Original Article

Association of the Plasma Platelet-Derived Microparticles to Platelet Count Ratio with Hospital Mortality and Disseminated Intravascular Coagulopathy in Critically Ill Patients

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Aim: The role of platelet-derived microparticles (PDMPs) in the crosstalk between coagulopathy and inflammation in critically ill patients remains unclear. The aim of this cohort observational study was to investigate the associations between the PDMP levels and hospital mortality or disseminated intravascular coagulopathy (DIC).

Methods: This study included 119 patients who were admitted to the ICU. The PDMP levels were measured using an enzyme-linked immunosorbent assay three times a week, for a total of 372 samples. We calculated the maximum (max) PDMP value, max PDMP/platelet (PDMP/Plts) ratio (converted to the PDMP levels per 10^4 platelets) and nadir platelet count during the ICU stay. Baseline patient data and scores, including the Japanese Association for Acute Medicine (JAAM) DIC score, were collected, and potential predictors were analyzed for possible associations with hospital mortality.

Results: The max PDMP/Plts ratio was significantly different comparing the survivors ($n=98$: median, 2.54) and non-survivors ($n=21$: median 17.59; $p<0.001$). There was a weak but statistically significant negative correlation between the max PDMP level and nadir platelet count ($r=-0.332$, $p<0.001$). The max PDMP level and max PDMP/Plts ratio were higher in the DIC group (81.48 and 9.27, respectively) than in the non-DIC group (34.88 and 2.35, $p=0.001$ and $p<0.001$, respectively). The max PDMP/Plts ratio was the only variable found to be independently associated with hospital mortality according to a multivariate logistic regression analysis.

Conclusions: PDMPs are involved in the development of DIC but are not related to hospital mortality. There is a good association between the PDMP/Plts ratio and hospital mortality and/or DIC in critically ill patients.

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Introduction

The interplay among activated platelets, neutro-

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phils, the endothelium and monocytes in critically ill patients can lead to endothelial injury and microvascular thrombosis[1]. This interplay essentially involves crosstalk between the processes of coagulopathy and inflammation. While this crosstalk has been reported to play a key role in the development of multiple organ dysfunction in critically ill patients[1], the mechanisms governing this phenomenon have yet to be established.

Microparticles (MPs) are small membrane vesi-

cles shed from different cell types, such as platelets, monocytes, erythrocytes, endothelial cells and granulocytes[2-4]. The release of MPs from these cells is stimulated by a state of cellular activation or apoptosis. MPs contain a cytosolic content surrounded by a phospholipid bilayer, exposing several transmembrane proteins that are specific to their parent cells[5, 6]. Platelet-derived MPs (PDMPs) are MPs released from platelets and have strong procoagulant properties, as they expose phosphatidylserine[7]. Sinauridze and coworkers reported that PDMPs have a 50- to 100-fold higher specific procoagulant activity than activated platelets[8]. Because the release of PDMPs is stimulated by the activation of platelets, PDMPs can be regarded as a marker of platelet activation[9]. In fact, clinical studies have shown that elevation of the circulating PDMP level is associated with clinical situations related to platelet activation, such as thrombotic disorders[10-19].

Aside from their procoagulant activity, the levels of circulating MPs are elevated during septic shock[14-18] and conditions of trauma[19, 20]. Therefore, the MP levels serve as a pathological marker of the procoagulant activity and MPs function as inflammatory vascular mediators[15]. Indeed, PDMPs are presumed to play an important role in the crosstalk between coagulopathy and inflammation.

Although the PDMP level can be used as an index of platelet activation, the association between the PDMP levels and outcomes of critically ill patients has not been evaluated. In the present study, we found out that the PDMP value per platelet ratio was associated with hospital mortality and coagulopathy in critically ill patients.

Subjects and Methods

Subjects

This prospective observational study was performed in the ICU of Shiga University of Medical Science from May 12, 2012 to January 18, 2013. Consecutive patients who were admitted to the 5-bed ICU were informed about this study, and 119 patients or their relatives provided their written informed consent to participate in this study. The exclusion criteria were an age of < 18 years and recent cardiovascular surgery. The patients were followed from the time of admission to the ICU until discharge from our hospital. The research protocol was approved by the ethics committee of our institution.

