Exploring Predictive Factors and Developing a Model to Estimate the Timing of Plasma Leakage in Dengue Hemorrhagic Fever: A Retrospective Observational Study

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A Retrospective Observational Study

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A thesis submitted in partial fulfillment of the requirement for the degree of

Master of Medical Statistics

DECLARATION

The work reported in this thesis project was carried out by me as a partial requirement for the Master of Medical Statistics degree. It has not been submitted for any other degree in this University or any other national or international institution.

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APPROVAL OF THE DISSERTATION

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Index Number : FGS/MMstat/2024/097

I certify that this dissertation is submitted as a partial fulfillment of the Master of Science in Statistics degree of the University of Kelaniya.

Name of the Coordinator : Dr. U.P Liyaganage

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ABSTRACT

Background:

Dengue hemorrhagic fever (DHF) is a major global health challenge. The most important event that determines disease severity and clinical outcomes in DHF is plasma leakage. Although plasma leakage is the core problem in dengue pathophysiology, its precise timing is often difficult to determine in routine clinical practice, because the timing of plasma leakage is determined by subjective clinical indicators and retrospective assessments. Current diagnostic approaches focus primarily on the presence of leakage rather than its temporal dynamics. However, accurate identification of the onset of plasma leakage is important because timely and appropriate fluid management during this critical phase is essential to prevent shock, fluid overload, and associated complications. Therefore, this study aimed to identify clinical, hematological, and biochemical predictors of plasma leakage onset and to develop a predictive model to estimate the timing of leakage in patients with DHF, to support more precise and individualized fluid management strategies.

Methods:

A retrospective observational study was conducted at the National Institute of Infectious Diseases, Sri Lanka, including 185 adult patients diagnosed with DHF between June and December 2025. Demographic, clinical, hematological, and biochemical data were extracted from medical records. Plasma leakage onset was defined using clinical, laboratory, and ultrasonographic criteria. Patients were categorized into early (≤72 hours), standard (73–120 hours), and late (>120 hours) leakers. Descriptive statistics, correlation analyses, and regression modeling were performed. Independent predictors of leakage timing were identified using multiple linear regression with backward elimination. Model assumptions were verified using normality testing and transformation where necessary.

Results:

The mean time to plasma leakage was 87.3 hours (SD 31.4), with considerable inter-individual variability. Only half of the patients developed plasma leakage during the typical window of days 4–5 of illness, while the remaining patients developed either early leaking (before day 4) or delayed leaking (after day 5). There was no significant association between demographic

characteristics or comorbidities with leakage timing. In contrast, hematological parameters demonstrated strong relationships with leakage onset. Time to lowest white blood cell (WBC) count ($r = 0.779$, $p < 0.001$) and time to lowest platelet count ($r = 0.758$, $p < 0.001$) had a strong correlation with time to leakage. Faster rates of platelets ($r = -0.687$, $p < 0.001$) and WBC counts drop ($r = -0.582$, $p < 0.001$) were significantly associated with earlier leakage. Biochemical markers such as liver enzymes, creatinine, sodium, and CRP were not independently associated with leakage timing.

Multivariable regression identified time to lowest WBC, time to lowest platelet count, and lowest platelet count as independent predictors of leakage timing, explaining 67.8% of the variance (adjusted $R^2 = 0.678$). Secondary models using time gaps between hematological nadirs and leakage onset further confirmed that dynamic changes in blood counts, rather than absolute values, were the strongest determinants of timing of plasma leakage.

Conclusion:

This study demonstrates that timing of plasma leakage in dengue is primarily determined by dynamic changes in hematological parameters rather than static clinical or biochemical values. Time to lowest white blood cell count and time to lowest platelet count are strongly correlated with time to leakage. Rapid declines in platelet and white blood cell counts were also strongly associated with earlier leakage. This highlights the importance of trend-based monitoring. The proposed time-based predictive model may be helpful in early risk stratification and guide timely fluid management. Incorporating dynamic hematological trajectories into routine care could improve clinical outcomes. It is recommended to conduct future prospective studies with serial sampling to validate and refine these findings.

Keywords: Dengue hemorrhagic fever; Plasma leakage; Platelet dynamics; White blood cell dynamics; Predictive modeling;

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Finally, I would like to acknowledge all the patients whose clinical data formed the foundation of this study.

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ABBRAVATIONS

ADE - Antibody Dependent Enhancement

ALT - Alanine Aminotransferase

ANOVA - Analysis of Varience

AST - Aspartate Aminotransferase

CRP - C Reactive Protein

DF - Dengue Fever

DHF - Dengue Hemorrhagic Fever

ERC - Ethics Review Committee

GGT - Gamma-Glutamyl Transferase

IBM SPSS - International Business Machines Statistical Package for the Social Sciences

ICU - Intensive Care Unit

IHD - Ischaemic Heart Disease

NSAID - Non-Steroidal Anti-Inflammatory Drugs

PCV - Packed Cell Volume

PLT - Platelets

SD - Standard Deviation

SQRT - Square Root

USS - Ultrasound Scan

UTI - Urinary Tract Infection

WBC - White Blood Cells

WHO - World Health Organization

Chapter 1: Introduction

Dengue fever, caused by the dengue virus transmitted by Aedes mosquitoes, represents a significant global health challenge due to its prevalence and impact (Bhatt et al., 2021). The European Centre for Disease Prevention and Control (2024) states that there was a significant surge in dengue cases worldwide in 2023, characterized by a significant increase in both the number and geographical extent of outbreaks, affecting regions previously unaffected by the disease. Since there is no effective therapeutic management in dengue fever, the management of dengue patients is limited to symptomatic relief, judicious fluid replacement, and close monitoring for warning signs which may predict progression to severe dengue (Malavige et al., 2023).

The beginning of a typical case of dengue is identified by febrile phase. Subsequently, some patients may progress into a critical phase characterized by increased capillary permeability which leads to the leakage of fluid into the interstitial space. This critical phase usually occurs around day 5–7 of fever and lasts for 48–72 hours (Rodrigo et al., 2021). Although the commonest period of leaking is between 4–5 days of fever onset, leakage can start any day from 2nd to 7th day of fever (Ministry of Health – Sri Lanka, 2019). Several studies have proven this wide variation in the onset of leakage, (Ranasinghe et al., 2018; Jayarajah et al., 2020; Srikiatkhachorn et al., 2007), indicating the complexity and heterogeneity of this clinical manifestation.

The plasma leakage process in Dengue Hemorrhagic Fever (DHF) happens in two distinct phases: the ascending limb and the descending limb (National Epidemiology Unit, 2012). Fluid leakage does not occur at a constant rate throughout these phases. During the ascending limb, fluid leakage gradually increases and reaches the peak, while during the descending limb, fluid leakage gradually decreases. (Borré-Naranjo et al., 2022). When plasma leakage occurs, it results in depletion of intravascular volume, leading to compromised organ perfusion. Effective management of intravascular volume during these phases is important to reduce complications. Aligning the rate of fluid administration with the rate of fluid leakage is crucial to achieve this. (Chan & Ooi, 2015).

The volume and rate of fluid administration can be guided by the hemodynamic condition of the individual, including factors such as peripheral pulse, blood pressure, capillary refill time, and urine output, and also by laboratory parameters such as hematocrit and platelet count (Borré-Naranjo et al., 2022). However, a significant determinant that influences the trajectory of fluid administration is the time period from the onset of plasma leakage. This is decided by the correct onset timing of leaking (Wickramasinghe, 2019). If an early leaker remains unidentified, clinicians may administer excessive fluids during the descending limb, misunderstanding that the patient is still in the ascending phase, if leaking onset was judged solely by the day of fever onset. Similarly, failure to recognize late plasma leakage may lead physicians to reduce fluid administration during the ascending phase. Therefore, it is crucial to estimate the optimal timing of leakage onset for effective fluid management (Chan & Ooi, 2015; Jayaweera et al., 2021).

Primarily, two clinical criteria are used to diagnose Dengue Hemorrhagic Fever (DHF): a rise in hematocrit by more than 20% from baseline, or the detection of plasma leakage (such as pericholecystic fluid, fluid in the hepatorenal pouch, pleural effusion, or ascites) via ultrasound scanning (USS) (Wickramasinghe, 2019). Although these criteria are routinely used for diagnosing DHF, they do not determine the timing of plasma leakage onset accurately.

In clinical practice, these same criteria are often used to estimate the time of leakage onset in addition to establishing the diagnosis of DHF. For example, some clinicians use the midpoint of the hematocrit rise as an approximate time for the leakage onset. However, since hematocrit is also influenced by factors such as fever, dehydration, and internal or external bleeding, relying only on hematocrit changes may not be sufficient to accurately identify or assess the severity of plasma leakage, (Srikiatkhachorn, 2009). Additionally, Fernando et al. (2020) showed that in many cases where plasma leakage was identified via USS, patients did not exhibit a hematocrit rise exceeding 20%.

Some clinicians also use the fever pattern to estimate the onset of leakage, since plasma leakage often begins as the fever subsides (Srikiatkhachorn, 2009). However, this approach is unreliable, as some patients may enter the critical phase while still having fever (Ministry of Health – Sri Lanka, 2019; National Epidemiology Unit, 2012).

In most cases, once a diagnosis of leakage is made, clinicians use ultrasound findings to retrospectively estimate the timing of onset. For example, gallbladder wall edema with pericholecystic fluid is thought to indicate early leakage, while fluid in the hepatorenal recess or pleural cavity suggests at least six hours of leakage. Bilateral pleural effusions and ascites are typically considered as markers of the late critical phase (Kodikara & Gamage, 2018). However, these interpretations are subjective and often made by non-radiology-trained personnel, leading to inter-observer variability and potential misinterpretation (Srikiatkhachorn et al., 2007; Ranasinghe et al., 2018). Furthermore, early ultrasound findings may persist into the recovery phase or reappear later, which can complicate efforts to determine the true onset of leakage (Yousaf et al., 2011).

These real-world complexities emphasize the limitations of current clinical methods and highlight the need to invent more objective, standardized, and reliable tools to accurately estimate the onset of plasma leakage in DHF. It is evident that currently there is no single criterion or biochemical marker to determine this time point with certainty, and current methods are largely subjective.

Therefore, this study aims to identify and analyze demographic, clinical, biochemical, and hematological factors that are correlated with the timing of plasma leakage onset in DHF patients. It further aims to develop a predictive model using these factors to estimate leakage onset timing. This model could assist clinicians to align fluid administration with actual leakage dynamics, and to potentially improve outcomes by reducing both under- and over-resuscitation. However, to date, no study has attempted to construct a model or scoring system specifically designed to estimate the timing of plasma leakage onset in DHF.

1.2 Objectives:

1.2.1 General Objectives

To Explore Predictive Factors and Develop a Model to Estimate the Timing of Plasma Leakage in Dengue Hemorrhagic Fever

1.2.2 Specific Objectives:

1. To explore and identify which demographic, clinical, Hematological and biochemical variables significantly influence the timing of plasma leakage in DHF patients
2. To develop a model predicting **leakage onset time** using admission demographic, clinical and biochemical parameters.
3. To determine specific cutoff values or ranges of quantitative biochemical markers that best predict the **onset of leakage**
4. To identify the **optimal blood sample collection time** to maximize the predictive accuracy of the leakage onset model.

Chapter 2: Literature Review

Dengue remains a critical health problem in endemic regions, with complications such as plasma leakage, hemorrhage, and shock that contribute significantly to its morbidity and mortality. Many studies have focused on identifying predictors of severe disease, especially during the febrile phase, in order to facilitate early intervention and risk stratification. These investigations mainly compared non-severe and severe dengue cases, aiming to detect early warning signs or biochemical changes that may occur prior to clinical deterioration (Lee et al., 2016; Yang et al., 2023; Medagama et al., 2020).

