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ORIGINAL ARTICLE

Estimation of a cut-off value for immature platelet fraction (IPF) in predicting platelet recovery in dengue patients with thrombocytopenia

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Abstract

Introduction: Thrombocytopenia is a common complication in dengue that sometimes necessitates platelet transfusion. Immature platelet fraction (IPF) measures immature platelets that indirectly reflect thrombopoiesis and is helpful in predicting platelet recovery. *Objectives:* This study aimed to evaluate the role of IPF% and identify its cut-off value in predicting platelet recovery in dengue patients with thrombocytopenia. *Materials and Methods:* Serial platelet count and IPF results were obtained from fifty-four confirmed dengue patients with platelet count <50x10^9/L. Median peak IPF% and number of patients with platelet recovery were determined. Receiver operating characteristic (ROC) curve is generated to identify the IPF% cut-off value to predict platelet recovery. *Results:* Median peak IPF% among dengue patients was 12.15% with 83.3% of them achieving platelet recovery after reaching the peak IPF%. There was a significant difference between median IPF% on day one of admission with peak IPF% among dengue patients. ROC curve analysis showed IFP% of 10.55% can be used to predict platelet recovery with a sensitivity of 69% and a specificity of 67%. *Conclusion:* IPF% is a reliable and useful parameter in predicting platelet recovery in dengue patients. This would assist the clinician in managing dengue patients especially those with severe thrombocytopenia without giving unnecessary platelet transfusion.

Keywords: Immature platelet fraction, Dengue fever, thrombocytopenia, Receiver operating characteristic, cut-off value

INTRODUCTION

Dengue fever is cause by Dengue Virus (DENV), a flavivirus, spread by Aedes mosquitoes, and because there are four different DENV serotypes, individual can get infected up to four times.[1] It is a systemic, dynamic disease with shifting clinical and laboratory parameters from day to day. The clinical spectrum can range from a mild febrile illness to a life-threatening haemorrhagic fever and shock syndrome. The main haematological abnormalities are leukopenia and thrombocytopenia.[2] Thrombocytopenia is a common symptom of moderate and severe dengue sickness, and linked to the severity of disease.[3]

DENV-associated thrombocytopenia has been linked to a variety of mechanisms, including bone marrow suppression and peripheral platelet destruction. Studies have shown that DENV-infected haematopoietic progenitors or bone marrow stromal cells lead to suppressing haematopoiesis, and anti-platelet antibodies is a cause of premature destruction of platelets in the peripheral circulation.[4] Excessive bleeding is one of the clinical signs of severe dengue, and it is responsible for a significant number of fatalities, even though it is relatively uncommon compared to plasma leakage.

The exact pathophysiological process of haemostatic including severe thrombocytopenia, prolonged coagulation profiles, reduced coagulation factors, abnormal fibrinolysis and reduced natural anticoagulant had been reported in dengue patients, and most of this derangement will normalise on its own without causing any clinically significant bleeding.[5]

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Dengue patients with thrombocytopenia used to be treated with platelet transfusion either as prophylaxis or therapeutic approach because thrombocytopenia is a common laboratory finding and thought to be an important cause of bleeding. It was well documented that transfusion is associated with risk of an allergic reaction, sensitisation of alloantibody, pulmonary complications and transmitting blood-borne infectious diseases.[6] Moreover, the development of severe bleeding in dengue is not prevented by platelet transfusion nor does it shorten the bleeding.[7] A recent study showed that prophylactic platelet transfusion was not superior to supportive care in preventing bleeding in adult dengue patients with thrombocytopenia, and it may be associated with side effects.[8] As more evidence emerged against this practice,[5,7,9] prophylactic platelet transfusion has become less common and is currently not routinely recommended in dengue management.