Blood Collection and Laboratory Analysis

A total of 372 blood samples were obtained via an arterial line from the 119 patients. Routine blood sampling for the blood cell count, blood chemistry, blood coagulation tests and a blood gas analysis was performed. Blood collection for measurement of the plasma PDMP concentration was performed three times a week in concurrence with sampling for the routine laboratory tests described above.

The plasma PDMP level was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Otsuka, Tokyo, Japan) in accordance with the manufacturer's instructions. Briefly, 2 ml blood samples were collected from an arterial line into vacutainers containing ethylenediaminetetraacetic acid - acid citrate dextrose solution (EDTA-ACD, NIPRO, Osaka, Japan). The samples were gently mixed by turning the tube upside down and then centrifuged at 8,000 g for 5 minutes at room temperature. Immediately after centrifugation, the upper layer of the supernatant was collected to avoid contamination of the platelets. The samples were stored at −80 ℃ until the analysis. According to the report by Osumi and coworkers who developed this kit, 1 U/ml of PDMPs in this ELISA kit was defined as the amount of PDMPs obtained from 24,000 solubilized platelets/ml[21].

The blood cell count, blood coagulation indices and blood chemistry parameters were measured according to routine laboratory methods. Blood coagulation indices were analyzed using STACIA™ (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). For the blood gas analysis, an ABL8000 FLEX™ (Radiometer, Denmark) was used. The platelet count was determined daily throughout the patient's ICU stay, and the nadir platelet count was defined as the minimum platelet count during the ICU stay.

Outcome Measurements

This study investigated the relationship between the PDMP levels and outcomes. The primary outcome was all-cause mortality at the time of discharge from the hospital. The secondary outcome was a diagnosis of disseminated intravascular coagulopathy (DIC) during the ICU stay. The diagnosis of DIC was established using the Japanese Association for Acute Medicine (JAAM) DIC criteria[22]. The maximum (max) JAAM DIC score during the ICU stay was recorded.

In this study, we converted the PDMP level to the PDMP level per 10^4 platelets (PDMP/Plts ratio) in order to eliminate the influence of the broad distribution of the platelet count resulting from various underlying patient- or disease-related factors. The max

PDMP value and max PDMP/Plts ratio for each patient were recorded during the ICU stay.

Statistical Analysis

The statistical analyses were performed with the IBM SPSS Statistics 22 software package (IBM Japan, Tokyo, Japan). Variables are expressed as the median (interquartile range [IQR]). The Mann-Whitney U test was applied for two-group unpaired comparisons, and proportions were compared with the chi-square test when necessary. A two-sided p-value of less than 0.05 was considered to indicate statistical significance. Correlations were assessed with Spearman's rank correlation test, and univariate logistic regression analyses were performed to examine the associations between mortality and each of the predictors separately. Factors initially considered in the univariate logistic analysis to be associated with hospital mortality ($p < 0.10$) were included in the multivariate regression analysis using forward stepwise elimination. A criterion of $p < 0.05$ for entry and $p \geq 0.10$ for removal was imposed, respectively, in this procedure. For the final model, we calculated the odds ratios (ORs) and 95% confidence intervals (95% CI) for all significant predictors at the $p < 0.05$ level. Receiver operating characteristic (ROC) curves were used to examine the ability of variables to predict hospital mortality. The ROC curve represented a plot of sensitivity vs. 1-specificity. The area under the curve (AUC) was calculated from the ROC curve, and the ROC curve analysis was used to identify parameters as potential markers of the prognosis.

Results

Patients

The main characteristics of the 119 analyzed patients are presented in **Table 1**. The median age was 67.0 years (range, 19 to 88 years). Twenty-one patients died during hospitalization (mortality rate, 17.6%). The number of patients with DIC, the max JAAM DIC scores during the ICU stay, the Acute Physiology and Chronic Health Evaluation (APACHE) II scores, the Sequential Organ Failure Assessment (SOFA) scores, the lactate levels, the thrombin-antithrombin complex (TAT) levels and the D-dimer levels on admission were significantly higher in the non-survivor group than in the survivor group. The nadir platelet count, platelet count and antithrombin III (AT3) levels on admission were significantly lower in the non-survivor group than in the survivor group.

