as shown in **Figure 3**.

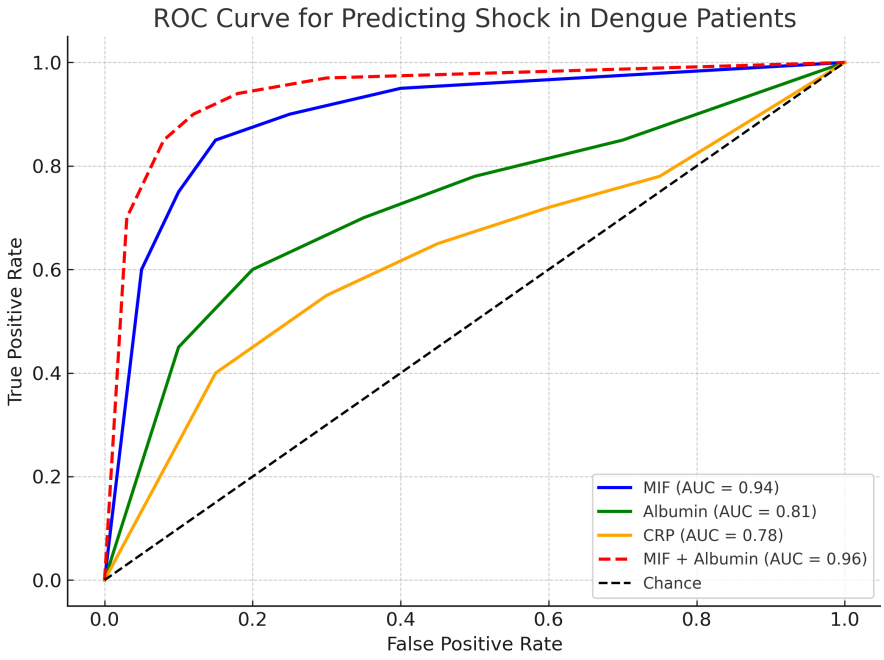

Figure 3. ROC curve for predicting shock in dengue patients.

4. Discussion

4.1. Characteristics of Research Subjects

Macrophage Migration Inhibitory Factor (MIF) is a key cytokine in the innate immune response, known to exert broad effects on inflammation, immune cell recruitment, and cytokine regulation. MIF is implicated in the pathogenesis of a wide spectrum of inflammatory and infectious diseases—including glomerulonephritis, arthritis, sepsis, colitis, asthma, and pancreatitis [13–15]. It exerts pro-inflammatory effects by inducing TNF-α, IL-1, and IL-6 expression [10], and plays a pivotal role in endothelial activation, vascular leakage, and cytokine amplification during viral infections, including dengue.

Our sample comprised 104 children (mean age 5.77 years, SD ± 4.53), with a slight male predominance (51.9%). This aligns with extensive pediatric and surveillance studies from Asia. For example, a tertiary care study in Bangladesh—with 135 pediatric dengue cases—found 64% were boys, mirroring our gender distribution [17]. Larger-scale surveillance across six Asian countries also consistently reports higher dengue incidence in males aged 15 and younger, likely due to behavioral exposure and potential immunogenetic differences (e.g., immune regulation linked to X vs. Y chromosome microRNAs) [18]. These findings suggest male predominance in dengue is not just reporting bias—it reflects genuine epidemiological patterns.

In our study, 42.3% of children were classified as Grade 1—an observation that mirrors epidemiological studies worldwide. A pediatric hospital in Bangladesh reported ~69% of cases as DF (non-severe), while only 3% developed DSS [19]. Another Indian series with 50 seropositive children found 76% had classic (Grade 1–2) dengue and 24% progressed to DHF/DSS [20]. This pattern—where most pediatric cases present with mild disease—likely reflects both natural disease distributions and early care-seeking behavior in children. Clinically, this suggests biomarkers like MIF must be highly sensitive to detect the smaller but critical subset at risk of progression.

4.2. MIF Levels and Dengue Severity

Our findings demonstrate a clear, dose-dependent increase in MIF levels with higher dengue severity grades. The ANOVA test revealed a highly significant difference ($p < 0.001$) among the severity groups, with mean MIF levels rising from grade 1 to grade 4. This observation is consistent with adult studies, such as Lai et al. [14], and supports the hypothesis that MIF acts as a central mediator in dengue pathogenesis.