The recovery from transient marrow suppression in dengue fever will usually occur within 10 days after infection even though the thrombocytopenia persists. Immature platelets are released into the peripheral bloodstream and mature within 24 hours during marrow recovery. Their presence in the blood can be used as a marker indicating bone marrow activity.[10]

Immature platelets are young and reticulated because they contain higher ribonucleic acid (RNA) than mature platelets and larger in size. They take up dyes more readily and this principle is used in modern haematology analysers to measure immature platelets in peripheral blood.[11] Immature platelet fraction (IPF) is measured as a percentage of the immature platelet over the total platelet population. Both absolute and percentage of IPF have been used in various researches related to thrombocytopenia.[11–15] Data has shown IPF is able to predict platelet recovery faster than the increment of platelet count and this is useful in the management of dengue particularly when the decision of platelet transfusion need to be made.[12,16,17]

Our study is focusing on the role of IPF% and identify its cut-off value in predicting platelet recovery in dengue patients with thrombocytopenia.

METHODS

Ethical considerations

Ethics approval was obtained from the National Medical Research Register (NMRR), Ministry of Health, Malaysia (NMRR -16-2675-33195) and Ethics Committee for research involving human subjects, Universiti Putra Malaysia. The research was conducted according to the Malaysian Good Clinical Practice Guidelines.

Study design and sample selection

This was a prospective cross sectional study design using serial FBC data in Hospital Tengku Ampuan Rahimah, (HTAR), Klang, Selangor, Malaysia. It was conducted in two phases: 1) validation of the local IPF% reference range. 2) Serial monitoring of IPF% from confirmed dengue patients. It was done from January 2017 to December 2018. FBC results from 30 healthy whole blood donors were taken to analyse the IPF% reference range for the local population.

For phase two, 54 subjects were recruited using purposive sampling, calculated based on the prevalence of dengue patients in Malaysia using the formula.[18]

The inclusion criteria include FBC results from patients who were positive for dengue serology (NS1 or IgM antibody or both), presented with or without dengue warning signs[19], platelet count of $<50x10^9$/L and did not received platelet transfusion during admission. We excluded FBC from dengue patients with other medical illness and sample more than 12 hours post venesection.

Data collection

i) Phase one
   Blood samples were obtained from 15 male and 15 female healthy blood donors who fulfilled the criteria for blood donation and after the written consent was sought. Peripheral blood samples (2.5cc) were collected in K_2EDTA and analysed using Sysmex XN-9000 (Sysmex, Kobe, Japan) within six hours of sampling. Platelet count and IPF% were obtained from the FBC results.
ii) Phase two study
   FBC results of the respondents were identified and chosen for daily monitoring of IPF% and platelet count. FBC were analysed using Sysmex XN- 9000 (Sysmex, Kobe, Japan). Demographic details of dengue patients, platelet count and IPF% were obtained from the hospital's laboratory information system. Platelet recovery was defined as an increase in platelet count of more than 20 x 10^9 cells/L in 48 hours.[20] Peak IPF% (maximum IPF% value) were identified for each patient.

Statistical analysis

The results were analysed using SPSS software version 24.

i) Determination of local reference range
The IPF% obtained from the normal population were tested for normality using the Shapiro Wilk test. Mean of IPF% were obtained and compared to the mean from the previous study using one sample t-test.[21]

ii) Analysis of respondent's data (dengue patients)
The demographic factors of the respondents were summarised using descriptive analysis. The IPF% obtained from the dengue patients were tested for normality using the Shapiro Wilk test. Mann-Whitney test was used to compare the median for IPF% between healthy individuals from phase one of the study with the median IPF% of dengue patients on the day of admission.

Median peak IPF% and achievement of platelet recovery after reaching peak IPF% were analysed using descriptive analysis. The median IPF% on the day of admission was compared with median peak IPF% using Wilcoxon Signed Ranks test. Receiver Operating Characteristic (ROC) curve was used to determine the cut-off value for peak IPF% in predicting platelet recovery.