Demographic factors such as younger age and female gender have shown varying associations with disease severity. However, their predictive power may differ across populations (Lam et al., 2015; Yang et al., 2023). Clinical signs including persistent vomiting, abdominal pain, mucosal bleeding, and hepatomegaly have been repeatedly associated with severe outcomes (Haridas et al., 2023; Medagama et al., 2020). Several scoring systems have been developed to use these features in actionable risk assessments. For instance, Djossou et al. (2016) and Gupta et al. (2020) proposed simple clinical scores incorporating vital signs and biochemical parameters, while Haridas et al. (2023) validated a severity prediction model using hospital-based data. Machine learning approaches have also shown to be successful; Madewell et al. (2025) demonstrated high predictive accuracy for severe dengue using integrated data models.

With regard to laboratory predictors, many biochemical and hematological markers have been associated with plasma leakage or progression to severe dengue. Rising hematocrit, thrombocytopenia, elevated liver enzymes (AST, ALT), and prolonged capillary refill time are among the classic indicators of vascular permeability and organ involvement (Fernando et al., 2016; Priyankara et al., 2024). Elevated venous lactate has emerged as a sensitive early marker of tissue hypoperfusion (Priyankara et al., 2024). New biomarkers such as angiopoietin-2, IL-10, and syndecan-1 are useful in early plasma leakage detection (Moallemi et al., 2025). Additionally, high serum ferritin and APRI scores have shown strong associations with disease

severity (Shukla et al., 2023; Jayachandran et al., 2024). Imaging-based assessments, particularly gallbladder wall thickness, also demonstrate high diagnostic value for predicting leakage (Shahsavand Davoudi et al., 2025).

Despite the huge number of research conducted to predict occurrence of severe dengue and plasma leakage, no study to date has specifically aimed to the timing of leakage onset once leakage is confirmed. Yet, defining the onset time of leakage is critically important—it guides fluid management strategies, influences decisions around escalation of care, and directly impacts patient outcomes. Current models focus on severity prediction during the febrile phase or classify patients into severe vs. non-severe categories, often overlooking the temporal dynamics of fluid extravasation (Premaratna et al., 2013; Lam et al., 2015).

Importantly, the existing literature collectively suggests that a number of physiological and biochemical changes occur around the period of leakage onset. These changes—such as shifts in hematocrit, lactate rise, transaminase elevations, and inflammatory biomarker surges—may therefore serve not only as markers of severity but also as potential indicators of when plasma leakage begins. However, the temporal association of these markers with leakage, has not been systematically evaluated.

This study aims to fill this gap by characterizing and modeling the precise timing of plasma leakage onset in patients already diagnosed with dengue hemorrhagic fever (DHF). Rather than simply identifying who will leak, this approach seeks to define when leakage starts, enabling a dynamic, time-sensitive understanding of dengue progression. By reinterpreting the well-established clinical and biochemical markers discussed in the literature, this study expects to generate new insights into the temporal prediction of leakage, eventuallysupporting more precise and individualized fluid management protocols.

Chapter 3: Methodology

3.1 Study Design

This study was conducted as a **retrospective observational analytical study** aimed at identifying predictors of onset of plasma leakage and developing a model to estimate the timing of plasma leakage in patients with Dengue Hemorrhagic Fever (DHF).

3.2 Study Setting and Duration

The study was conducted at the **National Institute of Infectious Diseases, Angoda, Sri Lanka**, a national tertiary care center for dengue management. The study included patients admitted with DHF between **15 June 2025 and 15 December 2025**, representing a six-month study period.

Data extraction was carried out between **October and December 2025** using hospital medical records and laboratory databases.

3.3 Study Population

3.3.1 Inclusion Criteria

Patients were included if they:

- Were aged **18 years or older**
- Had a diagnosis of **Dengue Hemorrhagic Fever (DHF)** based on the **1997 WHO classification**

3.3.2 Exclusion Criteria

1. DHF Patients with co-infections such as malaria, leptospirosis or secondary bacterial infection Eg: (UTI, Pneumonia).
2. Patients with uncontrolled chronic illnesses or advanced-stage chronic illnesses (e.g., chronic kidney disease stage IV, end-stage liver disease, chronic heart failure) that could confound the study results.
3. Patients with hematological conditions that affect hematological parameters (e.g., blood cancers, idiopathic thrombocytopenic purpura, polycythemia vera, myelodysplastic syndromes, myelofibrosis).
4. Pregnant women.
5. Patients who underwent platelet transfusions during the course of their illness.
6. ICU Care Requirements: Patients requiring intensive care support, including inotropes, blood products other than red cell packs, or ventilatory support.

3.4 Sample Size and Sampling Method

As this was an exploratory predictive modeling study, sample size estimation followed the rule-of-thumb for multiple linear regression: $\mathbf{N \geq 50 + 8m}$, where m represents the number of predictors. Sample size adequacy was guided by regression modeling principles (minimum of 10–15 observations per predictor variable), allowing sufficient power for multivariable analysis

Assuming up to 15 predictor variables, a minimum sample size of 170 patients was required. To account for missing data and ensure sufficient statistical power, a final sample size of 185 patients was included in the final analysis. Consecutive sampling was used, whereby all eligible patients admitted during the study period were included.

3.5 Data Collection

Data were collected retrospectively from bed head tickets. Collection was performed by the primary investigator with supervision from senior clinicians. Variables collected included:

Demographic and Clinical Data

- Age
- Sex
- Body weight
- Comorbidities (diabetes mellitus, hypertension, ischemic heart disease)
- Prior dengue infection
- NSAID use prior to admission

Clinical Features

- Date of fever onset
- Presenting symptoms (headache, myalgia, arthralgia, vomiting, abdominal pain, bleeding manifestations)
- Vital signs at admission (blood pressure, heart rate)
- Presence of abdominal tenderness

Laboratory Parameters

- Full blood count (including serial platelet and WBC measurements)
- Liver function tests (AST, ALT, GGT)
- Renal function tests (serum creatinine)
- Serum electrolytes (sodium, potassium)
- C-reactive protein (CRP)

Definition of Key Time Points

Fever Onset

Given the subjective nature of symptom recall, fever onset was standardized as **12:00 noon on the recorded day of fever onset** for all patients to ensure consistency in time-based calculations.

Plasma Leakage Onset

Plasma leakage onset was defined using clinical documentation supported by laboratory and imaging findings, including:

- Rising hematocrit (≥20% from baseline),
- Ultrasonographic evidence of pleural effusion or ascites,
- Clinical signs of plasma leakage such as narrowing pulse pressure or hypotension.

3.6 **Statistical Analysis**

Categorization of Plasma Leakage Timing

Patients were classified into three groups based on the time to plasma leakage:

- **Early leakers:** ≤72 hours
- **Standard leakers:** 73–120 hours
- **Late leakers:** >120 hours

All analyses were performed using **IBM SPSS Statistics version 21**.

Descriptive Analysis

Continuous variables were summarized using means and standard deviations or medians and interquartile ranges, as appropriate. Categorical variables were summarized as frequencies and percentages.

Assessment of Normality and Data Transformation

Normality of dependent continuous variables was assessed using the Kolmogorov–Smirnov test, Shapiro–Wilk test, and visual inspection of Q–Q plots. Time to plasma leakage demonstrated right-skewed distribution and was therefore transformed using a square root transformation to approximate normality. This transformed variable was subsequently used in correlation and regression analyses to satisfy parametric assumptions.

Comparative Analyses

- Categorical variables were compared using the **Chi-square test**.
- Continuous variables were compared using **one-way ANOVA** when normally distributed.

Correlation Analysis

Pearson's correlation coefficient (r) was used to assess relationships between continuous variables

Rate of Hematological Decline

Rates were calculated as:

- **Rate of platelet decline = (250 − lowest platelet count) / time to lowest platelet**
- **Rate of WBC decline = (11 − lowest WBC count) / time to lowest WBC**

Regression Modeling

Dependent Variable (Y): Time of leakage onset (hours from admission)

Independent Variables (X): Demographic data, Hematological and Biochemical markers.

Packed cell volume (PCV) and ultrasound (USS) findings were not included as predictor variables in this analysis, as they constitute part of the current criteria used to approximate the timing of plasma leakage. Instead, these parameters were applied as reference indicators for determining timing of leakage onset, while other hematological, and biochemical factors were assessed as potential predictors.

A multivariable linear regression model was developed to identify independent predictors of plasma leakage timing. Variables entered into the final model included following which shows statistical significant correlation.

- Time to lowest platelet count
- Time to lowest WBC count
- Lowest platelet count
- Lowest WBC count
- CRP

Backward elimination was used to derive the final model. Model adequacy was assessed using adjusted R^2, and diagnostic plots were examined to verify assumptions of linearity, homoscedasticity, and normality of residuals.

Adjustment for Subjectivity in Fever Onset

Because the reported onset of fever was subject to recall bias and was standardized to 12:00 noon for all patients, an additional analytical approach was employed to minimize this potential source of error. To reduce dependence on the subjective estimation of fever onset, an alternative time-based variable was constructed:

Time gap between lowest platelet and leakage = Time to lowest platelet − Time to leakage

Time gap between lowest platelet and leakage = Time to lowest platelet − Time to leakage

This derived variable represents the interval between hematological nadir and the onset of plasma leakage. This allows timing independent of the reported onset of fever. Subsequent analyses explored which clinical and laboratory parameters were associated with this interval, allowing assessment of disease dynamics without reliance on patient-reported symptom onset**.** This approach strengthened the analysis by reducing measurement bias and improving the temporal precision of associations between hematological changes and plasma leakage.

Statistical Significance

All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the **Ethics Review Committee** (ERC Reference No: **P/03/06/2025**). Administrative clearance was obtained from the **Director of the National Institute of Infectious Diseases, Angoda**. As this was a retrospective study using anonymous patient data, individual informed consent was waived.

Chapter 4: Results

4.1 Study Population and Baseline Characteristics

A total of 185 patients were included in the final analysis. The mean and median age of the study population were both 39 years, with ages ranging from 18 to 95 years. The gender distribution was as follows: Male: 90 (48.6%), Female 95 (51.4%)

Body weight was documented in 129 patients (69.7%), while it was missing in 56 patients (30.3%). Among those with recorded values, the mean body weight was 63.5 kg, with a median of 63 kg.

		Weight	Age	Admission Fever day
N	Valid	129	185	185
	Missing	56	0	0
Mean		63.53	39.09	3.51
Median		63.00	39.00	3.00
Mode		65	47	3
Std. Deviation		13.236	14.834	1.399
Minimum		30	18	1
Maximum		111	95	8

Table 1: Distribution of Weight, Age and Fever day on admission

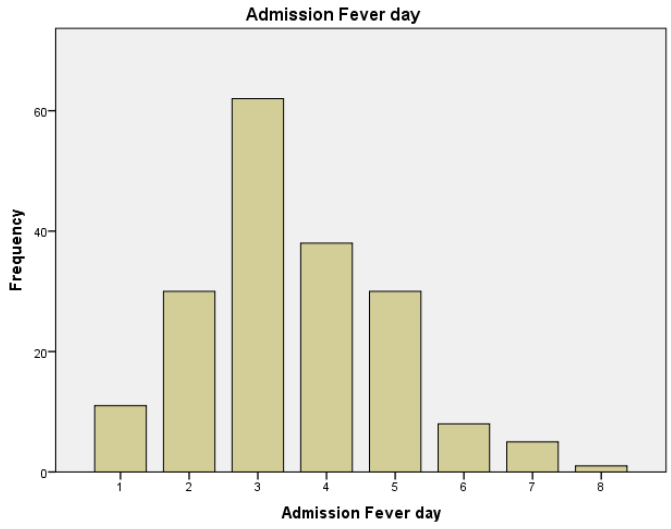

Figure 1: Distribution of dengue days at admission

4.2. Baseline comorbidities

Diabetes mellitus was present in 160 patients (86.5%) and absent in 25 patients (13.5%). Hypertension was present in 26 patients (14.1%) and absent in 159 patients (85.9%). Ischaemic heart disease (IHD) was present in 10 patients (5.4%), while 175 patients (94.6%) had no history of IHD.