Max PDMP Value and Max PDMP/Plts Ratio in the Survivors and Non-Survivors

The max PDMP value was 50.67 (IQR, 20.19 – 92.73) U/ml and 69.34 (IQR, 24.24 – 108.75) U/ml in the survivor and non-survivor groups, respectively, and this difference was not statistically significant ($p = 0.253$, **Fig. 1A**). However, there was a significant difference in the max PDMP/Plts ratio when comparing the survivors (median, 2.54; IQR, 0.90 – 5.98) and non-survivors (median, 17.59; IQR, 3.49 – 26.99, $p < 0.001$, **Fig. 1B**).

Nadir Platelet Count in the Survivors and Non-Survivors

The nadir platelet count was 148×10^9 (IQR, $93.3 \times 10^9 – 199 \times 10^9$) /L and 33×10^9 (IQR, $17.0 \times 10^9 – 63.0 \times 10^9$) /L in the survivor and non-survivor groups, and this difference was statistically significant ($p < 0.001$).

Correlation between the Nadir Platelet Count and Max PDMP Value

The max PDMP values were compared between the thrombopenia group (nadir platelet count $< 150 \times 10^9$ /L) and the non-thrombopenia group (nadir platelet count $\geq 150 \times 10^9$ /L) ($p = 0.001$, **Fig. 1C**). The max PDMP value was 76.94 (IQR, 28.24 – 102.94) U/ml in the thrombopenia group and 31.37 (IQR, 16.20 – 65.65) U/ml in the non-thrombopenia group, and the difference between the two groups was statistically significant ($p = 0.001$). The max PDMP/Plts ratio was 6.44 (IQR, 2.62 – 14.85) in the thrombopenia group and 1.31 (IQR, 0.79 – 2.78) in the non-thrombopenia group, which was also statistically significantly different ($p < 0.001$). There was a weak but significant negative correlation between the max PDMP value and the nadir platelet count ($r = -0.332$, $p < 0.001$, **Fig. 1D**).

Max PDMP Value and Max PDMP/Plts Ratio in the Patients with or without DIC

Of the 119 patients, six patients were excluded according to the JAAM DIC criteria, as thrombopenia due to other diseases was suspected (liver cirrhosis, $n = 3$; drug-induced thrombopenia, $n = 2$; heparin-induced thrombopenia, $n = 1$). Thirty-one patients fulfilled the JAAM DIC criteria during their ICU stay, and these patients were defined as the DIC group. The other 82 patients were defined as the non-DIC group. The max PDMP value was 81.48 (IQR, 39.93 – 108.24) U/ml in the DIC group and 34.88 (IQR, 18.55 – 77.19) U/ml in the non-DIC group, which was statistically significantly different ($p = 0.001$,

Table 1. Baseline characteristics of the patients. Variables are expressed as the median (interquartile range)