RESULTS

The data from healthy individuals in this study was normally distributed, so the mean value was used to compare with a mean IPF% value from a previous study done locally.[21] We found the mean and median IPF% were 1.99% (SD 0.68) and 2.0% respectively. The previous study showed the mean and median for IPF% were 1.80% and 1.5% respectively. There was no statistically significant difference (df: 1.526, p=0.138) between the mean IPF% value from our study with Ambayya.[21]

Patients' data

Fifty-four patients were enrolled and FBC results were monitored until platelet count showed increment. Table 1 showed the demographic data of the patients.

Most of the patients were in the age group of 15-29, n=16 (29.6%) male (72.2%) and Malay ethnicity (37.0%) The youngest patient was one year old and the oldest was 70 years old. Majority were adults (n=44, 81.5%) compared to children below 15 years old (n=10, 18.5%) The platelet counts during admission were ranging from 5 to 49 x 10^9/L. The IPF% were not normally distributed hence the median was used to describe the findings. The admission and peak IPF% were presented in table 2.

Table 3 showed the comparison of IPF% at admission and peak IPF% between adults and children. There was no statistically significant difference between the IPF% value from these two groups.

Table 1: The demographic data of the patients

Demographic factors		Frequency (n)	Percent (%)
Age group	1-14	10	18.6
	15-29	16	29.6
	30-44	14	25.9
	45-59	8	14.8
	60-74	6	11.1
Gender	Male	39	72.2
	Female	15	27.8
Ethnicity	Malay	20	37.0
	Chinese	7	13.0
	Indian	16	29.6
	Others	1	1.9
	Non-Malaysians	10	18.5
	Total	**54**	**100.0**

Table 2: Admission IPF% and peak IPF% for dengue patients

	Median	IQR
Admission IPF (%)	9.30	7.60
Peak IPF (%)	12.15	8.60

The median IPF% from the healthy individuals was compared with dengue patients on the day of admission. Among the dengue patients, the median IPF% on the day of admission was compared with the median peak IPF% (Table 4).

Twenty-six patients (48.2%) were monitored for 3 days, 14 patients for 4 days and remaining 14 monitored for 5 days. 45 (83.3%) patients recorded platelet recovery compared to 9 (16.7%) who failed to show platelet recovery after reached peak IPF%. Figure 2 illustrated the median platelet count and IPF% for 14 patients who were monitored for 5 days.

Based on the (ROC (Figure 3), the cut off value of peak IPF% in predicting platelet recovery is 10.55% with a sensitivity of 69% and specificity of 69% (95% CI : 0.489-0882) with the area under the curve (AUC) of 0.685

DISCUSSION

This study highlighted the potential use of IPF in the management of dengue patients by predicting platelet recovery. The measurement of immature platelets is a helpful marker of thrombopoietic activity as they are the most recently formed platelets released by megakaryocytes into the peripheral circulation.[11,13,22] Immature platelet fraction (IPF) is derived from automated measurement of immature platelets from the venous blood and its level will increase in peripheral platelets destruction or consumption.[16,23]

The reference range of IPF% was done for the Malaysian population in 2014 by a group of researchers.[21] Verifying the reference range was strongly suggested by the Clinical and Laboratory Standard Institute (CLSI) by collecting a minimum of 20 samples from qualified individuals.[24] Mean IPF% of healthy individuals in our study was 1.99% (reference range: 1.3 - 2.7), close to the mean IPF% value done previously which was 1.8% [21] (reference range: 0-4%) and comparatively showed no significant difference. We recruited healthy adults who had fulfilled the criteria as blood donors hence technically, the criteria used for this study was more comprehensive than the groups of subjects described by Ambayya.[21] However, in view of the huge number of subjects (2440) and large coverage of populations in Peninsular Malaysia, the IPF% reference range from Ambayya should be the reference point for other laboratories in Malaysia.