Comorbidity	**Present**	**Absent**
Diabetes Mellitus	25 (13.5%)	160 (86.5%)
Hypertension	26 (14.1%)	159 (85.9%)
Ischaemic Heart Disease	10 (5.4%)	175 (94.6%)

Table 2: Baseline comorbidities

A prior history of dengue fever was reported in 14 patients (7.6%), whereas 171 patients (92.4%) had no previous dengue infection. Use of non-steroidal anti-inflammatory drugs (NSAIDs) prior to admission was reported in 10 patients (5.4%), while 173 patients (94.6%) denied prior NSAID use.

4.2 Symptomatology of the patients

Here summarize the symptomatology of the patients

Symptom	**Present**	**Absent**
Headache	156 (84.3%)	29 (15.7%)
Nausea	132 (71.4%)	53 (28.6%)
Vomiting	79 (42.7%)	106 (57.3%)
Abdominal pain	45 (24.3%)	140 (75.7%)
Bleeding	5 (2.7%)	180 (97.3%)
Arthralgia	144 (77.8%)	41 (22.2%)
Myalgia	139 (75.1%)	46 (24.9%)

Table 3: Symptomatology of the patient cohort

4.3 Admission Examination Characteristics

Statistics

		Ad_SBP	Ad_DP	Ad_HR
N	Valid	185	185	185
	Missing	0	0	0
Mean		114.11	73.30	86.71
Median		110.00	70.00	85.00
Mode		120	80	80
Std. Deviation		17.066	11.858	14.661
Skewness		.138	.056	.423
Std. Error of Skewness		.179	.179	.179
Kurtosis		.302	-.037	.629
Std. Error of Kurtosis		.355	.355	.355
Minimum		70	40	55
Maximum		170	100	144

Table 4: Admission Examination findings of the patient cohort (Systolic blood pressure, Diastolic blood pressure and Heart Rate)

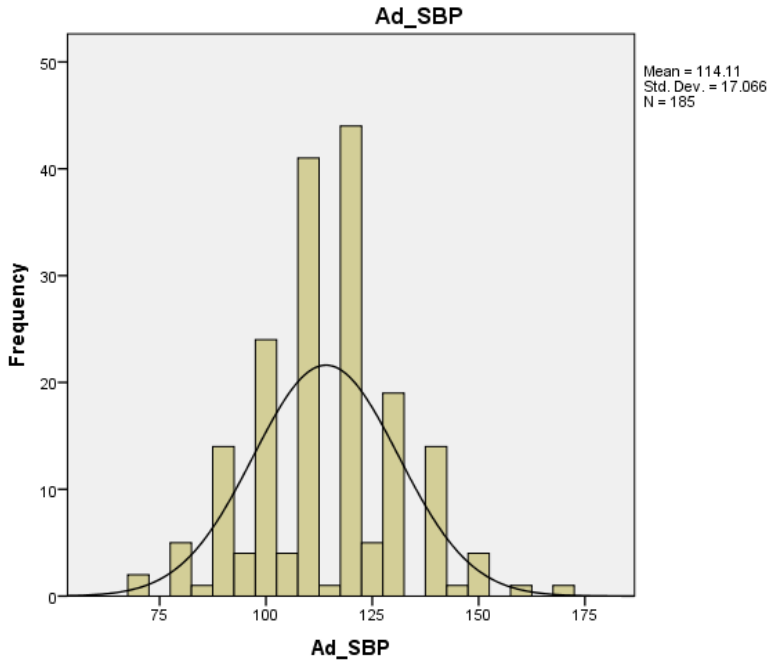

Figure 2: Distribution of Systolic Blood Pressure at admission

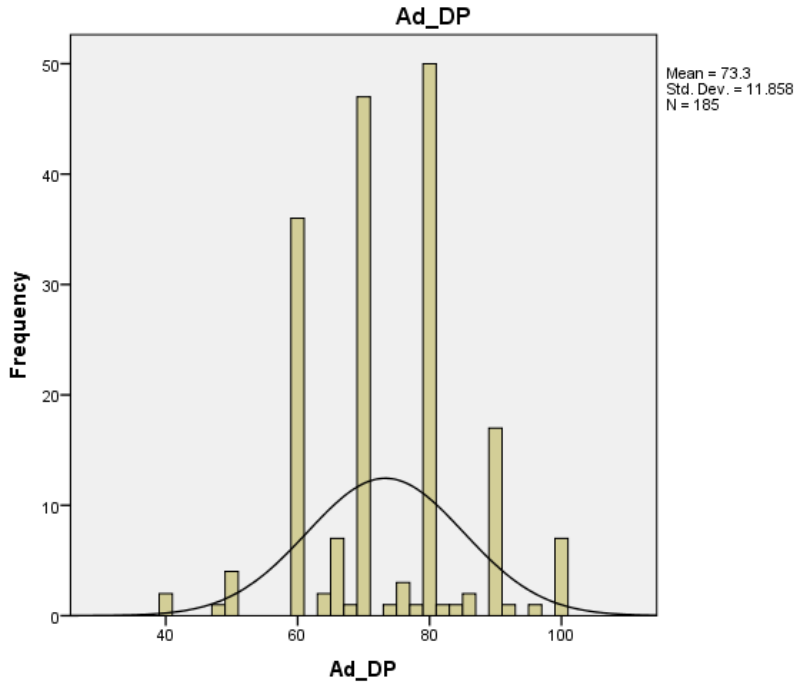

Figure 3: Distribution of Diastolic Blood Pressure at admission

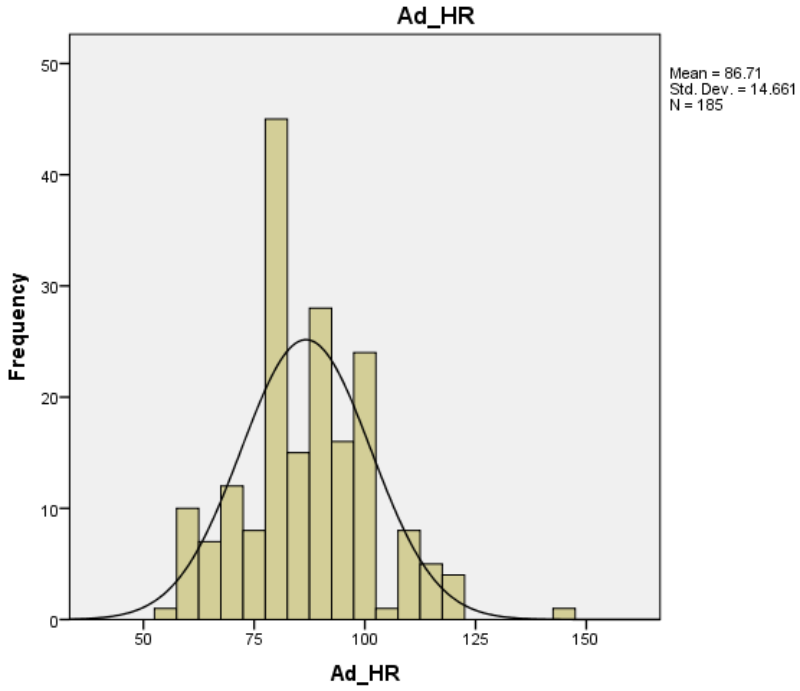

Figure 4: Distribution of Heart Rate at admission

Abdominal tenderness did not develop in 152 patients (82.2%), where as it developed in 33 patients (17.8%).

4.4 Results of Timing of Leaking Onset

The timing of plasma leakage was based on the clinically documented onset as determined by the treating medical team. Among the 185 patients included in the study, data on leakage onset were unavailable for two patients because their leaking onset time was not documented in bed head tickets and critical phase charts were missing.

The mean time to plasma leakage was 87.29 hours, with a median of 82 hours and a mode of 108 hours. The minimum observed time to leakage was 24 hours, while the maximum was 172 hours. The standard deviation was 31.36 hours, indicating substantial variability in the timing of leakage among patients. Assessment of data distribution showed that the variable was not normally distributed, despite a skewness value of 0.552 and kurtosis of −0.036.

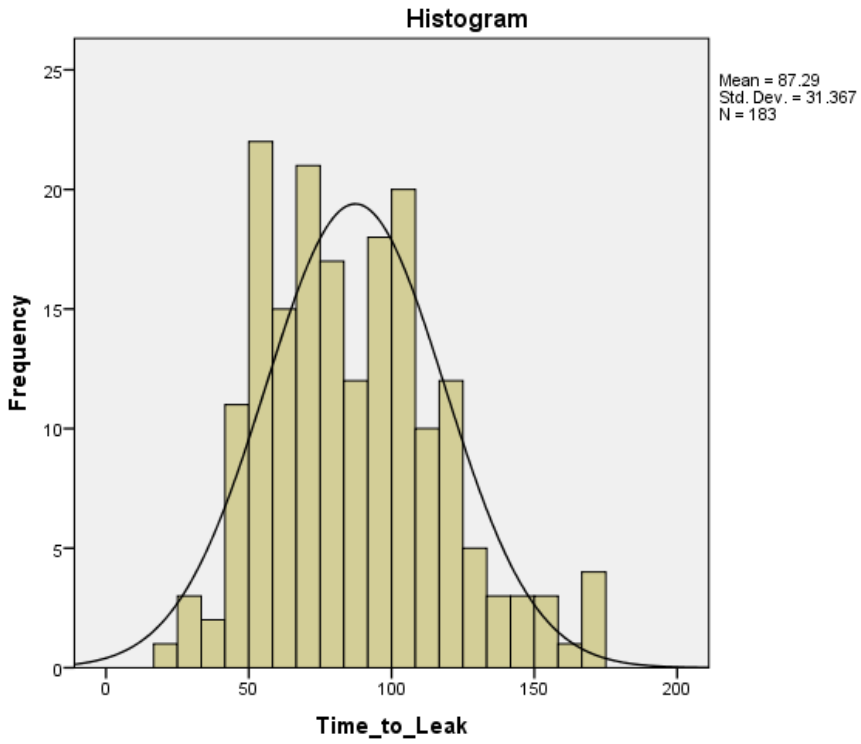

Figure 5: Distribution of onset of leaking

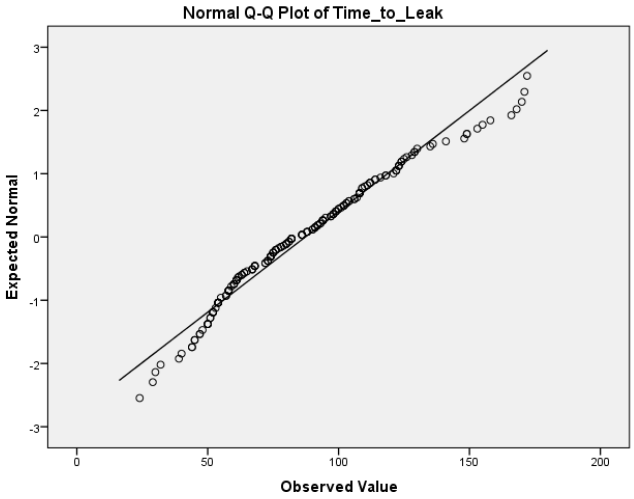

Figure 6: Distribution of onset of leaking in Q-Q Plot

The Q-Q plot demonstrate that the leaking time is not normally distributed Both Kolmogorov-Smirnov and Shapiro-Wilk is statistically significant, p = 0.03 and p = 0.001 respectively.