Variable	Total $n=119$	Survivors $n=98$	Nonsurvivors $n=21$	*P* value
Age (years)	67.0 (53.0 − 74.0)	66.5 (53.0 − 75.0)	68.0 (54.5 − 73.5)	0.842
Gender, F/M	43/76	35/63	8/13	0.837
ICU stay (days)	4.0 (2.0 − 9.0)	4.0 (2.0 − 7.3)	6.0 (3.0 − 15.5)	0.024*
DIC patients during the ICU stay	31 (26.0%)	17 (17.3%)	14 (66.7%)	<0.001*
Nadir platelet count (×10⁹/L)	127 (66 − 190)	148 (93 − 199)	33 (17 − 63)	<0.001*
The max JAAM DIC score during the ICU stay	2.0 (1.0 − 4.0): $n=13$	2.0 (1.0 − 3.0): $n=95$	7.0 (4.3 − 8.0): $n=18$	<0.001*
Data at admission to the ICU				
SIRS	87 (73.1%)	67 (68.4%)	20 (95.2%)	0.012*
APACHE Ⅱ score	12.0 (9.0 − 20.0)	11.5 (7.0 − 17.0)	20.0 (17.0 − 23.5)	<0.001*
SOFA score	4.0 (2.0 − 7.0)	4.0 (2.0 − 6.0)	12.0 (4.0 − 14.0)	<0.001*
PT-INR	1.32 (1.17 − 1.54)	1.30 (1.15 − 1.49)	1.44 (1.23 − 1.86)	0.056
Lac (mg/dl)	17.0 (11.0 − 31.0)	15.5 (9.0 − 28.0)	24.0 (17.5 − 57.5)	0.001*
AT3 (%)	65.5 (49.3 − 79.0)	68.0 (53.0 − 80.0)	57.0 (40.0 − 67.0)	0.013*
TAT (ng/ml)	8.25 (4.85 − 13.5)	7.30 (2.90 − 11.0)	13.20 (7.25 − 26.15)	0.006*
D-dimer (μg/ml)	5.10 (2.10 − 15.58)	3.80 (1.80 − 11.65)	17.70 (6.10 − 33.10)	<0.001*
Fibrinogen (mg/dl)	294.5 (202.5 − 406.0)	300.0 (209.0 − 406.0)	245.0 (174.5 − 399.5)	0.265
Cause of ICU admission				
Sepsis	26 (21.5%)	18 (18.4%)	8 (38.1%)	0.050
Post-operation	21 (17.6%)	19 (19.4%)	0 (0%)	0.030*
Cardiovascular disease	22 (18.5%)	21 (21.4%)	1 (4.8%)	0.074
Trauma	12 (10.1%)	11 (11.2%)	1 (4.8%)	0.372
Respiratory failure	5 (4.2%)	5 (5.1%)	0 (0%)	0.290
Cerebral stroke	5 (4.2%)	2 (2.0%)	3 (14.3%)	0.011*
Pancreatitis	3 (2.5%)	2 (2.0%)	1 (4.8%)	0.470
Others	25 (21.0%)	20 (20.0%)	5 (23.8%)	0.728
Underlying disease				
Hypertension	63 (52.9%)	56 (57.1%)	7 (33.3%)	0.056
Diabetes millitus	24 (20.2%)	22 (22.4%)	2 (9.5%)	0.239
Therapy				
Platelet transfusion	14 (11.8%)	4 (4.1%)	10 (47.6%)	<0.001*
Acetylsalicylic acid	27 (22.7%)	27 (27.6%)	0 (0%)	<0.001*
Clopidogrel sulfate	15 (12.6%)	15 (15.3%)	0 (0%)	0.070
Warfarin	10 (8.4%)	10 (10.2%)	0 (0%)	0.206
Statin	29 (24.4%)	27 (27.6%)	2 (9.5%)	0.098

SIRS: systemic inflammatory response syndrome, APACHE: Acute Physiology and Chronic Health Evaluation, DIC: disseminated intravascular coagulopathy, PT-INR: prothrombin time − international normalized ratio, Lac: lactate, AT3: antithrombin Ⅲ, TAT: thrombin-antithrombin complex, JAAM: Japanese Association for Acute Medicine, SOFA: Sequential Organ Failure Assessment.

Fig. 1E). The max PDMP/Plts ratio in the DIC group (median, 9.27; IQR, 4.87 − 18.74) was significantly higher than the ratio in the non-DIC group (median, 2.35; IQR, 0.86 − 4.73, *p* < 0.001, **Fig. 1F**).