Our data showed most of the patients were adult males. The higher incidence of dengue infection among adults compared to children was also reported in a few studies in Malaysia,[25–27] even though some data showed children have the highest proportion of dengue infection.[28–31] Our data were taken from dengue patients with

Table 3: Comparison between the adult and child group

Variable	Median (IQR)		z statistic	p value
	Adult	Children		
Admission IPF	9.55 (7.7)	6.15(8.2)	-1.292	0.196
Peak IPF	13.4 (8.9)	10.5(8.8)	-1.359	0.174

Table 4: Comparison of median IPF% among healthy individuals with dengue patients on the day of admission

Variable	Median (IQR)		z statistic	p value
	Healthy	Dengue		
IPF(%)	2.00 (1.2)	9.3 (7.6)	-7.114	0.001
Dengue	**Admission**	**Peak**		
IPF(%)	9.30 (7.60)	12.15(8.6)	-5.288	0.001

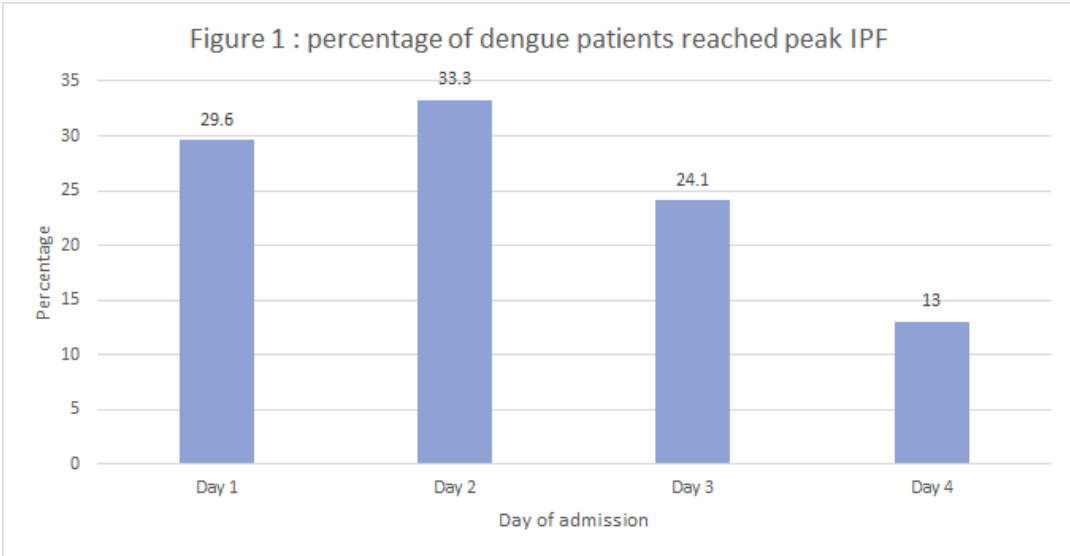


FIG. 1: Percentage of dengue patients who reached peak IPF% at different days of admission.

thrombocytopenia and it was reported that male and older age are among the risk factors to develop thrombocytopenia, and older patients are more likely to develop symptomatic dengue than younger individuals.[32,33] This may explain the demographic findings in our study. The slight dominance of Malay ethnicity is also comparable with a previous study done in the same location because Malays were the majority ethnic in the district and in concordance with the ethnic composition in Malaysia.[26]

Most of the studies evaluating IPF% in dengue infection were done according to age and no direct comparison of the IPF% value was done between the adult and paediatric population. Our study demonstrated the IPF% value increased on the day of admission for both children and adults infected with dengue. Even though the increments for adults were higher compared to children both at admission and when IPF% reached its peak, they were not statistically significant. Hence for this study, we