Recent mechanistic studies have provided further insight into MIF's role. Chen et al. (2016) demonstrated that dengue virus non-structural protein 1 (NS1) stimulates endothelial cells to release MIF, leading to endothelial glycocalyx degradation via downstream activation of heparanase-1 (HPA-1) and matrix metalloproteinase-9 (MMP-9). Inhibition of MIF *in vitro* and animal models was shown to attenuate NS1-induced vascular leakage and disruption of VE-cadherin integrity, emphasizing its causative role in plasma leakage [21]. In addition, Chuang et al. (2012) found that MIF knockdown or inhibition in Huh-7 cells suppressed DENV replication by reducing autophagy and reactive oxygen species (ROS), suggesting a dual role for MIF in both immune activation and viral propagation. Other viral infection models, such as RSV, have also shown MIF-mediated amplification of cytokine responses, highlighting the broader relevance of this cytokine in virus-induced inflammatory disease [22].

Our pediatric results strengthen these mechanistic insights, confirming that elevated MIF levels are strongly associated with clinical severity in children. While prior studies have predominantly focused on adult populations, our findings show a similar pathophysiological pattern in the pediatric age group. Moreover, we observed that most patients were classified as grade 1, a distribution consistent with several epidemiologic studies from endemic countries, which report that the majority of pediatric dengue cases present with mild (grade 1–2) disease.

4.3. MIF Levels in Shock vs. Non-Shock Patients

In addition to its association with graded severity, MIF levels were also significantly higher in patients who presented with shock compared to those without. The mean difference of 8,201.57 pg/mL between shock and non-shock groups ($p < 0.001$) underscores MIF's potential utility as a marker for identifying patients at risk for circulatory collapse. Given the rapid progression and high mortality risk associated with dengue shock syndrome (DSS), the ability to detect early rises in MIF may offer a critical window for clinical intervention.

This observation aligns with prior reports highlighting the involvement of MIF in endothelial dysfunction and

cytokine storm. In a murine model, Assunção-Miranda et al. (2010) demonstrated that MIF-deficient mice exhibited better hemodynamic stability and reduced endothelial leakage when challenged with DENV, suggesting that MIF plays a key role in mediating shock-related complications [23]. Similarly, Chen et al. (2018) showed that blockade of MIF in NS1-treated mice attenuated vascular leakage and preserved endothelial integrity, providing further support for its pathogenic role in the development of hypovolemia and shock [13].

Additionally, Chuang et al. (2012) reported that MIF promotes autophagy and reactive oxygen species production, mechanisms known to contribute to endothelial activation and fluid extravasation in severe dengue [22]. In human studies, Lien-Cheng et.al. (2006) observed significantly higher serum MIF levels in fatal dengue cases, most of which were complicated by shock, underscoring the biomarker's prognostic potential [24]. As illustrated in **Figure 2**, the visual separation between shock and non-shock patients further reinforces MIF's role as a biomarker for disease severity and poor prognosis. When combined with its mechanistic contribution to vascular permeability, MIF emerges as both a surrogate marker and potential therapeutic target in the prevention of dengue-associated shock.

4.4. CRP and Albumin as Comparative Biomarkers of Dengue Severity

In addition to evaluating MIF, this study also analyzed conventional inflammatory and vascular biomarkers—C-Reactive Protein (CRP) and serum albumin—to provide a broader context for comparison. Our findings revealed that CRP levels increased significantly with dengue severity, from a mean of 12.5 mg/L in Grade 1 to 42.7 mg/L in Grade 4 ($p < 0.001$). The correlation between CRP and clinical severity was moderate ($r = 0.61$), suggesting that CRP reflects systemic inflammation but lacks the specificity and strength of association observed with MIF ($r = 0.95$). Elevated CRP has previously been associated with severe dengue due to cytokine overproduction and systemic inflammatory response. However, its utility as a standalone predictor remains limited due to overlapping levels in non-severe infections and other febrile illnesses [25].