Association between the PDMP Levels and Hospital Mortality

We evaluated the association between hospital mortality and gender, age, underlying disease, max PDMP value, max PDMP/Plts ratio, nadir platelet count (<150×10⁹/L), max JAAM DIC score during the ICU stay and the following values on admission: prothrombin time international normalized ratio (PT-INR), lactate, AT3, D-dimer, TAT, fibrinogen, APACHE Ⅱ score and total SOFA score. The univariate logistic regression analysis demonstrated that higher PT-INR, lactate, max JAAM DIC score, APACHE Ⅱ score, total SOFA score and max PDMP/
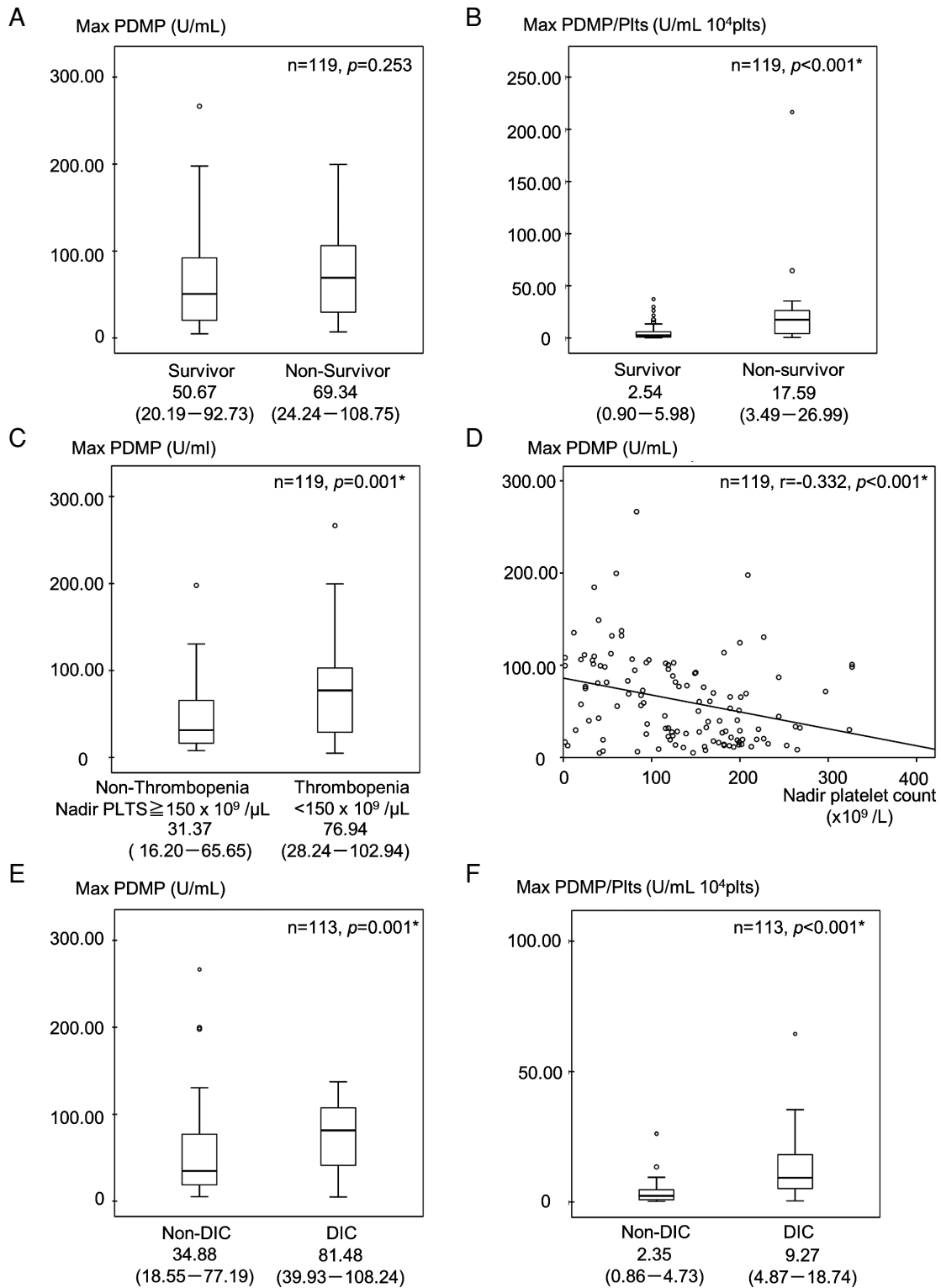
Fig. 1. The results are shown as whisker boxes (the horizontal line inside the box is the median, the upper and lower box limits are the 25-75th percentiles and the T-bars are the 10-90th percentiles)