As data is slight right deviated to make them more normal distribution Squre root of the leaking time is considered.

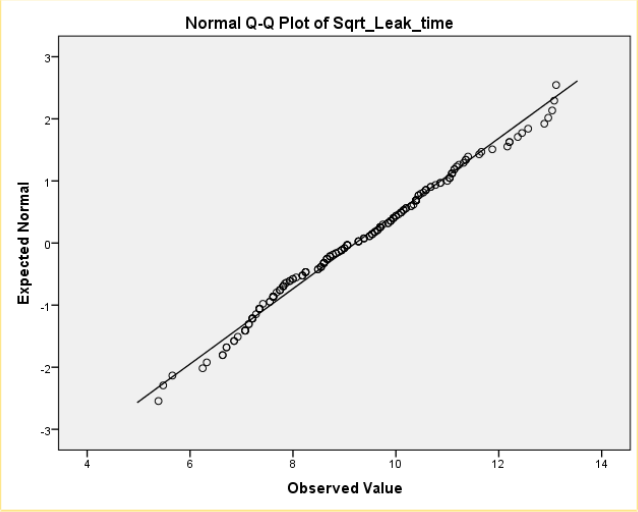

Figure 7: Distribution of Square root of onset of leaking in Q-Q Plot

The “square root” transformed leakage time demonstrated a normal distribution, with both the Kolmogorov–Smirnov and Shapiro Wilk tests showing no statistically significant deviation from normality (p = 0.200 and p = 0.099, respectively).

As previously described, patients were categorized into three groups based on the timing of plasma leakage: early leakers (≤72 hours), standard leakers (73–120 hours), and late leakers (>120 hours). Based on this classification, 63 patients (34.1%) were identified as early leakers, 91 patients (49.2%) as standard leakers, and 29 patients (15.7%) as late leakers.

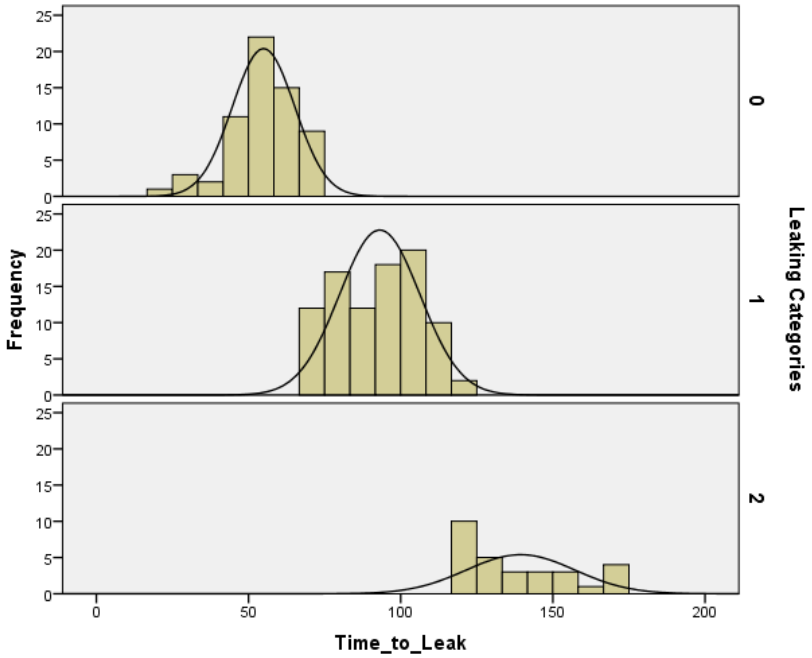

Figure 8: Distribution of onset of leaking time in 3 categories (0- early leakers, 1-standard leakers, 2-late leakers)

The mean time to plasma leakage differed clearly across the predefined leakage categories. Among early leakers, the mean time to leakage was 54.93 hours (SD = 10.27), with observed values ranging from 24 to 72 hours, reflecting relatively early transition to the critical phase. In the standard leaker group, the mean leakage time is 93.10 hours (SD = 13.27), with a range of 73 to 118 hours, representing the majority of patients and aligning with the typical timeframe described in classical DHF. Patients classified as late leakers demonstrated a substantially delayed onset of leakage, with a mean time of 139.38 hours (SD = 17.87) and a range of 121 to 172 hours. Overall, the pooled cohort had a mean leakage time of 87.29 hours (SD = 31.37), reflecting wide inter-individual variability in the timing of plasma leakage.

Time_to_Leak

Leaking Categories	Mean	N	Std. Deviation	Minimum	Maximum	Grouped Median
0	54.93	63	10.265	24	72	56.33
1	93.10	91	13.274	73	118	93.43
2	139.38	29	17.867	121	172	130.00
Total	87.29	183	31.367	24	172	83.78

Table 5: Summery of leaking onset time in each leaking categories (0- early leakers, 1-standard leakers, 2-late leakers)

4.5 Hematology Parameters

Baseline hematological parameters at their lowest recorded values demonstrated substantial variability across the study population. The mean lowest total white blood cell (WBC) count was 3.98 $\times 10^9$/L (SD 2.02), with values ranging from 1.05 to 11.90 $\times 10^9$/L. Neutrophil counts showed a mean nadir of 2.86 $\times 10^9$/L (SD 1.36), while lymphocyte counts reached a mean lowest value of 1.44 $\times 10^9$/L (SD 0.95). The lowest absolute neutrophil percentage had a mean of 48.99%, whereas the lowest lymphocyte percentage averaged 34.30%. Among other leukocyte subsets, the mean lowest monocyte count was 0.50 $\times 10^9$/L, with a corresponding percentage of 13.46%, while eosinophils demonstrated marked suppression, with a mean lowest absolute count of 0.05 $\times 10^9$/L and percentage of 1.33%. Basophil counts were also low, with a mean nadir of 0.08 $\times 10^9$/L and a percentage of 2.03%. Considerable inter-individual variability was observed across all hematological parameters, as reflected by wide ranges and standard deviations.

Statistics

		Lowest_WBC	Lowest_Neu_ab	Lowest_Neu_Per	Lowest_Lym_ab	Lowest_Lym_Per	Lowest_MXD_ab	Lowest_MXD_Per	Lowest_Eosin_ab	Lowest_Eosi_Per	Lowest_Mono_ab	Lowest_Mono_Per	Lowest_Baso_Per	Lowest_Baso_ab
N	Valid	184	169	166	166	166	107	107	58	58	58	58	58	58
	Missing	1	16	19	19	19	78	78	127	127	127	127	127	127
Mean		3.9832	2.8554	48.9922	1.4360	36.0301	.5654	14.2720	.05	1.33	.50	13.46	2.03	.08
Median		3.4100	1.6400	49.5000	1.2000	34.3000	.4000	13.4000	.03	.85	.36	10.10	1.65	.07
Mode		3.20	1.30[a]	46.40	.70[a]	45.30	.30	10.20	0	0	0	16	1	0
Std. Deviation		2.01863	13.58725	13.90582	.94807	11.75718	.45828	5.55864	.056	1.353	.405	18.435	1.873	.074
Minimum		1.05	.00	2.00	.30	8.50	.10	5.10	0	0	0	3	0	0
Maximum		11.90	178.00	86.40	5.20	64.80	2.00	32.90	0	5	2	147	11	0

a. Multiple modes exist. The smallest value is shown

Table 6: distribution of the lowest recorded white WBC counts and their respective subcategories

The lowest recorded platelet count among the study population demonstrated marked variability. The mean lowest platelet count was 26.45 $\times 10^9$/L (SD = 17.37), with a median value of 22.00

$\times 10^9$/L. The minimum observed platelet count was 3 $\times 10^9$/L, while the maximum reached 105 $\times 10^9$/L, reflecting a wide range in the severity of thrombocytopenia among patients.

As platelet and white blood cell counts demonstrated a U-shaped trajectory over the course of illness, a quadratic model was applied to estimate platelet levels at the time of plasma leakage. The predicted platelet count at the onset of leakage was calculated for each patient using the equation:

Predicted platelet = β_0 + β_1(Time to leakage) + β_2(Time to leakage^2).

Using this approach, the mean predicted platelet count at the time of plasma leakage was **50.15 $\times 10^9$/L** (SD = 15.02). The minimum predicted platelet count at leakage was **13.11 $\times 10^9$/L**, while the maximum was **95.78 $\times 10^9$/L**. These findings indicate substantial inter-individual variability in platelet levels at the onset of plasma leakage, reinforcing that a fixed platelet threshold may not reliably predict leakage onset. Instead, dynamic platelet trends appear to be more informative than absolute platelet values at a single time point.

Predicted platelet counts at the onset of plasma leakage varied across the three predefined leaking categories. Among early leakers (≤72 hours), the mean predicted platelet count at leakage was **46.6 $\times 10^9$/L** (SD = 12.8), with values ranging from **13.1 to 77.1 $\times 10^9$/L**. In the standard leaker group (73–120 hours), the mean platelet count at leakage was slightly higher at **51.0 $\times 10^9$/L** (SD = 15.5), with a range of **19.0 to 95.8 $\times 10^9$/L**. Late leakers (>120 hours) demonstrated the highest mean platelet count at leakage, **55.1 $\times 10^9$/L** (SD = 16.5), with values ranging from **31.3 to 89.6 $\times 10^9$/L**.

This progressive increase in platelet levels from early to late leakers suggests that patients who develop plasma leakage earlier in the disease course tend to do so at lower platelet counts. In contrast, those with delayed leakage exhibit relatively preserved platelet levels at the time of leakage. These findings support the concept that the timing of platelet decline, rather than an absolute platelet threshold, may be a more critical determinant of plasma leakage onset.

Entering_plt

Leaking Categories	Mean	N	Std. Deviation	Minimum	Maximum	Grouped Median
0	46.6009	63	12.81248	13.11	77.14	48.5550
1	51.0466	91	15.52095	19.00	95.78	48.7860
2	55.0744	29	16.50781	31.25	89.60	56.7059
Total	50.1544	183	15.01996	13.11	95.78	49.2727

Table 7: Platelet count at onset of leaking in each categories of leakers (0- early leakers, 1-standard leakers, 2-late leakers)

A one-way analysis of variance (ANOVA) was performed to compare the mean platelet count at the onset of plasma leakage across the three leakage categories (early, standard, and late leakers). The analysis demonstrated a statistically significant difference in mean platelet levels between the groups ($F = 3.578$, $p = 0.030$). This indicates that platelet counts at the time of leakage varied significantly according to the timing of leakage onset. Patients who developed plasma leakage earlier tended to have lower platelet counts compared to those who leaked later, suggesting that earlier leakage is associated with more profound thrombocytopenia.

4.6 Biochemistry

Biochemical analysis demonstrated significant variability across inflammatory and hepatic markers during the course of illness. Transaminase levels were notably elevated, with mean ALT and AST values of 146.9 U/L and 98.2 U/L respectively, indicating frequent hepatic involvement in dengue infection. Although bilirubin and albumin values were available only in a limited number of patients, they largely remained within near-normal ranges, suggesting preserved hepatic synthetic function in most cases. Serum sodium levels were mildly reduced on average, consistent with the known tendency toward hyponatremia in dengue. Potassium levels remained largely within normal physiological limits. C-reactive protein (CRP) levels were elevated in many patients, reflecting an inflammatory response, though with wide inter-individual variation. Overall, these findings highlight that biochemical abnormalities in dengue are heterogeneous, with liver enzyme elevation and inflammatory markers being the most prominent features, while severe derangements in renal or electrolyte parameters were less frequent in this cohort.