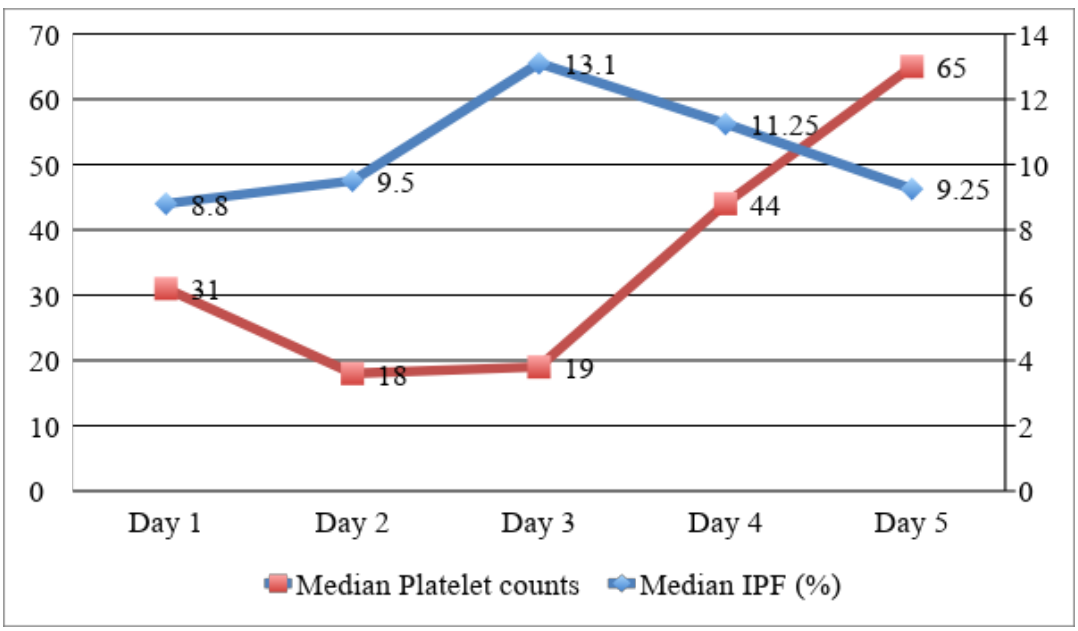
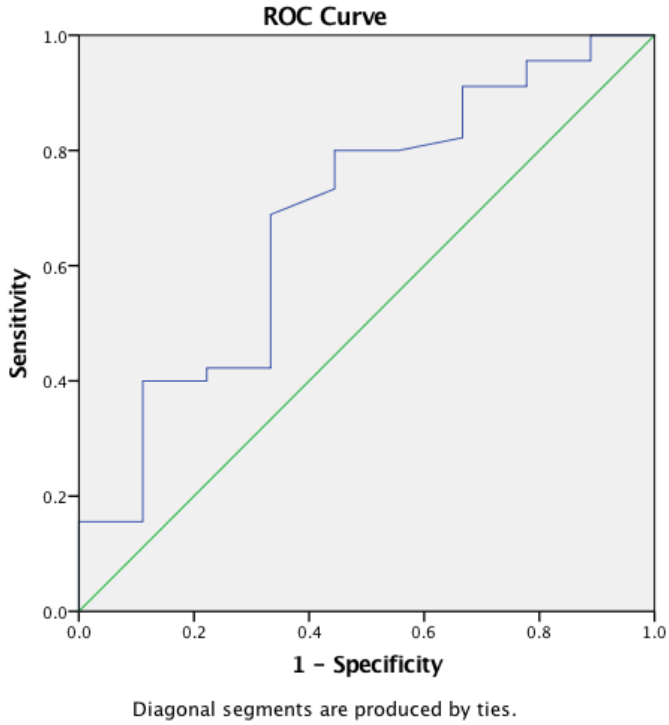


FIG. 2: Pattern of IPF% and platelet count.



