ORIGINAL ARTICLE

Platelet Parameters and Interleukin 6 as Predictors of Disease Severity and Outcomes Amongst Hospitalised Coronavirus Disease-19 Patients

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ABSTRACT

Introduction: COVID-19 has been declared a pandemic by the WHO. Most mortality cases were linked to the presence of cytokine storms induced by the virus. Platelet parameters and IL-6 may provide significant results in the disease severity and input in a COVID-19 patient's management. This study aimed to determine the usefulness of platelet parameters and IL-6 with disease severity and outcomes amongst COVID-19 patients admitted to Hospital Kuala Lumpur (HKL). **Methods:** A retrospective study utilising clinical data of confirmed COVID-19 cases. Demographic data, platelet parameters on admission, serum IL-6 level, and treatment outcomes were retrieved and analysed. **Results:** 283 patients' data were analysed. The mean age of patients was 54.10 ±14.9 years old. Sixty percent of the patients were with comorbidities and (n=65, 23%) of them had succumbed to the disease. Males and females were equally affected and (n=139, 49.1%) were Malays. Ethnicity was an independent predictor for COVID-19 severity. A significant association was found between platelet count, MPV, and IL-6 with COVID-19 severity and outcomes. PDW was not associated with disease outcomes (p=0.236). Comorbidity and platelet count were independent predictors of COVID-19 death. A multivariate analysis of patients' platelet count, MPV, and IL-6 level using binary logistic analysis showed that platelet count of the non-survivor group significantly decreased by 0.004, compared to the survivor group. **Conclusion:** Combining a readily available routine blood investigation of low platelet count, raised MPV, and IL-6 level signifies an increased risk of COVID-19 severity, and thus, warrants close clinical attention in reducing mortality.
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Keywords: Platelet; Mean Platelet Volume; Platelet Distribution Width; Interleukin-6; COVID-19

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 coronavirus, was first detected in December 2019 in Wuhan, China, and quickly spread throughout the world. On March 11, 2020, the World Health Organisation (WHO) announced the novel coronavirus (COVID-19) outbreak as a global pandemic (1). This virus is very virulent and can undergo genetic mutations over time, producing more mutant forms with potentially distinct characteristics from its original strains and the infected person will have severe symptoms and high mortality (2). Malaysia's Covid-19 census recorded 36,198 deaths up until August 2022, with 4.78 million reported cases. In severe cases, patients may have severe pneumonia and might develop septic shock with acute respiratory distress syndrome (ARDS), which may require intensive care unit admission (3).

Full blood count (FBC) is a rapid, affordable, and generally available routine test in all medical centres. It results provide a lot of information on managing COVID-19 patients. Platelet parameters (platelet count, mean platelet volume (MPV), and platelet distribution width (PDW) are available information in the FBC result. MPV measures the average size of platelets and PDW measures platelet anisocytosis. Besides in haemostasis and coagulation process, platelets are also important in innate immunity and inflammatory responses. Excess production of cytokine and acute phase reactant in COVID-19 may affect megakaryopoiesis in the bone marrow and the release of immature platelets into peripheral circulation and changing the platelet parameter indices (4).

The host's immune system releases various cytokines

as part of the inflammatory process in COVID-19 infection. IL-6 is produced by stromal cells where its secretion is stimulated by proinflammatory cytokine. In COVID-19 infection, due to the pathogenesis of the disease itself which causes a hypoxia state and cytokine storm, both platelet parameters and serum IL-6 level will be affected. IL-6 marker is very useful for disease monitoring due to the availability of targeted therapy. Patient management guidelines were updated by WHO and IL-6 receptor blockers (tocilizumab and sarilumab) were added in the management of severely or critically ill with COVID-19, especially when treated with corticosteroids (5). To our knowledge, IL-6 test not routinely done in government hospitals and no study on IL-6 amongst COVID-19 patients has been done in Malaysia yet. The objective of our study was to determine the association and significance of platelet parameters and IL-6, and to elucidate their predictive nature in COVID-19 patients during hospital admission. Therefore, we aimed to determine the usefulness of monitoring patients' platelet parameters and IL-6 levels in managing COVID-19 patients.