Statistics

		ALT	AST	Bilirubin	GGT	Albumin	Scr	Na	K	CRP
N	Valid	185	185	2	0	2	185	178	176	183
	Missing	0	0	183	185	183	0	7	9	2
Mean		146.9838	98.2270	1.1250		35.7500	84.2192	137.2022	4.2084	25.5748
Median		102.0000	66.0000	1.1250		35.7500	82.5300	137.0000	3.8000	17.1400
Mode		72.00[a]	23.00[a]	.54[a]		29.90[a]	92.59[a]	137.00[a]	3.90	2.57[a]
Std. Deviation		138.62663	93.09446	.82731		8.27315	17.70133	4.29342	4.93874	27.66672
Minimum		14.00	13.00	.54		29.90	47.54	118.00	2.50	1.40
Maximum		959.00	595.00	1.71		41.60	133.49	146.00	69.00	160.16

a. Multiple modes exist. The smallest value is shown

Table 8: Biochemistry of the patient cohort

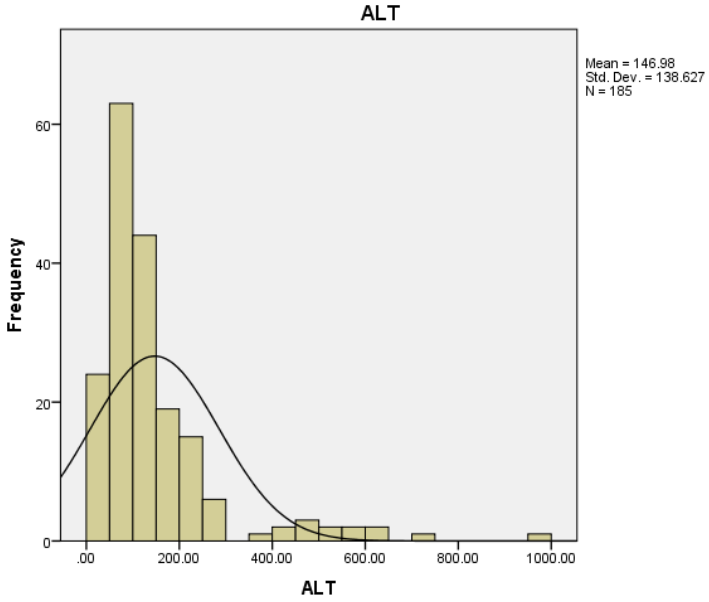

Figure 9: ALT change in the dengue cohort

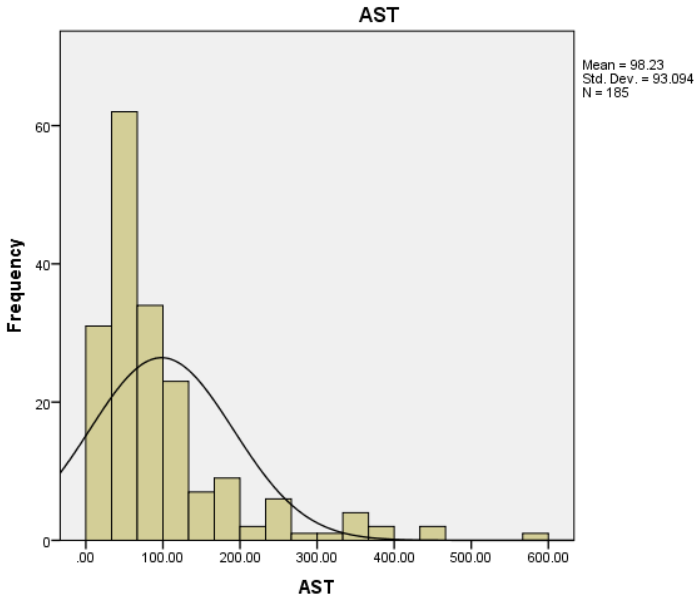

Figure 10: AST change in the dengue cohort

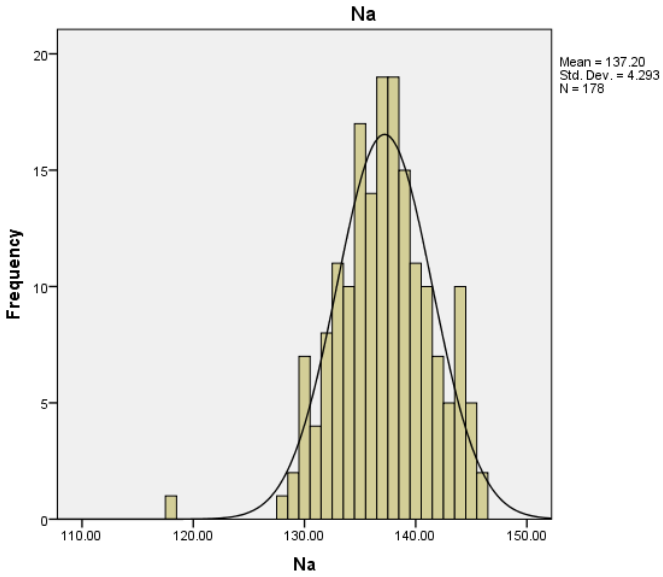

Figure 11: Na change in dengue cohort

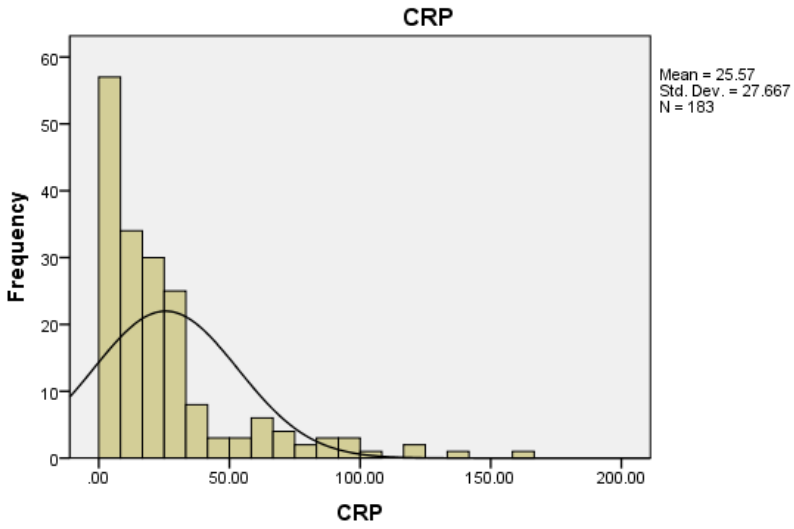

Figure 12: CRP change in dengue cohort

4.7 Associations with Onset of leaking time

Associations between demographic variables and leakage categories were assessed using Pearson's chi-square test, with a significance level set at 95%. No statistically significant associations were observed between leakage category and gender ($p = 0.629$), prior history of dengue infection ($p = 0.638$), or prior non-steroidal anti-inflammatory drug (NSAID) use ($p = 0.271$). These findings suggest that demographic characteristics and prior dengue exposure did not significantly influence the timing of plasma leakage in this cohort.

The association between presenting symptoms and categories of plasma leakage was evaluated using Pearson's chi-square test, with a significance threshold of 95%. No statistically significant associations were identified between leakage category and any of the reported symptoms. Specifically, headache ($p = 0.372$), nausea ($p = 0.377$), vomiting ($p = 0.355$), abdominal pain ($p = 0.464$), bleeding manifestations ($p = 0.788$), arthralgia ($p = 0.464$), and myalgia ($p = 0.404$) were not significantly associated with the timing of plasma leakage. These findings indicate that common clinical symptoms at presentation were poor predictors of early or late plasma leakage in this cohort.

Parameter	P value
Gender	P = 0.629
Prior DF	P = 0.638
NSAID Use	P = 0.271
Headache	P = 0.372
Nausea	P = 0.377
Vomitting	P = 0.355
Abdominal pain	P= 0.464
Bleeding	P= 0.788
Arthralgia	P= 0.464
Myalgia	P = 0.404

Table 9: P value of observed parameters

Clinical examination findings were analyzed in relation to the timing of plasma leakage. No significant association was observed between abdominal tenderness and leakage category ($p = 0.834$). Similarly, neither systolic nor diastolic blood pressure at admission showed a significant association with the timing of leakage ($p = 0.350$ and $p = 0.147$, respectively).

In contrast, admission heart rate demonstrated a statistically significant association with leakage timing ($p = 0.03$). Pearson correlation analysis revealed a weak negative correlation ($r = -0.108$). This indicates that higher heart rates at presentation were associated with a slightly earlier onset of plasma leakage. However, the strength of this association was weak, suggesting that, it is not a strong independent predictor of leakage timing.

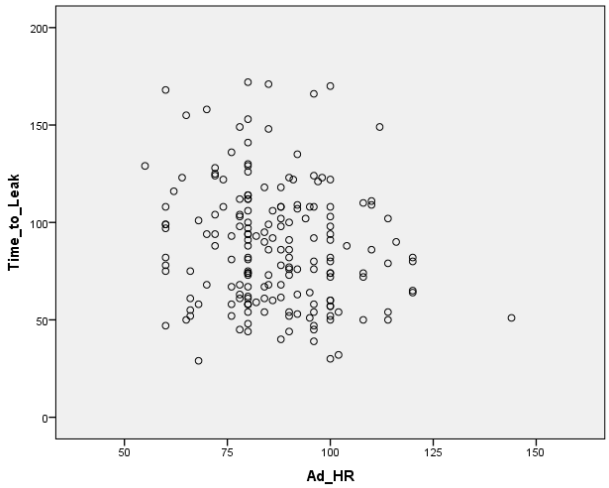

Figure 13: Scatter Plot of Admission heart rate and Time to leak

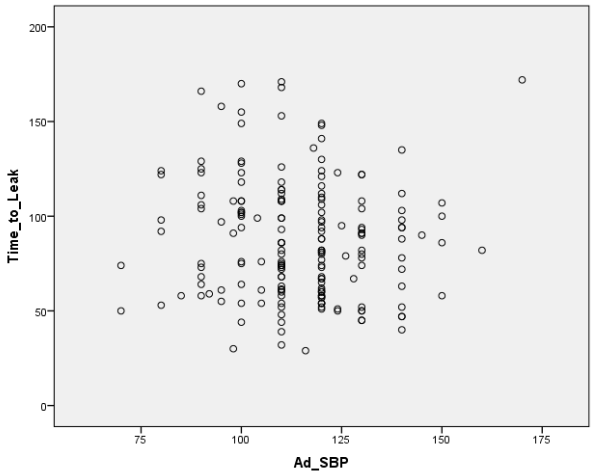

Figure 14: Scatter Plot of Admission Systolic blood pressure and Time to leak

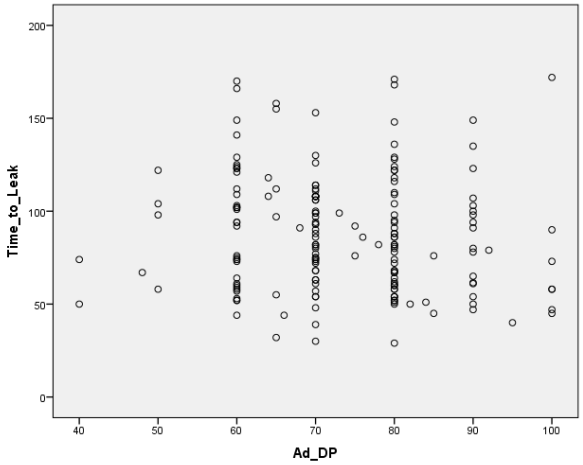

Figure 15: Scatter Plot of Admission Diastolic blood pressure and Time to leak

A one-way analysis of variance (ANOVA) was performed to compare key biochemical parameters across the three leakage categories (early, standard, and late leakers). No statistically significant differences were observed in liver enzyme levels, including alanine aminotransferase (ALT) ($F = 0.031$, $p = 0.969$) and aspartate aminotransferase (AST) ($F = 0.021$, $p = 0.980$), indicating comparable hepatic involvement across the groups. Similarly, inflammatory and metabolic markers showed no significant variation between groups, with C-reactive protein (CRP) demonstrating no significant difference ($F = 2.134$, $p = 0.121$). Serum sodium levels also did not differ significantly among the groups ($F = 0.446$, $p = 0.641$). Serum creatinine level also did not reach statistical significance ($F = 2.632$, $p = 0.075$). Overall, these findings suggest that biochemical parameters, including liver enzymes, inflammatory markers, electrolytes, and renal function indices, were not significantly associated with the timing of plasma leakage in this cohort.