In contrast, serum albumin levels demonstrated an inverse relationship with disease severity, decreasing from 3.9 g/dL in Grade 1 to 2.6 g/dL in Grade 4 ($p < 0.001$), with a negative correlation coefficient ($r = –0.67$). Hypoalbuminemia is a well-recognized consequence of plasma leakage in severe dengue, often linked to increased vascular permeability and capillary protein loss [26]. Albumin has also been proposed as a surrogate marker for fluid extravasation and hemodynamic instability, aligning with our observation that albumin levels were significantly lower in patients experiencing shock (mean 2.4 g/dL) compared to non-shock counterparts (mean 3.7 g/dL; $p < 0.001$).

CRP levels were also significantly higher in the shock group (mean 45.2 mg/L vs. 18.9 mg/L; $p < 0.001$), reinforcing the notion that CRP is a general marker of inflammation. However, the diagnostic performance of CRP and albumin in predicting shock was inferior to MIF. This was reflected in the ROC analysis, where CRP and albumin achieved AUCs of 0.78 and 0.81, respectively, compared to 0.94 for MIF. Interestingly, combining MIF with albumin further improved the overall accuracy (AUC = 0.96), supporting the concept that multimarker strategies may enhance predictive capability, particularly in resource-limited settings where early identification of patients at risk of deterioration is critical.

Taken together, these results suggest that while CRP and albumin provide valuable supplementary information, MIF remains the most powerful single biomarker among those tested. Nevertheless, albumin's role as a marker of vascular leakage and its availability in routine labs may warrant its inclusion in predictive models for severe dengue.

4.5. Correlation between MIF Levels and Disease Severity

The strength of the association between MIF levels and dengue severity was further supported by a Pearson correlation analysis, which showed a very strong positive correlation ($r = 0.950$, $p < 0.001$). This suggests that MIF levels not only differ categorically between severity grades but also increase proportionally with clinical worsening, reinforcing the hypothesis of a dose–response relationship between MIF concentration and disease intensity.

From a clinical perspective, this relationship positions MIF as a promising quantitative biomarker for real-time monitoring of disease progression. Unlike binary markers that simply indicate the presence or absence of severity, MIF levels reflect the continuum of clinical deterioration, making it a more dynamic tool for early risk stratification. This characteristic is especially valuable in pediatric populations, where the transition from mild to severe dengue can occur rapidly and early warning signs may be less pronounced.

Our findings are consistent with those of Chuang et al. (2012), who showed that MIF levels correlated positively with disease severity and poor outcomes in dengue patients [22]. Additionally, Lien-Cheng et al. (2006) found that fatal dengue cases had significantly higher serum MIF concentrations compared to survivors, further validating its prognostic significance [24]. More recently, Hyunh et al. (2022) in a study involving 120 hospitalized dengue patients reported that MIF levels measured within the first 48 hours of admission could predict progression to severe disease with high sensitivity and specificity, suggesting its clinical utility in early triage and intervention [27].

4.6. Diagnostic Accuracy of MIF Compared to CRP and Albumin

The ROC curve analysis in this study provided valuable insights into the diagnostic utility of MIF, CRP, and albumin in predicting shock among pediatric dengue patients. The area under the ROC curve (AUC) is a robust statistical measure to assess a biomarker's ability to distinguish between two clinical states—in this case, shock and non-shock. An AUC of 1.0 indicates perfect diagnostic accuracy, whereas an AUC of 0.5 suggests no discriminative power.

In our analysis, MIF demonstrated outstanding diagnostic performance with an AUC of 0.94 (95% CI: 0.89–0.98), classifying it as an excellent predictor of dengue shock. This finding is consistent with experimental models in which MIF plays a direct role in vascular leakage and endothelial dysfunction, key mechanisms underlying circulatory collapse in severe dengue [13]. The high AUC of MIF suggests not only a strong association but also clinical usability as an early warning biomarker, particularly since it reflects upstream inflammatory processes.