A: Max PDMP values in the survivors and non-survivors. B: Max PDMP levels per 10^4 platelet (PDMP/Plts ratios) in the survivors and non-survivors. C: Max PDMP values in the non-thrombopenia group (nadir platelet count during the ICU stay ≥ 150,000/μl) and the thrombopenia group (nadir platelet count during the ICU stay < 150,000/μl). D: Correlation between the max PDMP value and nadir platelet count during the ICU stay. E: Max PDMP values in the non-DIC group and DIC group. F: Max PDMP/Plts ratios in the non-DIC group and DIC group.
PDMP: platelet-derived microparticle, Max: maximum, DIC: disseminated intravascular coagulopathy

Table 2. Independent predictors of hospital mortality according to the univariate logistic regression analysis

Variables	Odds ratio	95%CI	*P* value
Gender	1.108	0.419 – 2.930	0.837
Age	1.001	0.970 – 1.034	0.936
Max PDMP	1.006	0.997 – 1.016	0.167
Max PDMP/Plts	1.122	1.060 – 1.188	<0.001*
Nadir platelet count ($<150 \times 10^9$/L)	4.080	1.280 – 13.001	0.017*
The max JAMM DIC score	1.944	1.482 – 2.552	<0.001*
Data at admission			
PTINR	3.554	1.080 – 11.70	0.037*
Lac	1.029	1.009 – 1.049	0.004*
AT3	0.973	0.949 – 0.997	0.027*
D-dimer	1.029	1.010 – 1.049	0.003*
TAT	1.036	0.993 – 1.081	0.098
Fibrinogen	0.998	0.994 – 1.001	0.998
APACHE II score	1.139	1.061 – 1.223	<0.001*
SOFA score	1.305	1.161 – 1.467	<0.001*
Underlying disease			
Hypertension	0.375	0.139 – 1.011	0.053
Diabetes mellitus	0.364	0.079 – 1.683	0.196

PT-INR: prothrombin time - international normalized ratio, Lac: lactate, AT3: antithrombin Ⅲ, TAT: thrombin-antithrombin complex, JAAM: Japanese Association for Acute Medicine, APACHE: Acute Physiology and Chronic Health Evaluation, SOFA: Sequential Organ Failure Assessment, CI: confidence interval.

Table 3. Independent predictors of DIC according to the multivariate logistic regression analysis

Variables	Odds ratio	95%CI	*P* value
Max PDMP/Plts	1.206	1.012 – 1.437	0.036*
Max JAAM DIC score	2.627	0.963 – 7.165	0.059
AT3 at admission	1.149	0.994 – 1.328	0.060
APACHE II score	1.192	0.968 – 1.468	0.099

JAAM: Japanese Association for Acute Medicine, CI: confidence interval.

Plts ratio values, lower AT3 values on admission, and thrombopenia were associated with a significantly greater hazard of death (**Table 2**).

These 11 variables ($p<0.10$ in the univariate logistic analysis) were entered in a multivariate logistic regression analysis, in which hospital mortality was the independent variable. The variables in the final model are shown in **Table 3**. This model revealed that only the max PDMP/Plts ratio (OR, 1.206; 95% CI, 1.012 – 1.437, $p=0.036$) was independently associated with hospital mortality. The correct classification rate for the model was 87.3%.

Max PDMP/Plts Ratio for Predicting Hospital Mortality

ROC curves were drawn to evaluate the max PDMP/Plts ratio that could be measured using only a platelet analysis and this value was compared to common scoring systems calculated from multiple factors (APACHE II, SOFA and max JAAM DIC score). The AUC was calculated as 0.769 ± 0.067 (95% CI, 0.639 – 0.900, $p<0.001$) for the max PDMP/Plts ratio, 0.812 ± 0.048 (95%CI, 0.717 – 0.907, $p<0.001$) for the APACHE II score, 0.780 ± 0.062 (95% CI, 0.659 – 0.902, $p<0.001$) for the SOFA score and 0.826 ± 0.070 (95%CI, 0.689 – 0.964, $p<0.001$) for the max JAAM DIC score. The optimal cutoff values and positive likelihood ratio for hospital mortality are shown in **Table 4**. A ROC curve analysis was performed to compare the AUC for the max PDMP/Plts ratio with that for the APACHE II score, SOFA score and max JAAM DIC score. There were no significant