Scatter plot analysis demonstrated no meaningful association between time to plasma leakage and lowest neutrophil count, lowest lymphocyte count, or their respective percentages. The distribution of data points showed no clear linear or nonlinear pattern.

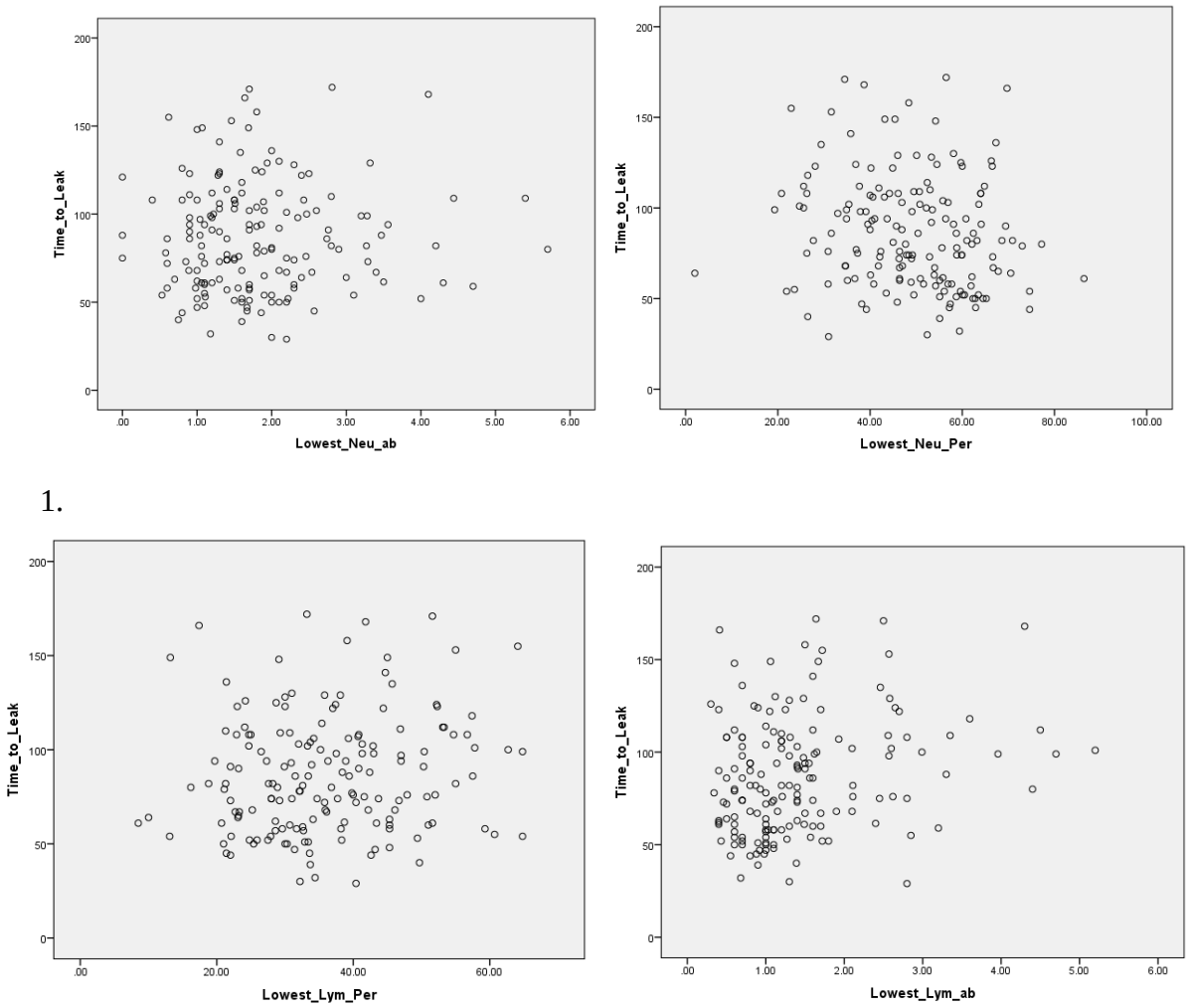

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Figure 16: Scatter Plots of Lowest Neutrophil and Lymphocytes to Time to leak

Correlation analysis demonstrated a statistically significant association between time to plasma leakage and both the lowest platelet count and the lowest white blood cell (WBC) count. Time to leakage showed a weak but significant positive correlation with the lowest platelet count ($r = 0.196$, $p = 0.008$). This indicates that patients with lower platelet nadirs tended to develop plasma leakage earlier. Similarly, a significant positive correlation was observed between time to leakage and the lowest WBC count ($r = 0.195$, $p = 0.008$). Similarly, this suggests that earlier leukopenia was also associated with earlier onset of plasma leakage. Although scatter plot visualization did not demonstrate a strong linear pattern, these statistically significant correlations indicate that lower nadir values of both platelets and WBCs are temporally associated with earlier plasma leakage.

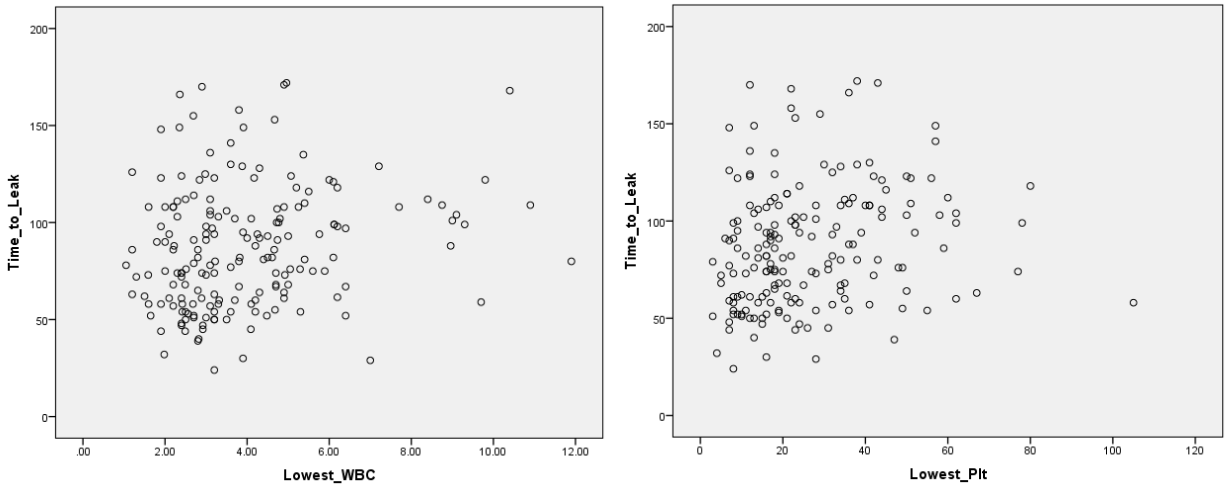

Figure 17: Scatter Plots of Lowest WBC and Platelet to Time to leak

The analysis demonstrated a **statistically significant positive correlation** between *time to plasma leakage* and *predicted platelet at the onset of leaking platelet count* ($r = 0.269$, $p < 0.001$). This indicates that patients who developed plasma leakage later in the course of illness tended to have **higher platelet counts at the onset of leakage**, whereas those who developed leakage earlier tended to have **lower platelet counts** at the time leakage occurred.

Although the strength of the correlation is modest, the direction of the association is clinically meaningful. It suggests that **earlier leakage is associated with more profound thrombocytopenia at the time of leakage**, whereas patients who progress more slowly toward the critical phase retain relatively higher platelet counts when leakage begins.

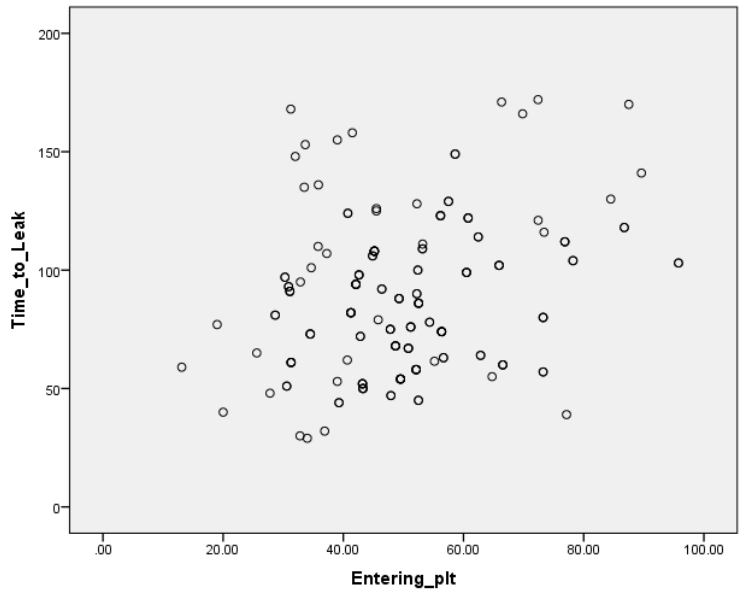

Figure 18: Scatter Plot of predicted platelet at the onset of leaking to Time to leak

Association with Time to lowest counts and leaking time

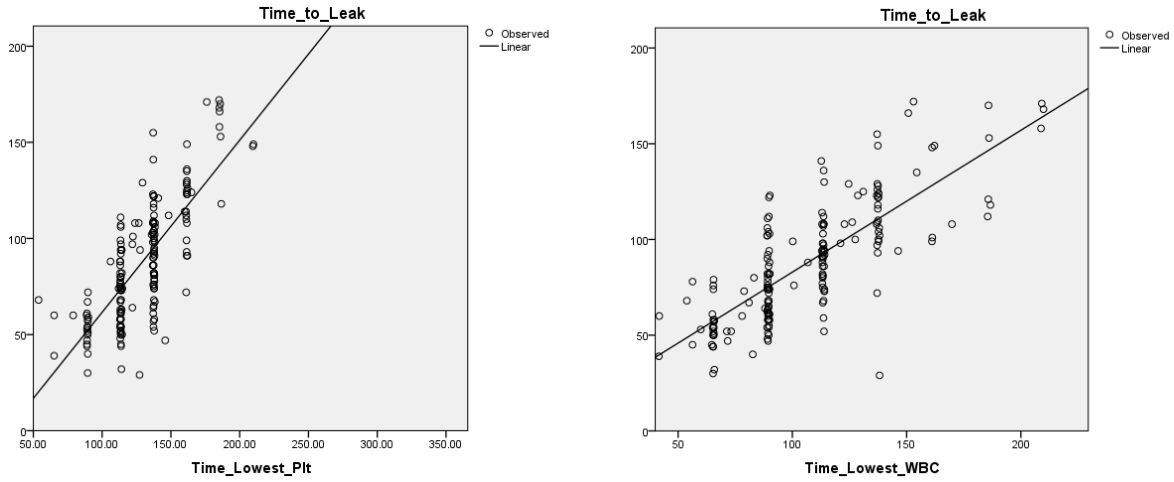

Figure 19: Scatter Plots of Time to lowest platelet and Time to lowest WBC to Time to leak

A strong temporal association was observed between the timing of hematological nadirs and the onset of plasma leakage. The time to lowest white blood cell (WBC) count demonstrated a strong positive correlation with the time to plasma leakage ($r = 0.779$, $p < 0.001$), indicating that patients who reached their lowest WBC later in the disease course also experienced a later onset

of plasma leakage. Conversely, patients who developed leukopenia earlier tended to progress to plasma leakage earlier.