MATERIALS AND METHODS

A retrospective study was done at Hospital Kuala Lumpur (HKL), which is the largest hospital under the MOH. It is a government tertiary hospital with 83 wards and 2300 beds. Since the COVID-19 outbreak, this hospital became the COVID-19 hospital for monitoring and managing COVID-19 patients. This study was conducted after obtaining an ethical approval by Medical Research Ethics and Committee (MREC), MREC Ref. No: NMRR-21-1836-61224 (IIR)) and Clinical Research Centre (CRC) Hospital Kuala Lumpur. The sample size was calculated based on mean difference specific study's objectives using the formula from OpenEpi (www.openepi.com). From the calculation, the minimum sample size required was 112. In this study, the sample size was increased for better analysis. The final total sample size with complete data for analysis was n=283. The clinical data of all confirmed COVID-19 cases aged 18 years old and above admitted to HKL between 1 June 2021 and 31 December 2021 and fulfilled the study inclusion criteria were randomly selected. The inclusion criteria of patients to be selected were: Reverse-transcriptase-polymerase chain reaction (RT-PCR) confirmed COVID-19 cases, aged 18 years old and above, admitted to HKL between 1st June 2021 until 31st December 2021, and available documented FBC results on admission and IL-6 during hospital admission. The exclusion criteria were confirmed COVID-19 cases with incomplete data and patients who were admitted to HKL due to other illnesses and were incidentally diagnosed with COVID-19 in the ward.

Patients' demographics and other clinical data including comorbidities, platelet parameters at admission, and IL-6 during hospital admission were retrospectively retrieved from the laboratory information (LIS) system. A blood sample on admission to the emergency department was taken and subjected to FBC analysis within four hours of sample collection using Sysmex XN-1000 and Sysmex XN-3000 analysers from Japan. In the ward, another blood sample was drawn and subjected to IL-6 assay by Electrochemiluminescence Immunoassay (ECLIA) method using Roche Cobas e602 analyser, Switzerland. The retrieved clinical data and laboratory investigation results were compiled and subjected to statistical analysis.

Statistical analysis

The data were analysed using IBM SPSS Statistics version 7.0 and presented as mean (SD) for parametric data or median (IQR) for non-parametric data. One-way ANOVA and Kruskal-Wallis test were used to describe and determine the platelet parameters and IL-6 in various COVID-19 stages. The association between platelet parameters, IL-6, and outcomes (non-survivors and survivors) was analysed using Independent t test and Mann-Whitney U test. Binary logistic regression analysis was done to look for independent predictors for COVID-19 disease severity and outcomes. The chi-square test was used to determine mortality outcomes (non-survivors and survivors) in patients with comorbidities and without comorbid conditions.

RESULTS

There were 283 patients (males and females equally involved) and the mean age was 54.10±14.9 years old. Malay was the major ethnicity (n=139, 49.1%). About (n=172, 60.8%) of the patients had comorbidities. Seventy-seven percent, n=220 of total patients were in the severe group during admission. Of the total patients, (n=65, 23%) did not survive. Their demographic data are summarised in Table I.

Table II summarises the platelet parameters and serum IL-6 levels according to various COVID-19 categories. The platelet count and MPV were normally distributed and the results are presented in mean ± SD. In the non-survivor group platelet count, MPV and IL-6 showed significant findings. The platelet count was significantly lower in the non-survivor group at 192.46±72.83x109/L. MPV and serum IL-6 levels were higher in the non-survivor group.

Table III shows the statistical analysis of all variables, at $p < 0.25$, by univariate analysis, which was subjected to multivariate logistic regression analysis. For COVID-19 disease severity, the categorical variables in the regression analysis included age, ethnicity, and gender. The analysis showed only

Table I : Demographic data of COVID-19 patients treated in HKL

Parameters	n(%)
Age (years)	
Mean (SD)	54.10(14.9)
18-39	52(17.7)
40-59	123(43.5)
60 above	108 (38.2)
Gender	
Male	141(49.8)
Female	142(50.2)
Race	
Malay	139(49.1)
Chinese	68(24)
Indian	29(10.2)
Others	47(16.6)
Covid category (on admission)	
Category 3 (mild)	63(22.3)
Category 4 (severe)	173(61.1)
Category 5 (severe)	47(16.6)

ethnicity to be an independent predictor for COVID-19 severity. Chinese and Indian patients were 0.252 and 0.246, respectively, less likely to develop severe COVID-19 infection compared to Malay patients. Multivariate analysis of the continuous variables, i.e., platelet count and IL-6, was done using binary logistic analysis. The analysis showed that none of these continuous variables are independent predictors of COVID-19 severity. For COVID-19 disease outcomes, the categorical variables in the regression analysis included ethnicity and comorbidities. The analysis showed only comorbidities to be an independent predictor for COVID-19 outcomes (death). Patients with comorbidities were 2.243 more likely to have a poor outcome (death) compared to patients without comorbidities. Multivariate analysis of the continuous variables included platelet count, MPV, and IL-6 using binary logistic analysis. The analysis showed platelet count of the non-survivor group significantly decreased by 0.004, compared to the survivor group. This result shows that severe infection can to lead thrombocytopenia.