	Area under the Curve (AUC)	95% confidence interval	
		Lower	Upper
IPF%	0.685	0.489	0.882

FIG. 3: ROC curve of peak IPF% in predicting platelet recovery.

took the median IPF% of all patients for further analysis. The IPF% of dengue patients in this study were significantly higher than the reference interval. Most IPF% studies in dengue patients also showed similar findings in both adults and paediatric patients.[17,34,35]

Moderate thrombocytopenia is an usual finding associated with dengue, the cause for which are multifactorial including early transient marrow suppression with damage to megakaryocytes[36], platelet aggregation to endothelial cells targeted by dengue viruses, haemophagocytosis[37], and immune destruction of platelets with dengue antibody complexes being found on their membranes.[38] Recent data identified a strong association between platelet activation and the platelets depletion from circulation in patients with dengue. This phenomenon started a few days after the fever when the patients were usually admitted to the hospital with thrombocytopenia (less than 50000/L).[39] In this study, our patients were selected when the platelet count is less than 50x 10^9/L, hence this explains the increment of IPF% as it measures the platelet production in the bone marrow when there is peripheral destruction. Most patients achieved the peak IPF% at the early days of admission and it was significantly higher than the median IPF% at admission. This correlates with the critical phase of dengue when the patients usually will be admitted and the platelet activation and destruction is happening.[18]

It is a well-known fact that IPF% in cases with peripheral destruction will increase as the platelet count decreases especially in patients with PC less than 40 x10^9/L.[16] We discovered the same phenomena as illustrated in 14 patients who were monitored for five days (figure 2). The median peak IPF% was recorded at day three of admission, corresponding with the lowest platelet count. Chuansumrit in a study evaluating IPF% in paediatric patients showed a similar pattern.[17]

Thrombocytopenia in dengue infection frequently occurred at the end of the febrile phase or beginning of the critical phase. It is one of the potential indicators of clinical severity.[40] In less severe cases, the platelet count recovered

spontaneously after fluid or electrolyte therapy and often preceded by the recovery of white cell count.[18] However, bleeding manifestations were highly variable and did not always correlate with degree of thrombocytopenia as it happened even with normal platelet counts. The correlation between thrombocytopenia and bleeding was more common in severe dengue infection than non-severe dengue infection. The mechanism for bleeding manifestations is multifactorial and factors such as thrombocytopenia, coagulation defects, vasculopathy and hepatic derangement act simultaneously. Therefore, other causes of bleeding need to be excluded before transfusing platelets.[18] Modified WHO bleeding scale played a role in managing dengue patients and aided the decision for blood and blood products transfusion.[41] Earlier study reported platelet transfusion was required in high-risk dengue patients with platelet count less than 20x10^/L. The ones with moderate risk only receive platelet transfusion if they have bleeding manifestation and other comorbidity that enhance the bleeding risk.[42] However, later evidence showed that some dengue patients responded to platelet transfusion but it did not prevent its progression into severe bleeding besides increased the risk of severe adverse reaction.[9] There is also no role of routine prophylactic platelet transfusion as it has undesirable effects on patients such as paradoxical fall in platelet counts and increased hospital stay.[43]

Platelet transfusion in dengue patients with thrombocytopenia remained a debate. For patients with low bleeding score and stable haemodynamics, serial monitoring of the platelet is mandatory to see the trend. Platelet transfusion can be avoided if we have a parameter that can predict platelet recovery especially in stable dengue cases. In this situation, IPF% is proven to be a useful parameter to predict platelet recovery and prevent platelet transfusion as shown by our data and few others.[14,17,20,44]