Table 4. Area under the curve (AUC), cut-off value and positive likelihood ratio calculated according to the receiver operating characteristic (ROC) curve

Variables	AUC	SE	P value	95%CI	Cut off value	Specificity	Sensitivity	Positively likelihood ratio
Max PDMP/Plts	0.769	0.067	<0.001*	0.639−0.900	6.54	0.806	0.667	3.438
Max JAAM DIC score	0.826	0.070	<0.001*	0.689−0.964	5.0	0.905	0.778	8.189
APACHE II score (at admission)	0.812	0.048	<0.001*	0.717−0.907	15.5	0.745	0.810	3.176
SOFA score (at admission)	0.780	0.062	<0.001*	0.659−0.902	6.5	0.775	0.667	2.964

AUC: area under the curve, SE: standard error, CI: confidence interval, APACHE: Acute Physiology and Chronic Health Evaluation, SOFA: Sequential Organ Failure Assessment, JAAM: Japanese Association for Acute Medicine.

differences between the max PDMP/Plts ratio and these scores (vs. APACHE II score: $p=0.509$, vs. SOFA score: $p=0.875$, and vs. the max JAAM DIC score: $p=0.448$).

Discussion

In the present study, the max PDMP/Plts ratio during the ICU stay was higher in the non-survivors than in the survivors. Moreover, the max PDMP value and max PDMP/Plts ratio were higher in the patients with DIC during their ICU stay than in those without DIC, and the max PDMP/Plts ratio was associated with hospital mortality and DIC during the ICU stay. This is the first report to demonstrate the association between the PDMP levels and patient outcomes and coagulopathy in critically ill patients.

The optimal method for measuring PDMPs remains controversial[2]. Flow cytometry (FCM) is considered the "gold standard" for PDMP analyses due to its ability to evaluate the size, surface antigens and quantity of PDMPs. In 2010, the quantification of PDMPs using FCM was standardized by the International Society for Thrombosis and Haemostasis (ISTH)[23]. According to the ISTH, PDMPs are defined as being between 0.5 and 1.0 μM in size and as annexin V + and CD41 + double positive particles. However, PDMP assays may consist of quantifying assays, functional assays and morphologic assays. Although the ISTH report was a first step towards achieving standardization of assay methods for evaluating PDMPs, studies continue to use different assay types[24-32]. Strasser and coworkers showed a good correlation among three different methods of characterizing PDMPs and suggested that an analysis method other than FCM should be used concurrently to detect MPs when the PDMP size is below the limit of detection on FCM[24]. Duarte and coworkers revealed

that the circulating levels of PDMPs found to be either CD31 +/CD42 + or CD31 +/CD42b +/ annexin V + were significantly increased in subjects with bronchial asthma compared with those without asthma[25]. Woth and coworkers showed that different surface antigens of PDMPs are elevated in subjects with or without fungal infection (CD42a)[28]. Therefore, the measurement method recommended by the ISTH, which uses only a single antigen (CD41), might be too limited for assessing PDMPs.

The ELISA method has advantages and disadvantages. Obtaining an "accurate" evaluation of PDMPs is difficult due to many concerns. Moreover, the size, roles and concentrations of PDMPs in the plasma of healthy individuals have not been adequately determined[29, 33]. Therefore, PDMP analyses should be interpreted with caution, taking into account the pitfalls of each method and purpose of the specific clinical investigation. Although FCM is a reliable method for determining the levels of surface antigens and evaluating the number of particles, it is impossible to measure the amount of surface antigens and evaluate the functional activity when using this method. In addition, FCM cannot be used to accurately enumerate MPs smaller than 0.5 μM[33]. The ELISA method used in the present study, on the other hand, employed two monoclonal antibodies against platelet glycoproteins (GP), CD42b and CD42a (GP Ib and GP IX). This method has stable reproducibility[2, 10, 21, 34]. The functional activity of PDMPs can be evaluated using this ELISA method by measuring the amount of antigens. Furthermore, both standardized size PDMPs ranging from 0.5 to1.0 μM and smaller particles (or larger particles) that cannot otherwise be detected with FCM, are detected using this assay. Although the ELISA method cannot detect the size or count the number of particles, these capabilities are advantages of this method for evaluating PDMPs.