The analysis of the mortality outcomes (non-survivors and survivors) in patients with comorbidities and without comorbid conditions showed a significant association between comorbidities and disease outcomes (p= 0.014). Twenty-three percent, n=65 of patients did not survive the COVID-19 infection and (73.8%, n=48) of them had comorbidities.

DISCUSSION

Demographic distribution, disease severity and outcomes amongst COVID-19 patients

In this study, the mean age of patients was 54.10±14.9 years old with the majority (n=123, 43.5%) of patients ranging from 40 to 59 years old. In a study conducted by Rahman et al. (2021), involving 306 patients, the mean age of the admitted COVID-19 patients was 48.79±8.53 years old (7). Malaysia's COVID-19 census showed that most COVID-19 patients were between 18-59 years old (https://covidnow.moh.gov.my/). The data support our finding that (n=185, 61.2%) of the patients admitted to HKL due to COVID-19 were from the same age group. This finding also could be probably because only patients of 18 years old and above were treated in HKL. Lim et al. (2020b) reported that their larger sample size of study comprising of 5889 cases involving COVID-19 cases aged ≥ 12 years old showed a median of 34 years old.

In agreement with our findings, Malays were predominantly infected with COVID-19 and admitted to the health care centre compared to Chinese, Indians, and other populations (8). This could be due to Malays being the predominant ethnicity in the Malaysian population (9). Besides that, our findings also noted that the Chinese and Indian populations were less likely to develop severe infections possibly due to early presentation to the hospital. This study noted that patients' comorbidities increased with severity and mortality rate. About (n=172, 60.8%) of total patients who were admitted due to COVID-19 had comorbidities and most of the patients were diagnosed with Category 4 on admission. Twenty-three percent of the patients had not survived the infection, as summarised in Table I. There is a strong correlation between comorbidities with COVID-19 disease severity and outcomes (8,10). Our study found (n=91, 32.2%) had at least one comorbid and these comorbidities also contributed to mortality, and about (n=48, 73.8%) of the non-survivor patients had comorbidities. A regression analysis study also showed comorbidities has a significant predictor for COVID-19 outcomes where patients with comorbidities were 2.243 more likely to have a poor outcome (death).

Platelet parameter changes in COVID-19 patients

This study mainly focused on platelet parameters. Platelets have important roles in innate immunity and inflammatory responses. Excess production of cytokines and acute phase reactants will affect megakaryopoiesis in the bone marrow and release of immature platelets into circulation (4). This condition will change the platelet indices. Platelet indices such as platelet count, MPV, and PDW are considerably

Table II : Platelet parameters (Platelet, MPV, PDW) and IL-6 levels of COVID-19 categories and outcomes (non-survivors and survivors)

Parameters / COVID-19 categories	Overall n=283	CAT 3 n (%) = 63(22.3)	CAT 4 n(%) =173(61.1)	CAT 5 n(%) =47(16.6)	p-value*	OUTCOMES		Mean diff. (95% CI)[a]	p-value*
						Non-Survivors	Survivors		
	Mean (SD) [a]Median(IQR)	Mean (SD) [a]Median(IQR)	Mean (SD) [a]Median(IQR)	Mean (SD) [a]Median(IQR)		Mean (SD) [a]Median(IQR)	Mean (SD) [a]Median(IQR)		
Platelet (normal range: 150-410x10^9/L)	227.76 (104.65)	211.38 (96.78)	238.10 (111.10)	211.66 (85.59)	0.114	192.46 (72.83)	238.29 (110.38)	-45.827 (-74.490, -17.165)	0.002*
MPV (normal range: 9.2-12.8 fL)	10.86 (1.03)	10.738 (1.0480)	10.929 (1.0565)	10.796 (0.9231)	0.404	11.11 (0.96)	10.79 (1.05)	0.3219 (0.0364, 0.6075)	0.027*
PDW	[a]12.20 (3.3)	[a]11.8(3.0)	[a]12.4(3.5)	[a]11.9(2.7)	0.495	[a]12.40(3.0)	[a]12.0(3.4)		0.236
IL-6 (Normal range: <7pg/ml)	[a]25.78 (78.50)	[a]28.49 (109.15)	[a]20.24 (51.13)	[a]53.85 (133.28)	0.001*	[a]44.96 (154.94)	[a]22.28 (60.19)		<0.001*

PDW = Platelet Distribution Width, MPV = Mean Platelet Volume, IL-6 = Interleukin 6

[a] Median (IQR) *Statistically significant at p-value<0.05

altered in COVID-19 infection and can thus be utilised as biomarkers (4,11). In critically ill patients, thrombocytopenia is a common laboratory abnormality that is associated with poor clinical outcomes and increased mortality. Thrombocytopenia has been observed in COVID-19 patients, and lower platelet counts are associated with worse clinical outcomes (12). Thrombocytopenia at admission in COVID-19 patients was associated with a 4.24-fold increased risk of inpatient mortality (13).