About 83% of dengue patients in our study achieved platelet recovery after reaching peak IPF% compared to 16.7% who didn't achieve platelet recovery. Dadu found that almost 94% of the dengue patients showed platelet recovery within 24-48 hour of the rise of IPF% and all patients showed recovery within 24 hour of the falling of IPF%.[34] However, the peak IPF% value and the platelet recovery in that study was not clearly defined. In another study, Suman showed serial monitoring of IPF% in different risk categories of dengue patients showed an increasing IPF% trend on the first five days after admission and reduced afterwards.[20] This study did not mention the peak IPF% value but they showed the change of risk category based on the platelet count from high risk to low risk to indicate platelet recovery. In another recent study, 85% of the patients had IPF% more than their upper limit of IPF%(> 8%) and it showed positive correlation with the platelet count on day two and three of illness.[44] They also noted that when the IPF% was above the normal limit, the platelet count showed an increasing trend. Dengue patients with platelet count of < 50 x 10^9/L were also found to have increased in IPF% which was also demonstrated in this study.[11] This illustrated the inverse relationship of IPF% with the platelet counts. Higher IPF% was also noted in severe dengue compared to non-severe dengue cases thus suggesting the severity of peripheral platelet destruction.[10]

Based on our AUC for the ROC curve, the cut-off IPF% value of 10.55% could predict platelet recovery for dengue patients with the sensitivity and specificity of 69 % and 67%. respectively. When the value of peak IPF% is more than 10.55%, the platelet count of dengue patients will increase >20 x 10^9/L within 48 hours. Dadu demonstrated 93.75% of patients achieved platelet recovery within 48 hours when IPF% was more than 10%, but the platelet recovery in that study was not clearly defined.[34] Another study demonstrated that when IPF% was more than 10.6%, there was a 100% chance of platelet recovery using the similar definition of platelet recovery like ours, even though they discovered a significant improvement in platelet values within 48 hours if the IPF% is more than 6.1%.[20]

Similar findings are also noted in paediatric patients with dengue infection even though there were some variations in IPF% value as a cut-off point. One study found IPF% more than 10% after defervescence period was a predictor of platelet count will increase to > 60x10^9/L. Almost 73% and 100% of the patients will achieve the targeted platelet count within 48 hours and 72 hours respectively when using IPF% of 10% as a cut-off point.[17] Recent data also showed IPF% is a useful tool to differentiate causes of thrombocytopenia and the increased IPF% level is around 10% if the platelet count reduced to less than 40 x 10^9/L.[16]

Even though there are differences in the results due to variation in methodology and statistical analysis of the related IPF% studies, there is a growing body of evidence that IPF% is a valuable

parameter to predict platelet recovery and disease progression in dengue patients. It would be exciting if the standardised cut off value of IPF% can be determined in future research and IPF% can be integrated into the management of dengue patients with thrombocytopenia. This will subsequently shorten the hospital stay in cases of stable dengue infection and help clinicians in better managing the patients.

LIMITATIONS

Our study was limited by a small sample size and only included the dengue cases in one hospital in a state in Malaysia. Hence the results may not represent the whole Malaysian population. The duration of ward admission was also not similar for all patients. In tertiary hospital with high occupancy rate like the location of our study, clinicians tend to discharge the patient early in the admission days if they were stable with increasing trend of platelet count even though it was below 50x10^9/L. All these factors may influence the outcome of the results. Despite these limitations, our study provides further evidence of the usefulness of IPF% in managing dengue patients with thrombocytopenia and gives insight to the cut-off point of IPF% to predict platelet recovery.

CONCLUSION

Our study verified the reference range of IPF% done previously in the local setting. IPF% value of dengue patients during admission was significantly higher than the normal population and most of them reached IPF% peak value early in the days of admission. The cut-off point IPF% value of 10.55% can predict platelet recovery with acceptable level of sensitivity and specificity. IPF% is a promising parameter to predict platelet recovery in dengue patients, earlier than the platelet count, and this can assist clinicians to effectively manage dengue patients with thrombocytopenia. Further validation using larger samples with standardised operational definitions is required to determine the most useful cut-off IPF value to predict platelet recovery in this population.

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Authors' contribution: F.I., J.A., S.M.N., and S.Z.M. conceived and planned the experiments. J.A. carried out the experiments and analysed the data, supervised by F.I, S.M.N and S.Z.M. J.A wrote the first draft of the manuscript then reviewed and edited by F.I. All authors provided feedback to the written manuscript.

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