Similarly, a strong positive correlation was observed between the time to lowest platelet count and the onset of plasma leakage ($r = 0.758$, $p < 0.001$). This suggests that patients with an earlier platelet nadir were more likely to develop plasma leakage earlier, whereas those with delayed platelet decline experienced later leakage.

Association between the Rate of Hematological Decline and Leaking Onset

To assess the relationship between the rate of hematological decline and the timing of plasma leakage, rates of platelet and white blood cell (WBC) reduction were calculated assuming a constant linear decline.

A strong negative correlation was observed between time to leakage and rate of platelet decline ($r = -0.687$, $p < 0.001$), indicating that patients with a faster rate of platelet fall developed plasma leakage earlier. Similarly, time to leakage showed a moderate-to-strong negative correlation with rate of WBC decline ($r = -0.582$, $p < 0.001$), suggesting that a more rapid reduction in WBC count was also associated with earlier onset of plasma leakage. Additionally, the rate of platelet decline and rate of WBC decline were positively correlated with each other ($r = 0.560$, $p < 0.001$), indicating that patients with rapid platelet decline also tended to experience rapid WBC decline.

These findings indicate that faster hematological deterioration is strongly associated with earlier onset of plasma leakage. The close relationship between platelet and WBC decline further supports the presence of a coordinated pathophysiological process during the critical phase of dengue infection.

Importantly, these results suggest that dynamic trends, rather than single-point laboratory values, may provide superior prognostic information for anticipating plasma leakage.

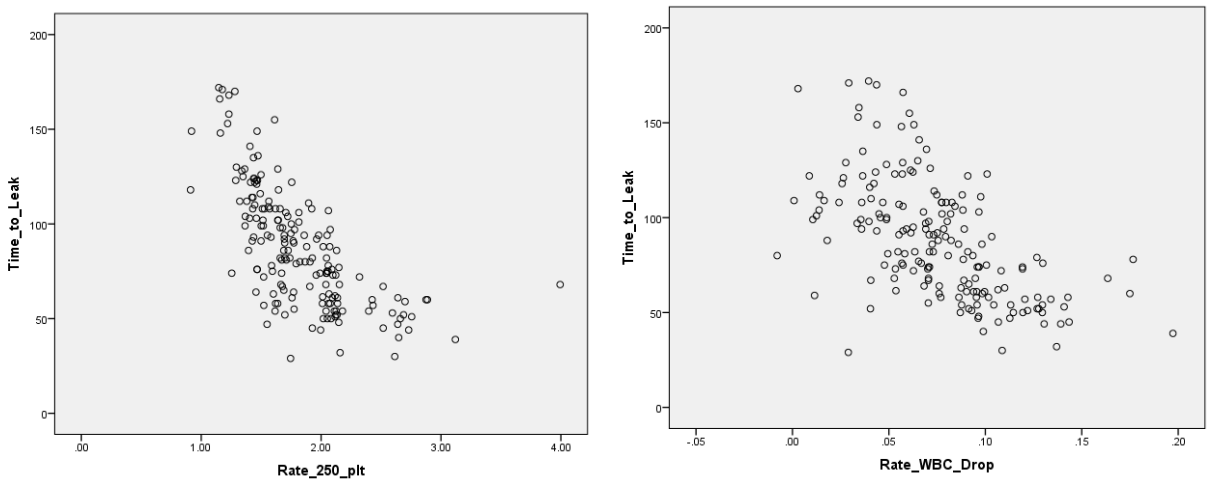

Figure 20: Scatter Plots between Rate of platelet decline and Rate of WBC decline to Time to leak

Model Development

A multiple linear regression analysis was performed using the backward elimination method to identify factors independently associated with **Square root of leaking time (SQRT leak time).** Initially, five clinically relevant variables (time to lowest platelet count, time to lowest WBC, lowest WBC, lowest platelet count, and CRP) were entered into the model. Through stepwise backward elimination, non-significant variables were removed, and the final model retained **time to lowest WBC, time to lowest platelet count, and lowest platelet count** as independent predictors.

The final regression model was statistically significant, explaining **67.8% of the variance** in SQRT leak time (adjusted $R^2 = 0.678$). Time to lowest WBC demonstrated the strongest association with SQRT leak time ($\beta = 0.454$, $p < 0.001$), followed by time to lowest platelet count ($\beta = 0.420$, $p < 0.001$). Lowest platelet count also remained a significant predictor, though with a smaller effect size ($\beta = 0.132$, $p = 0.003$). All retained predictors were positively associated with SQRT leak time, indicating that longer time to nadir counts and lower platelet levels were associated with increased leaking onset time.

Final Regression Model (Backward Elimination)

Using **unstandardized coefficients**, the final linear regression equation is:

Sqrt (Leaking Onset time) = 3.049 + 0.023(TLWBC) + 0.026(TLplt) + 0.013(Lplt)
Leaking Onset time (Hours) = [3.049 + 0.023(TLWBC) + 0.026(TLplt) + 0.013(Lplt)]^2
Adjusted R^2 = 67.8%

Sqrt (Leaking Onset time) = Square root of the Leaking onset time
TLWBC = Time to lowest WBC
TLplt = Time to Lowest Platelet
Lplt = Lowest platelet count

In the initial regression analysis, time-dependent variables were calculated from the onset of fever. However, the onset of fever was subjectively defined and taken as 12:00 PM on the relevant day for all patients, which introduced potential measurement bias. To minimize this subjectivity, a revised outcome variable was derived. The **time gap between onset of plasma leakage and the time to lowest platelet count** was calculated for each patient using the following formula:

Time gap between onset of plasma leakage and the time to lowest platelet count = *time to lowest platelet - time to onset of leaking*

Subsequently, regression analysis was repeated using this newly defined time gap as the dependent variable to identify factors associated with the progression from plasma leakage to platelet nadir. The predictors were evaluated using the backward elimination method to determine independent factors influencing this interval. This refined modeling approach reduced dependence on subjective time assumptions.

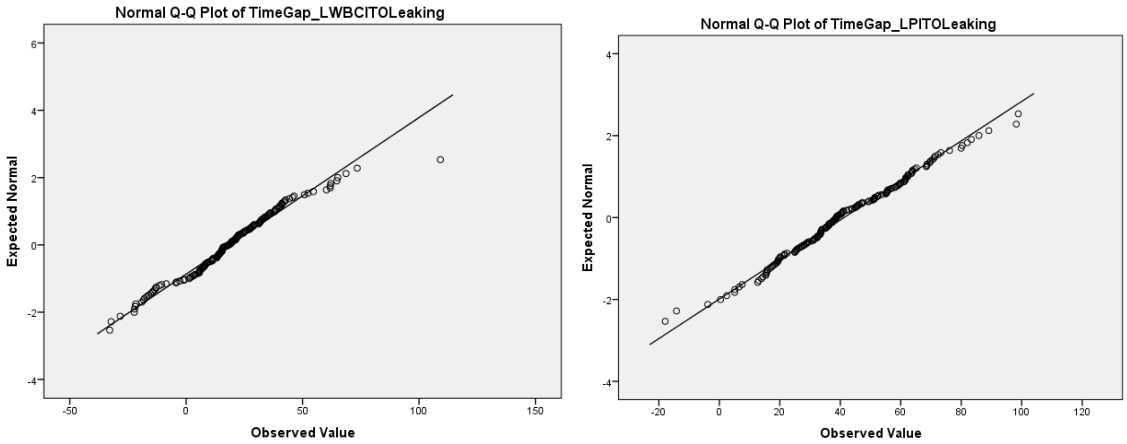

Figure 21: Q-Q Plots of Time Gap between time to Lowest platelet and Lowest WBC to leaking onset

Normality of the derived time-gap variables was assessed using the Kolmogorov–Smirnov test and the Shapiro-Wilk test. The untransformed **time gap between lowest platelet count and onset of leaking** showed a significant deviation from normality on the Kolmogorov-Smirnov test (D = 0.072, p = 0.027), although the Shapiro-Wilk test did not indicate significant non-normality (W = 0.993, p = 0.545). In contrast, the **time gap between lowest WBC and onset of leaking** demonstrated significant non-normality on both the Kolmogorov–Smirnov test (D = 0.074, p = 0.021) and the Shapiro–Wilk test (W = 0.974, p = 0.003).

Given the evidence of non-normal distribution, particularly for the WBC time-gap variable, a **square-root transformation** was applied to both time-gap variables to satisfy the assumptions required for linear regression analysis. Following transformation, reassessment of normality indicated acceptable distribution for both variables. The square-root-transformed **time gap between lowest WBC and onset of leaking** showed non-significant results on both the Kolmogorov-Smirnov test (D = 0.046, p = 0.200) and the Shapiro-Wilk test (W = 0.989, p = 0.299). Similarly, the square-root–transformed **time gap between lowest platelet count and onset of leaking** demonstrated non-significant Kolmogorov-Smirnov (D = 0.050, p = 0.200) and Shapiro-Wilk test results (W = 0.983, p = 0.068).

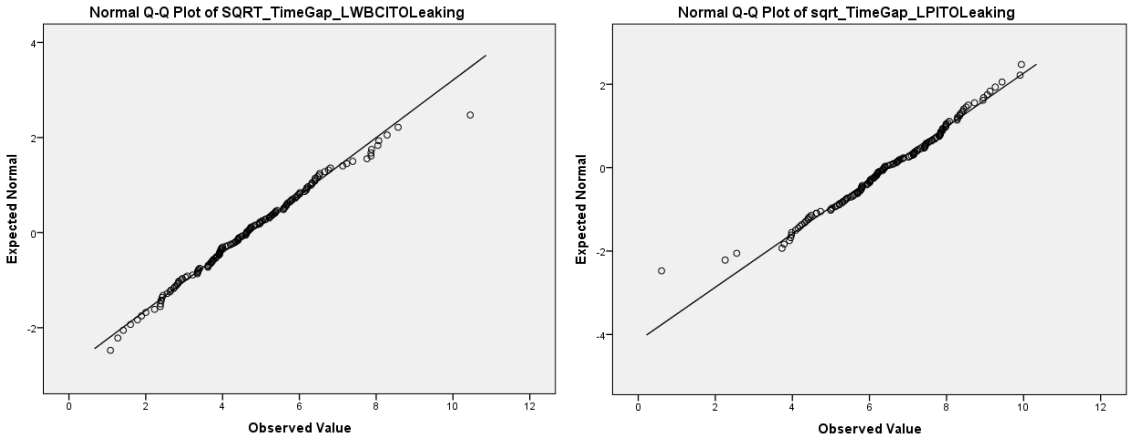

Figure 22: Q-Q Plots of Square roots of Time Gap between time to Lowest platelet and Lowest WBC to leaking onset

Multiple linear regression analyses were conducted to identify hematological predictors of time to leak, assessed separately for leukocyte (WBC) and platelet (PLT) related leakage outcomes. The dependent variables were square-root–transformed time gaps to leakage to satisfy assumptions of normality.

WBC-related time to leak

In the initial model, lowest platelet count, lowest WBC count, rate of platelet drop, and rate of WBC drop were entered simultaneously. Among these variables, only the **rate of platelet drop** (B = 1.192, p = 0.008) and the **rate of WBC drop** (B = −31.948, $p < 0.001$) were statistically significant predictors. Lowest platelet count and lowest WBC count were not significantly associated with time to leak ($p > 0.05$).

After stepwise removal of non-significant predictors, the final model retained **rate of platelet drop** (B = 1.153, β = 0.285, p = 0.001) and **rate of WBC drop** (B = −27.355, β = −0.579, $p <$ 0.001) as independent predictors. A faster decline in WBC was associated with a shorter time to leak, whereas a higher rate of platelet drop was associated with a longer time to leak.