Our study showed platelet count and MPV had significant changes in correlation to mortality outcomes with p< 0.05. Platelet count of the non-survivor group also significantly decreased by 0.004, compared to the survivor group. A study has reported on a cohort of 3915 hospitalised COVID-19 patients, whose platelet indices at admission were collected and analysed, and it was discovered that platelet count, size, and maturity are linked to increased critical illness and leads to mortality in COVID-19 hospitalised patients (12). A meta-analysis of 31 trials involving 7613 participants found that severe COVID-19 infection is related to a lower platelet count and a threefold increase in the chance of getting severe COVID-19 infection (14). In severe COVID-19 patients, there were reduced platelet counts when compared to non-severe COVID-19 patients. Non-survivors exhibited significantly lower platelet counts than survivors. Thrombocytopenia may be a risk factor for the progression of COVID-19 to a more severe state. Further research on platelet count and MPV level in various COVID-19 severity is required for better disease understanding, prognosis prediction, and optimum management.

Interleukin 6 as a biomarker of COVID-19 disease severity and outcomes

Serum IL-6 is an emerging biomarker and a key mediator in COVID-19 infection (15). IL-6 is a good inflammatory indicator that is useful in COVID-19 monitoring and prognosis. This study focused on IL-6 because this inflammatory marker has a unique role in the cytokine storm occurring in COVID-19 patients. The information can also be used as a pharmacological target because IL-6 receptor blockers (tocilizumab and sarilumab) are a class of medicines that are lifesaving in patients who are severely or critically ill with COVID-19, especially when administered alongside corticosteroids (5). Findings in this study showed serum IL-6 levels have a significant association with the severe category of the disease, with p < 0.001. Elevated serum IL-6 levels observed in severe infections are in agreement with other studies (15–18). Clinicians can use this information to predict patients' condition and outcome and help them to decide on further management. Although IL-6 is a good marker for COVID-19, the test is not available in all hospitals and is very expensive compared to other inflammatory markers, like ferritin and CRP, which are easily available in all centres. All these parameters (platelet parameters and IL-6 level) can be used to predict outcomes and decision-making for active and aggressive management when a patient is admitted to the hospital.

CONCLUSION

COVID-19 is very contagious and had caused high mortalities worldwide. The virus can mutate to other strains and give a significant challenge to the

Table III : Binary logistic regression analysis for COVID-19 disease severity and outcomes

(A) COVID-19 Severity					
Parameters	**B**	**S.E**	**AOR**	**95% CI**	***p-value**
Age (years)					
18-39			1		
40-59	-0.355	0.475	0.701	0.276-1.781	0.456
>60	-0.801	0.476	0.449	0.177-1.141	0.092
Ethnicity					
Other			1		
Malay	-1.035	0.566	0.355	0.117-1.076	0.067
Chinese	-1.377	0.598	0.252	0.078-0.815	0.021*
Indian	-1.402	0.673	0.246	0.066-0.921	0.037*
Gender					
Male			1		
Female	-0.292	0.310	0.747	0.407-1.372	0.347
Platelet count	0.000	0.002	1.000	0.997-1.003	0.893
IL-6	0.000	0.000	1.000	1.000-1.000	0.248
(B) COVID-19 Death					
Parameters	**B**	**S.E**	**AOR**	**95% CI**	***p-value**
Comorbidities					
No			1		
Yes	0.808	0.318	2.243	1.202-4.186	0.011*
Ethnicity					
Other			1		
Malay	-0.319	0.401	0.727	0.331-1.596	0.427
Chinese	-0.069	0.442	0.933	0.393-2.217	0.875
Indian	-0.925	0.643	0.397	0.112-1.400	0.151
Platelet	-0.004	0.002	0.996	0.993-1.000	0.046*
MPV	0.142	0.148	1.152	0.862-1.542	0.339
IL-6	0.000	0.000	1.000	1.000-1.000	0.162

*statistically significant at p<0.05; AOR=adjusted odd ratio, B=regression coefficient, S.E=standard error

healthcare system. Patients can present a wide range of symptoms and can as well be asymptomatic. A lot of blood parameters and demographic factors have been investigated to look for associations and independent predictors for the severe stage and outcomes. This study showed a significant association between platelet parameters and IL-6 with the disease severity and outcomes. Platelet count, MPV, and IL-6 were affected by COVID-19 infection. Changes in platelet parameters and IL-6 provide important information to clinicians and give a clue on how aggressive treatment should be given to the patients.

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