In the present study, there was a significant association between hospital mortality and the PDMP/Plts ratio. The meaning of the PDMP/Plts ratio should be considered. A higher PDMP/Plts ratio might be a consequence of the association between thrombopenia and mortality, as previously reported[35-37]. The PDMP/Plts ratio is elevated as a result of a decreased platelet count. However, it is assumed that the PDMP level changes with the platelet count because PDMPs are released by platelets. Many previous reports have shown that there is a positive correlation between the platelet count and PDMP level as measured by ELISA and FCM in healthy individuals[38, 39]. Moreover, Joop and coworkers used FCM and suggested that the PDMP level is simply a reflection of the platelet count[16]. Therefore, according to the findings of several studies, the PDMP/Plts ratio should be a constant value, regardless of the presence of thrombopenia[40-42].

In our study using ELISA, the thrombopenia group has significantly elevated PDMP values, and there was a weak but significant negative correlation between the max PDMP value and the nadir platelet count. Therefore, the higher PDMP/Plts ratios in the non-survivors reflect a decreased platelet count as well as increased PDMPs. In addition, thrombopenia was not found to be an independent variant related to hospital mortality according to the multivariate logistic regression analysis in this study.

An elevated PDMP/Plts ratio may represent a state of activated platelets or activated form of PDMPs. The reason why our study did not show a positive correlation between the PDMP level and platelet count might be related to inflammation. For example, Andoh and coworkers reported finding no positive correlations between the PDMP level and platelet count in patients with inflammatory bowel disease[13]. In contrast, there was a positive correlation between the PDMP level and platelet count in the patients with inflammatory disease, as measured using FCM[16]. According to these studies, the amount of antigens per particle might be increased in subjects with inflammatory diseases, because the ELISA method is able to measure the amount of antigens and because the FCM method can be used to measure the number of particles. Therefore, the finding of an elevated PDMP/Plts ratio in this study has several implications. First, the number of particles too small to be detected with FCM may be increased. Second, the amount of antigens (GP Ib and GP IX) per particle is increased in subjects with DIC or a poor outcome. In both cases, the PDMP/Plts ratio in this study appeared to be a marker of platelet activation. If the amount of antigens per particle is increased in the context of stimulation or inflammation, then our results are consistent with those of other studies that reported an elevated PDMP/Plts ratio in subjects with inflammatory diseases[13, 43]. This hypothesis may account for why a healthy individual's PDMPs do not have adverse effects. Further research is needed to investigate this hypothesis.

Several limitations of our study should be mentioned. First, the manner in which blood was drawn could not be standardized. In this study, blood samples were drawn from critically ill patients via arterial catheters. Second, the ELISA kit used in this study cannot be used to identify surface markers of PDMPs other than GPIb and GPIX. Therefore, these data should be interpreted carefully because the PDMP level measured with this ELISA kit does not represent all types of PDMPs. In fact, one study that used antibodies with FCM reported that subpopulations of PDMPs reflect the platelet activation status better than the total count of PDMPs[44]. Finally, the changes in the PDMP level or PDMP/Plts ratio over time were not evaluated in the present study. However, we previously reported that the average PDMP/Plts ratio of each patient during the ICU stay is also a good predictor of hospital mortality in critically ill patients[45]. As this study was performed in a single center and the patients' backgrounds were not uniform, our results may not be applicable to other institutions. Furthermore, a subanalysis of patients was not performed according to the specific disease, as the groups would have been comprised of a relatively small number of patients. A further prospective study is thus needed to clarify the association between the changes in the PDMP level, coagulopathy and organ dysfunction over time.

In conclusion, we demonstrated that the max PDMP/Plts ratio during the ICU stay is higher in non-survivors than in survivors and that the PDMP/Plts ratio is associated with hospital mortality and coagulopathy in critically ill patients. Measuring the PDMP level is useful for monitoring coagulopathy and evaluating the severity of disease in critically ill patients by assessing platelet activation.

Conflicts of Interest

There are no conflicts of interest to declare in association with this study.

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