The final WBC model explained a **modest proportion of variance**, with an **adjusted R^2 of 22.1%**, indicating limited but statistically meaningful predictive value.

Sqrt (Time Gap of Leaking Onset time to lowest WBC) = 4.573 + 1.153(Rate of plt drop) – 27.355(Rate of WBC drop)

Time Gap of Leaking Onset time to lowest WBC = [4.573 + 1.153(Rate of plt drop) – 27.355(Rate of WBC drop)]^2

Adjusted R^2 = 22.1%

Platelet-related time to leak

For platelet-related leakage, lowest platelet count, lowest WBC count, rate of platelet drop, and rate of WBC drop were entered into the regression model. All four predictors were statistically significant.

Lower minimum platelet count was associated with a shorter time to leak (B = −0.038, β = −0.405, $p < 0.001$), while higher minimum WBC count was associated with a longer time to leak (B = 0.285, β = 0.353, $p = 0.004$). In contrast, a faster rate of platelet decline was associated with a shorter time to leak (B = −2.115, β = −0.531, $p < 0.001$), whereas a faster rate of WBC decline was associated with a longer time to leak (B = 34.941, β = 0.761, $p < 0.001$).

This model demonstrated slightly stronger explanatory power than the WBC model, with an adjusted R^2 of 23.1%**.**

Sqrt (Time Gap of Leaking Onset time to lowest Platelet) = 7.337 - 2.115(Rate of plt drop) + 34.941(Rate of WBC drop) – 0.038(Lowest platelet) + 0.285(Lowest WBC)

Time Gap of Leaking Onset time to lowest Platelet = [7.337 - 2.115(Rate of plt drop) + 34.941(Rate of WBC drop) – 0.038(Lowest platelet) + 0.285(Lowest WBC)]^2

Adjusted R^2 = 22.1%

Comparison with previous model

Although both the WBC-related and platelet-related models identified statistically significant predictors, their explanatory power was relatively limited. This contrasts with the **previous model of overall time to leak**, which demonstrated a **strong adjusted R^2 of 67.8%**, indicating substantially better model fit and suggesting that additional factors beyond isolated hematological parameters contribute to leakage timing.

Chapter 5: Discussion

During 1997, WHO categorized the dengue into dengue fever and dengue hemorrhagic fever (DHF). In this classification, plasma leakage has being a mandatory criterion for DHF diagnosis (Srikiatkhachorn et al., 2011). However, the revised 2009 classification introduced the categories into dengue with and without warning signs, and severe dengue. Although the definition changes in 2009, plasma leakage remains a central pathological event defining the disease severity and clinical deterioration. Therefore plasma leakage is embedded within the definition of severe dengue alongside severe bleeding and organ dysfunction (Srikiatkhachorn et al., 2011).

In clinical practice, plasma leakage is often recognized only after it has already occurred. In addition, it is difficult to accurately determine the true onset of leakage. This challenge arises because many of the clinical and radiological indicators used to identify plasma leakage are inherently subjective and often detected retrospectively. As a result, the precise timing of transition from the febrile to the critical phase is frequently uncertain. Timely recognition of plasma leakage is essential to prevent progression to shock and organ dysfunction. Therefore, there is a critical need for objective and reproducible markers that can define the onset of plasma leakage more accurately. This study was therefore designed to address this gap by exploring temporal and hematological predictors that may allow earlier and more reliable identification of plasma leakage.

In this cohort of 185 patients with dengue hemorrhagic fever, plasma leakage occurred at a mean of approximately 87 hours from illness onset, with considerable inter-individual variability. Our

study shows that only approximately 50% developed leakage between 72 and 120 hours (days 4–5) which is considered standard time. Nearly one-third of patients developed leakage within the first 72 hours and rest leakage occur after day 5. This shows the heterogeneous and unpredictable nature of dengue leakage onset.

Our study shows some interesting findings about the factors affecting the leakage onset. Demographic characteristics such as age, sex, and body weight showed no association with the time of onset of leaking. Similarly, common comorbidities, including diabetes mellitus, hypertension, and ischemic heart disease did not influence the time of onset of leakage. Clinical symptoms at presentation, including headache, myalgia, vomiting, abdominal pain, and bleeding, also failed to predict the timing of plasma leakage. Admission vital signs similarly showed minimal predictive value. Although a weak inverse association was observed between heart rate and time to leakage, the effect size was small and unlikely to be clinically meaningful. Collectively, these findings suggest that demographic and early clinical assessment alone is insufficient for timing of the plasma leaking.

In contrast to clinical features, hematological parameters demonstrated strong and consistent associations with the timing of plasma leakage. A strong association was observed between the timing of platelet and white blood cell (WBC) nadirs. The timing of these nadirs showed strong positive correlations with the timing of plasma leakage. This shows that earlier hematological nadirs were associated with earlier leakage onset.

Furthermore, this study reveals that the rate of hematological decline is also an important factor. Faster declines in platelet and WBC counts were strongly associated with earlier plasma leakage. This highlights that the velocity of hematological deterioration is more informative than the absolute values alone.

A modest but statistically significant positive correlation was also observed between predicted platelet count at the onset of leakage and the timing of leakage. Patients who leaked earlier exhibited more profound thrombocytopenia at the time of leakage, whereas those with delayed leakage retained relatively higher platelet counts. This suggests that rapid disease progression leads to both earlier endothelial dysfunction and more severe platelet depletion.

In this study, multivariable regression modeling again demonstrated that changes in hematological parameters were more informative predictors of plasma leakage timing than absolute laboratory values alone. The primary regression model, incorporating time to lowest WBC, time to lowest platelet count, and lowest platelet count, explained a substantial proportion of the variability in leakage onset (adjusted $R^2 = 67.8\%$). Among these predictors, time to lowest WBC showed the strongest association with leakage timing, followed by time to lowest platelet count.

To address the potential bias related to subjective fever onset timing, an alternative modeling strategy was applied. Here the time gap between leakage onset and hematological nadirs was considered. After transformation to satisfy normality assumptions, regression analyses demonstrated that rates of change in hematological parameters were key determinants of leakage timing.

In the WBC-based model, a faster decline in WBC was associated with earlier leakage, while a slower platelet decline was associated with delayed leakage. Similarly, in the platelet-based model, faster platelet decline and lower platelet nadir were associated with earlier leakage, whereas higher WBC levels and slower WBC decline were associated with delayed leakage. Although these models showed modest explanatory power (adjusted $R^2 \approx 22–23\%$), they consistently highlighted the importance of dynamic hematological trajectories rather than absolute counts.

Overall, these findings suggest that the kinetics of leukocyte and platelet decline play a more critical role in predicting plasma leakage than isolated laboratory values.

To date, most dengue studies have focused on classifying disease severity rather than examining the temporal dynamics of leakage onset. Few studies have specifically evaluated predictors of when leakage occurs. A prospective cohort study from China involving 135 dengue patients demonstrated that platelet nadirs typically occurred during the decline phase and were not significantly influenced by age or comorbidities, findings that are consistent with our results (Guo et al., 2025). However, that study did not quantify the temporal relationship between platelet nadir and plasma leakage.

Other studies have reported conflicting findings regarding leukopenia as a predictor of severe dengue. Some have found no significant association between declining WBC counts and progression to the critical phase, while others reported leukopenia as an early warning sign (Rodrigo et al., 2021). Our findings help to resolve these inconsistencies, by demonstrating that the timing and rate of WBC decline, rather than absolute WBC values, are most relevant to predicting plasma leakage. In addition, these previous studies have typically categorized patients into broad disease phases without defining precise temporal thresholds. In contrast, our approach quantifies time-to-event relationships, offering a more clear understanding of disease dynamics.

The observed associations can be explained by known mechanisms of dengue pathophysiology which has both similar mechanism for thrombocytopenia, Leukopenia and plasma leaking (Ministry of Health, 2022). There are multiple mechanisms that explain thrombocytopenia in dengue. Dengue virus impairs megakaryopoiesis through direct infection and apoptosis of megakaryocytes, suppresses platelet production, and promotes immune-mediated platelet destruction via antibody-dependent enhancement (ADE). Activated platelets release pro-inflammatory mediators that further amplify endothelial dysfunction and vascular permeability.(Bhatt et al., 2021; Khazali et al., 2024) Similarly, leukopenia results from bone marrow suppression, peripheral destruction, and immune-mediated apoptosis. The synchronized decline in platelet and WBC counts observed in this study reflects a coordinated immunopathological process (Bhatt et al., 2021).

Plasma leakage itself is driven by cytokine-mediated endothelial disruption, characterized by increased vascular permeability and hemoconcentration. (Moallemi et al., 2025; Rodrigo et al., 2021). The strong association between hematological decline and leakage onset in our cohort supports the concept that these processes evolve in parallel rather than independently

Our study findings have several important implications for dengue management. First, reliance on static laboratory thresholds may delay recognition of patients entering the critical phase. Instead, monitoring the rate of change in platelet and WBC counts provides earlier and more clinically actionable information.

Second, the identification of a predictable temporal relationship between hematological decline and leakage supports to develop the dynamic risk prediction models. Such models could improve

triage decisions, optimize inpatient monitoring, and reduce unnecessary hospital admissions, particularly in resource-limited settings.

Third, the observation that earlier leakage is associated with more severe thrombocytopenia suggests the need for closer clinical observation during the early febrile phase, especially in patients showing rapid hematological deterioration.

Limitations

While informative, this study also has several limitations. First, this was a single-center retrospective study, which may limit the generalizability of the findings to other populations, other healthcare settings. The collected data and therefore the results are not compared with specific dengue viral serotypes.

The timing of fever onset was based on patient recall and was standardized to a fixed reference time, which may have introduced imprecision in defining the true onset of illness. This causes imprecision in exact timing of plasma leakage. In addition, there were missing data for certain variables, such as body weight and certain blood investigations which could not be retrieved as this is a retrospective study.

The timing of laboratory values were based on the time of results was reported rather than the exact time of blood sample collection. This may introduce a delay of approximately 1–2 hours, potentially affecting the precision of relationships between hematological changes and leakage onset. In addition, the lowest recorded laboratory values were used to represent nadir levels. However, in true physiological terms, the actual lowest values may have occurred between sampling points and therefore may not have been captured. Similarly, rates of platelet and WBC decline were assumed to be linear, whereas in reality, hematological changes may follow non-linear trajectories. Therefore, more frequent sampling would be needed to accurately characterize these dynamic patterns.

Additionally, certain biochemical parameters of potential relevance, such as serum albumin were not routinely measured in the study setting and therefore adequate data was not available for analysis.

Future prospective studies with standardized and accurately timed fever onset, more frequent serial blood sampling with time of collection, inclusion of viral load / serotype, and validation across diverse populations are recommended to strengthen predictive modeling of plasma leakage in dengue.

Conclusion

In summary, this study demonstrates that the onset of plasma leakage in dengue is driven predominantly by the dynamic temporal behavior of hematological parameters rather than by baseline demographic or clinical characteristics. The findings highlight that early and rapid declines in platelet and white blood cell counts are strongly associated with earlier onset of plasma leakage. This study revealed the importance of monitoring trends of laboratory parameters over time rather than relying on single absolute values. This study supports a paradigm shift toward dynamic, time-based risk stratification in dengue management..

Furthermore, the identification of relationships between hematological nadirs and time to nadirs with time of plasma leakage provides a framework for developing predictive models. This model could assist clinicians to align fluid administration with actual leakage dynamics, and to potentially improve outcomes by reducing both under- and over-resuscitation. Overall, these findings support the value of monitoring dynamic biomarker changes and highlight their role in improving early identification and management of severe dengue

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