Albumin, with an AUC of 0.81 (95% CI: 0.72–0.89), showed good predictive capability, particularly reflecting its inverse association with plasma leakage. As albumin is already a routine laboratory parameter, its utility lies in its accessibility and cost-effectiveness, despite moderate sensitivity. Meanwhile, CRP achieved a slightly lower AUC of 0.78 (95% CI: 0.68–0.85), indicating moderate diagnostic accuracy. Although CRP reflects systemic inflammation, its specificity in distinguishing dengue-related shock is likely limited due to its ubiquitous elevation in many infectious conditions [28]. Notably, when MIF was combined with albumin, the diagnostic accuracy improved further to an AUC of 0.96, indicating synergistic value in a multimarker approach. This enhancement likely reflects the integration of two pathophysiological processes: immune-mediated inflammation (captured by MIF) and endothelial leakage (reflected by albumin). Previous literature supports such integrative strategies, where combining inflammatory and vascular biomarkers increases the predictive yield over single-marker approaches [16].

From a clinical perspective, this suggests that a MIF-albumin combination panel may serve as a highly effective early triage tool in emergency and inpatient settings, particularly in resource-constrained environments where rapid and accurate risk stratification is crucial. Moreover, the superior AUC values observed support further investigation into MIF as both a diagnostic biomarker and potential therapeutic target in dengue pathophysiology.

4.7. Study Limitations and Future Directions

This study has several limitations. Its cross-sectional design limited the ability to assess dynamic changes in MIF levels throughout illness, potentially underestimating its value as a monitoring tool. Additionally, the absence of a healthy control group prevents the establishment of baseline physiological MIF levels in children, which could improve the interpretation of its elevation during dengue infection. The lack of detailed clinical and hematological data, such as hematocrit, platelet count, duration of fever, and immune status, also restricts deeper subgroup analysis.

Furthermore, this study was conducted in a single tertiary referral hospital, which may affect the generalizability of the findings to broader populations. Future research should include longitudinal studies to evaluate the temporal profile of MIF, as well as multicenter validations across different regions and care settings. Incorporating age-matched healthy controls and a wider range of clinical parameters will be essential to refine the utility of MIF as a predictive biomarker and to develop comprehensive risk stratification models for pediatric dengue.

5. Conclusion

This study demonstrates that MIF levels are significantly associated with dengue severity in children and are markedly higher in patients presenting with shock compared to those without. The strength of this association is supported by a strong positive Pearson correlation ($r = 0.950$, $p < 0.001$), indicating a proportional relationship

between increasing MIF levels and clinical severity. This dose–response pattern reinforces the role of MIF as a dynamic indicator of disease intensity.

In ROC analysis, MIF showed superior diagnostic performance (AUC = 0.94) compared to conventional biomarkers such as CRP (AUC = 0.78) and albumin (AUC = 0.81). Furthermore, combining MIF with albumin enhanced predictive accuracy (AUC = 0.96), suggesting synergistic value in integrating markers of inflammation and vascular leakage.

These findings highlight MIF as a promising biomarker for early risk stratification in pediatric dengue, with potential application in diagnostic algorithms and clinical decision-making. Its strong association with both severity and shock also raises the possibility of MIF as a therapeutic target in future dengue management. Multimarker approaches, including MIF and albumin, may be particularly valuable in resource-limited settings for identifying high-risk patients early and guiding timely interventions.

Author Contributions

Conceptualization, A.I..; methodology, A.I.; software, A.I. and I.K.; validation, A.I. and I.K.; formal analysis, A.I. and I.K.; investigation, A.I. and I.K.; resources, A.I. and I.K.; data curation, A.I. and I.K.; writing—original draft preparation, A.I., I.K., and A.A.; writing—review and editing, A.I., I.R.D., and A.A.; visualization, A.I.; supervision, A.I. and I.R.D.; project administration, A.I. and I.R.D.; funding acquisition, A.I. All authors have read and agreed to the published version of the manuscript.

Funding

This work was partially supported by Universitas Brawijaya.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of SAIFUL ANWAR GENERAL HOSPITAL with Approval No: 400/196/K/3/302/2017

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available due to institutional regulations, but are available from the corresponding author on reasonable request**.**

Acknowledgments

The author thanks colleagues from the Department of Child Health, Faculty of Medicine, Brawijaya University/ Saiful Anwar General Hospital for their invaluable collaboration. Special thanks are also due to the Dean of Faculty of Medicine, Brawijaya University for partially supporting the funding of this study.

Conflicts of Interest

The authors declare no conflict of interest.

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